ROMÂNIA
UNIVERSITATEA DIN BUCUREŞTI
GRĂDINA BOTANICĂ „D. BRANDZA”

ACTA HORTI BOTANICI BUCURESTIENSIS
43

editura universității din bucurești®
2016

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

CONTENTS

Scientific papers

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 5-18
DOI: 10.1515/ahbb-2016-0001

ALIEN PLANT SPECIES TURNOVER IN CONSTANŢA HARBOR (ROMANIA) IN THE LAST DECADE

MEMEDEMIN Daniyar[1*], ANASTASIU Paulina[2], PREDA Cristina[1], NEGREAN Gavril[2], COGĂLNICEANU Dan[1]

Abstract: Previous studies indicate Constanţa Harbor as an important entrance point of alien species in Romania, defining it as an ideal place for studying alien plant species. The monitoring of vegetation over a decade (2004-2014) underlines the turnover in specific composition of alien species, many of them with invasive character. The species accumulation curve is not nearing a plateau for native species, suggesting that the inventory is incomplete, but indicates an almost complete inventory for alien species. This result is supported by the incidence-based estimators of species richness that indicate an incomplete inventory, but show a higher percentage of missing native species compared to alien species. The estimated invasion rate during for the studied period was 2 species year^{-1}. Our study highlights the efficiency of long term extensive monitoring program, as a measure for rapid detection of invasive species.
Key words: species turnover, alien plant species, harbors.

Received 02 February 2016 *Accepted 22 February 2016*

Introduction

One of the European Union strategic objectives for 2020 is represented by prevention and management of alien species (EC 2011 in Gallardo & Aldridge 2013). Becoming invasive (sensu Richardson et al. 2000) alien species can cause ecosystem degradation, loss of biodiversity and homogenization of regional biotas (Pysek & Richardson 2010, Vila et al. 2011, Macer et al. 2012, Jeshke et al. 2014).

Changes in environmental conditions and dispersal limitations generates species turnover in space and time (Whittaker 1972). The response of plant communities to this kind of changes can be quantified analyzing species turnover (Ulrich et al. 2014).

Transport corridors and especially hubs – harbors, airports, train stations – are the main routes for entrance and spreading of alien species (Bax et al. 2003, Anastasiu et al. 2011). Environmental instability and maritime traffic make the harbors more susceptible to invasion by exotic species (Çinar et al. 2006). This hypothesis was confirmed by previous studies in Constanţa harbor (Costea 1996, Anastasiu et al. 2009, 2011) that reported a high proportion of alien plant species therefore entail a continuous and sustained research of this area.

[1] University "Ovidius" of Constanța, Faculty of Natural Sciences and Agricultural Sciences, Universității Alley nr. 1, 900470 - Constanța, Romania
[2] University of Bucharest, Faculty of Biology and Botanic Garden "D. Brandza", 1-3, Intr. Portocalelor, Sector 6, 060101 – Bucureşti, Romania
[*]Correspondence: daniyar_memedemin@yahoo.com

Historical traffic values, current data and prognosis for the next years for Constanţa harbor (Fig. 1) show that despite a slowdown caused by the economic crisis, the predicted recovery period followed by rapid growth represents a major risk and is correlated with a higher probability of alien species emergence and an increasing invasion rate.

The aim of this study is to evaluate species turnover and estimate the rate of invasion, based on a decade of monitoring alien plant species in the harbor.

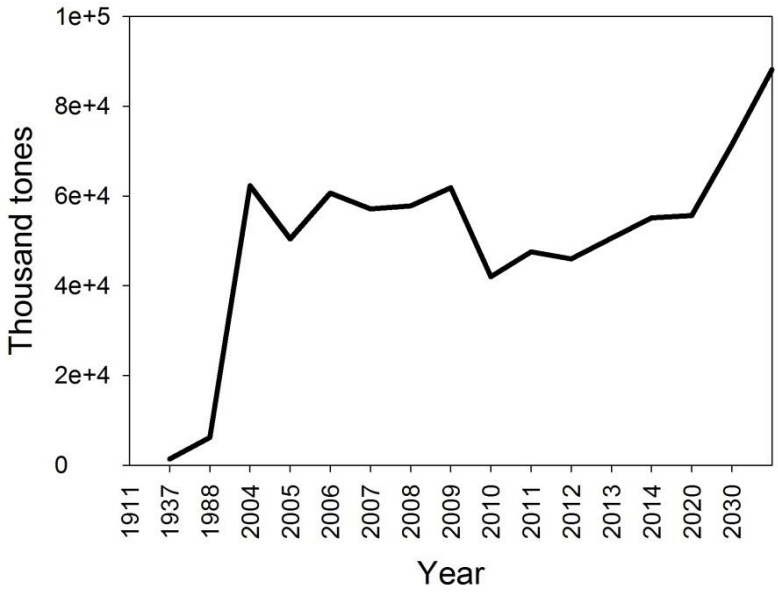

Fig. 1. Traffic values in Constanţa harbor (source: Constanţa Port Administration - http://www.portofconstantza.com/).

Material and methods

Study area. Constanţa harbor is the largest port at the Black Sea and the second, taking into account the capacity, from Europe. Together with two smaller satellite harbors, Midia and Mangalia, the Port of Constanţa (Fig. 2) can be considered the main eastern gate of Europe, both for goods, but also for alien species accidentally transported.

With an area up to 3,626 hectares, from which 1,094 inland and 2,532 waterway, the harbor has 140 functional berths, from a total number of 156.

Vegetation inventory. Based on a previous study that was considered a baseline for our inventory, we conducted repeated visits to the harbor during 2004-2014. The inventory was done along linear transects. This method was favored by existence of extended communication network (roads, railroads), which were used by following their lines. For comparing the data with previous studies (Anastasiu et al. 2011), selected areas were monitored, respectively 11 areas, seven of them in old harbor – area with most alien species abundance (Fig. 3a), three areas in oil products terminal and ore

berths – area with constant traffic (Fig. 3b), respectively one area in Constanţa Sud – Agigea – the container terminal – area with the most intense traffic (Fig. 3c).

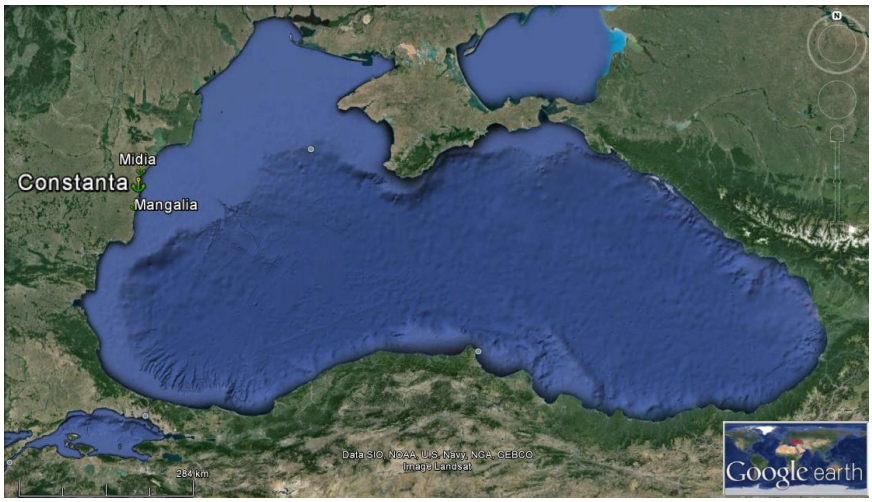

Fig. 2. The location of Constanţa harbor and its satellites, Midia and Mangalia on the western side of Black Sea. Map data: Google, SIO, NOAA, U.S. Navy, NGA, GEBCO. Image: Landsat.

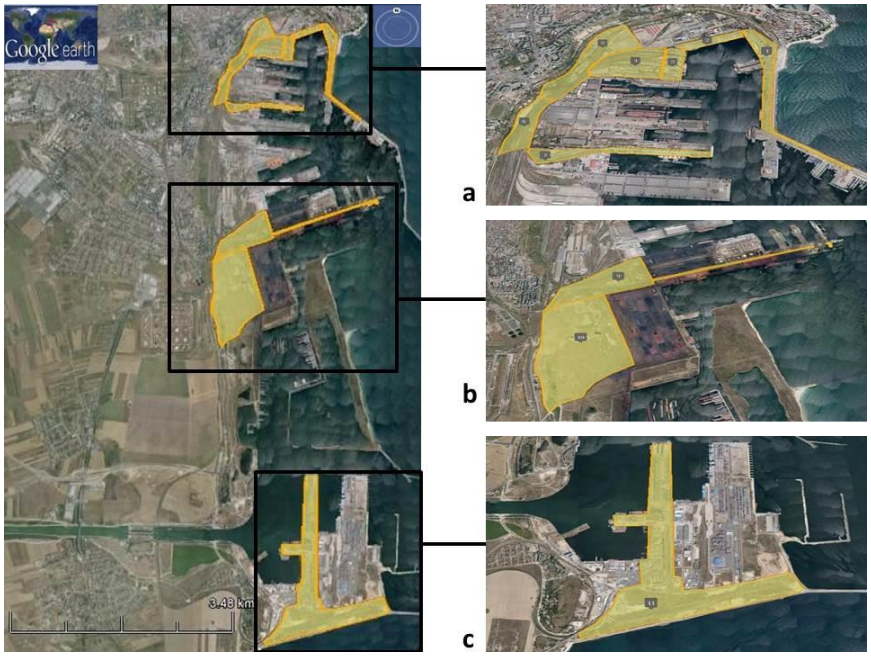

Fig. 3. Study areas in Constanţa Harbor. Map data: Google, SIO, NOAA, U.S. Navy, NGA, GEBCO. Image: Terra Metrics.

We extended the monitoring to the northern satellite Midia harbor, due to large areas of disturbed lands with high amount of alien species identified here. We did not consider the southern satellite harbor of Mangalia, because of the low traffic.

To present the invasive status for alien plant species, definitions provided by Richardson et al. (2000) were used:

Casual alien plants – Alien plants that may flourish and even reproduce occasionally in an area, but which do not form self-replacing populations, and which rely on repeated introductions for their persistence;

Naturalised plants – Alien plants that reproduce consistently and sustain populations over many life cycles without direct intervention by humans (or in spite of human intervention); they often recruit offspring freely, usually close to adult plants, and do not necessarily invade natural, seminatural or human-made ecosystems.

Invasive plants – Naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from parent plants and thus have the potential to spread over a considerable area.

Data analysis. Due to the specific activities in the harbor during the study period access was at times restricted in areas visited previously. Also the management of the vegetation in the port was not consistent over time. Thus the transects used in the inventory varied between visits. To limit the bias we decided to pool the data for each year, and use as sampling year the species inventoried during a year, disregarding the length of the transects and the number of visits.

Estimators of species richness and species accumulation curves were used to estimate if our inventory was complete and were computed using EstimateS 9.1 (Colwell 2013). We used the following species richness incidence-based estimators: Chao 2 - wich uses the number of uniques (species detected in only one sample) and duplicates (species detected in only two samples) (Chao 1987); ICE - Incidence Coverage-based Estimator of species richness, assumes that the detection probabilities vary among species) (Lee & Chao 1994); Jackknife 1 (First-order jackknife estimator) use the frequency of uniques and Jackknife 2 (Second-order jackknife estimator) uses the frequency of uniques and duplicates to estimate the number of undetected species (Burnham & Overton 1978). Bootstrap richness estimator is a reliable method related to the jackknife and has a wider applicability (Smith & van Belle 1984). We used the Bootstrap estimator as reference since it is more stable and less influenced by sample size (Smith & van Belle 1984).

Species turnover was calculated as β diversity (Magguran 2004), using Wilson and Shmida (1984) equation: $\beta_T = \frac{[g(H)+l(H)]}{2S_j}$

where g(H) = the number of species gained; and l(H) = the number of species lost.

Invasion rates were estimated by dividing the number of established non-indigenous species discovered over a decade by the length of that time interval (Ricciardi 2006).

Results and discussion

Between 2004 – 2014 we identified 526 plant species (see Annex), 412 of them native and 114 alien species (Fig. 4). We can see high species occurrence between 2004-2009. An explanation for this may be due to changes after 2010 in Constanţa harbor administration, management measures regarding natural vegetation within an

industrial area being regulated by authorities with responsibilities in emergency situations (e.g. fire). Thus, after 2010 some of the areas investigated were mowed, so the identified species list was shorter.

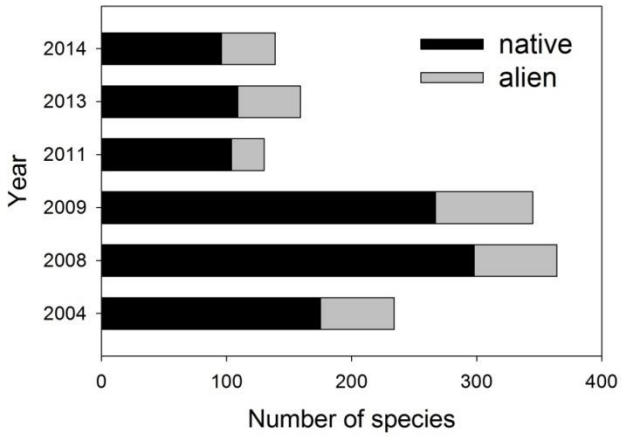

Fig. 4. Plant species from Constanţa Harbor during 2004-2014.

From 412 native species identified, 238 of them (57.76%) are incidental species, and only 67 are constant species, being inventoried in almost every year.

Regarding the alien species, from 114 species identified (Fig. 5), 14 are naturalized (12%), 57 casual (50%) and 43 (38%) of them having characteristics of invasive species. From the total number of alien species, 60 of them are incidental and only 23 species were constantly inventoried.

Our long term monitoring program enabled the identification of a new alien species for the flora of Romania, *Conyza sumatrensis* (Retz) E. Walker (Anastasiu & Memedemin 2012), providing evidence for the utility and efficiency of such a program.

Species accumulation curve (Fig. 6) for native species shows an incomplete inventory, this fact being explained by the dynamic environment of the harbor under permanent human pressure. The species accumulation curve for alien species indicates an almost complete inventory, much closer to the plateau. The patterns correspond with field observations.

The estimators of species richness support the conclusions obtained from analyzing the species accumulation curves and indicate that the inventory is still incomplete for both native and alien species, but the differences are higher for native as compared to alien species (Table 1).

Both native and alien species showed a high species turnover during the period of study. The β diversity index as a measure of species turnover had similar values for both native and alien species during the study period: 0.553 for alien species and 0.549 for native species. A positive turnover deviation indicates higher species turnover than expected by chance (Wang et al. 2013).

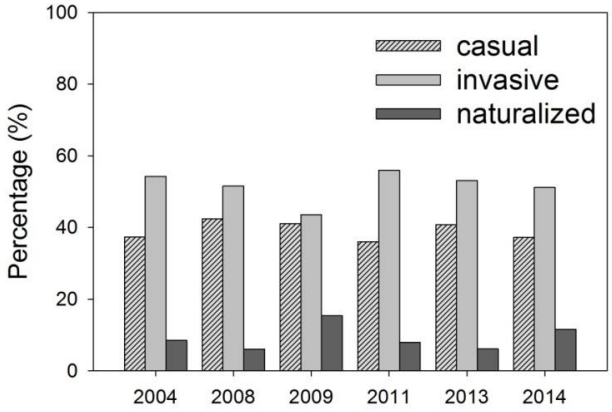

Fig. 5. Percentage of casual, invasive and naturalized plants per year.

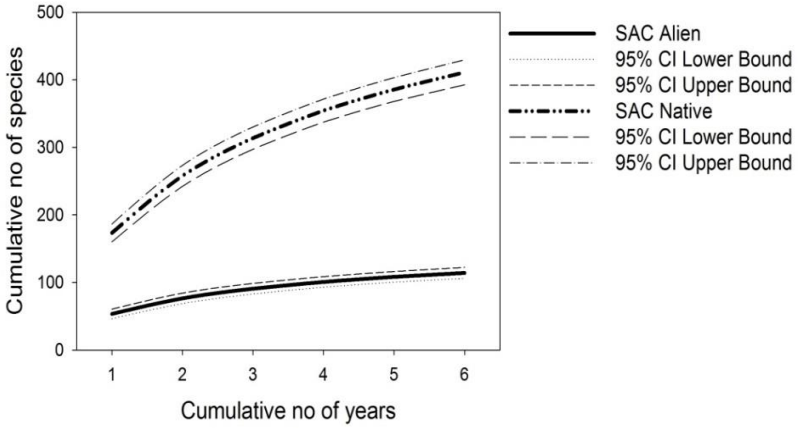

Fig. 6. Species Accumulation Curve with 95% confidence interval.

Table 1. Incidence based estimators of species richness computed separately for native and alien plant species for Constanţa harbor

Estimators	**Native species**		**Alien species**	
Observed species number	413	% increase	114	% increase
Chao 2	522	26	133	17
ICE	558	35	142	24
Jackknife 1	538	30	143	25
Jackknife 2	594	44	153	34
Bootstrap	471	14	128	12

The estimators were computed considering as sample unit years (n=6).

Invasion rate is in direct connection with rate of discovery of alien species, so an increasing discovery rate indicates that a region is becoming more invaded (Ricciardi 2006). In our case, the invasion rate, measured by dividing the number of established alien species inventoried over a decade (2004-2014) by the length of time (i.e. 10 years), was 2 species year^{-1}, having thus one new alien species every 26 week.

Comparing with one of the most invaded estuary, and possibly the most invaded aquatic ecosystem in the world – San Francisco Bay and Delta (Cohen & Carlton 1998) which has a new alien species identified every 14 week, we can conclude that, regarding alien plant species, Constanţa harbor has a medium invasion rate.

Taking into consideration the prognosis of increasing traffic in the next years, we can anticipate a stable estimated invasion rate at least. This fact sustains the necessity of monitoring and implementing an early detection and rapid response program in order to prevent, control and if possible to eradicate the invasive alien plant species.

Conclusions

Our study confirms that Constanţa harbor functions as main entrance and reservoir for alien species. Despite the long-term inventory of plants in the harbor this is still incomplete due to the dynamic environment and the frequent changes in land use that facilitate the persistence of plants. The high probability of new alien species emergence imposes the continuous monitoring of harbor areas. We also recommend the use of species accumulation curves and estimators of species richness as valuable tools to evaluate the success of a monitoring program, based on repeated inventories.

Acknowledgements. The present study was partly funded by Consiliul Naţional al Cercetării Ştiintifice din Învăţamântul Superior (CNCSIS) PNII-IDEI 273/2007 grant to Dan Cogălniceanu, PNII-IDEI 611/2008 to Paulina Anastasiu, and Esenias-Tools funded by EEA FM (2009-2014). Access permits were provided by the Constanţa Port Administration. Special thanks to Marius Skolka, Marius Făgăraş and Ioana Sîrbu that helped with fieldwork.

References

Anastasiu, P. & Memedemin, D. (2012). *Conyza sumatrensis*: a new alien plant in Romania. *Botanica Serbica*, 36(1), 37-40.

Anastasiu, P., Negrean, G., Samoilă, C., Făgăraş M. & Cogălniceanu, D. (2009). A major gateway and reservoir for alien plant species – Constanţa Harbour (Romania). *Acta Horti Botanici Bucurestiensis,* 36, 1-25.

Anastasiu, P., Negrean, G., Samoilă, C., Memedemin, D. & Cogălniceanu, D. (2011). A comparative analysis of alien plant species along the Romanian Black Sea coastal area. The role of harbours. *Journal of Coastal Conservation*, 15, 595-606.

Bax, N., Williamson, A., Aguero, M., Gonzalez, E. & Geeves, W. (2003). Marine invasive alien species: a threat to global biodiversity. *Marine policy*, 27(4), 313-323.

Burnham, K.P. & Overton, W.S. (1978). Estimation of the size of a closed population when capture probabilities vary among animals. *Biometrika*, 65, 625–633.

Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, 43, 783–791.

Çinar, M.E., Katagan, T., Öztürk, B., Egemen, Ö., Ergen, Z., Kocatas, A., Onen, M., Kirkim, F., Bakir, K., Kurt, G., Dagli, E., Kaymakci, A., Acik, S., Dogan, A. & Ozcan, T. (2006). Temporal changes of soft-bottom zoobenthic communities in and around Alsancak Harbor (Izmir Bay, Aegean Sea), with special attention to the autecology of exotic species, *Marine Ecology*, 27(3), 229-246.

Cohen, A. N., & Carlton, J. T. (1998). Accelerating invasion rate in a highly invaded estuary. *Science*, 279(5350), 555-558.

Constantza Port Administration (2009). Constantza Port Handbook 07-08.

Costea, M. (1996). The recording of some new adventive taxa for Romania in the harbour Constanţa. *Rev. Roum. Biol. Veg.*, 41, 91–96;

Gallardo, B., & Aldridge, D.C. (2013). Priority setting for invasive species management: risk assessment of Ponto-Caspian invasive species into Great Britain. *Ecological Applications*, 23(2), 352-364.

Jeschke, J.M., Bacher, S., Blackburn, T.M., Dick, J.T., Essl, F., Evans, T., Gaertner, M., Hulme, P.E., Kuhn, I., Mrugala, A., Pergl, J., Pyšek, P., Rabitsch, W., Ricciardi, A., Richardson, D.M., Sendek, A., Vila, M., Winter, M. & Kumschick, S. (2014). Defining the Impact of Non-Native Species. *Conservation Biology*, 28(5), 1188-1194.

Lee, S.M. & Chao, A. (1994). Estimating population size via sample coverage for closed capture-recapture models. *Biometrics*, 50, 88–97.

Magurran, A.E. (2004). *Measuring Biological Diversity*. Padstow, UK: Blackwell Publishing.

Marcer, A., Pino, J., Pons, X. & Brotons, L. (2012). Modelling invasive alien species distributions from digital biodiversity atlases. Model upscaling as a means of reconciling data at different scales. *Diversity and distributions*, 18(12), 1177-1189.

Pyšek, P., & Richardson, D.M. (2010). Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources*, 35, 25-55.

Ricciardi, A. (2006). Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions*, 12, 425–433.

Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D. & West, C.J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distribution*, 6, 93-107.

Smith, E. P. & van Belle, G. (1984). Nonparametric Estimation of Species Richness. *Biometrics*, 40(1), 119–129.

Ulrich, W., Soliveres, S., Maestre, F.T., Gotelli, N. J., Quero, J.L., Delgado-Baquerizo, M., … Zaady, E. (2014). Climate and soil attributes determine plant species turnover in global drylands. *Journal of Biogeography*, 41(12), 2307–2319.

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y.& Pyšek, P. (2011). Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology letters*, 14(7), 702-708.

Wang, S., Wang, X., Guo, H., Fan, W., Lv, H., & Duan, R. (2013). Distinguishing the importance between habitat specialization and dispersal limitation on species turnover. *Ecology and Evolution*, 3(10), 3545–3553.

Wilson, M. & Shmida, A. (1984). Measuring beta diversity with presence-absence data. *Journal of Ecology*, 72, 1055-1064.

Whittaker, R. H. (1972). Evolution and measurement of species diversity. *Taxon*, 213-251.

Colwell, R.K., 2013. Estimates: Statistical Estimation of Species Richness and Shared Species from Samples. Version 9. 2013. User's Guide and application. Retrieved January 25, 2016 from: http://purl.oclc.org/estimates

Constantza Port Administration. Annual reports 2004 – 2014. Retrieved January 11, 2016 from: http://www.portofconstantza.com

https://www.google.com/earth/

Annex. List of alien species identified in Constanţa Harbor

Species	Family	Invasive status	Origin	Persistance (years)
Acer negundo L.	Aceraceae	I	North America	5
Aesculus hippocastanum L.	Hippocastanaceae	C	Balcanic Peninsula	3
Ailanthus altissima (Mill.) Swingle	Simaroubaceae	I	China	6
Alcea rosea L.	Malvaceae	I	Crete Isl & Eastern Europe	2
Alopecurus myosuroides Huds.	Poaceae	I	Atlantic-mediteranean	1
Amaranthus albus L.	Amaranthaceae	I	North America	4
Amaranthus crispus (Lesp. Et Thevenau) N. Terracc.	Amaranthaceae	I	South America	1
Amaranthus hybridus L.	Amaranthaceae	I	America	1
Amaranthus lividus L.	Amaranthaceae	N	Mediteranean	3
Amaranthus palmeri S. Watson	Amaranthaceae	N	North America	4
Amaranthus powellii S. Watson	Amaranthaceae	N	North America	1
Amaranthus retroflexus L.	Amaranthaceae	I	North America	5
Amaranthus rudis Sauer	Amaranthaceae	N	North America	2
Ambrosia artemisiifolia L.	Asteraceae	I	North America	6
Ambrosia trifida L.	Asteraceae	I	North America	6
Amorpha fruticosa L.	Fabaceae	I	North America	4
Anethum graveolens L.	Apiaceae	C	SW Asia	2
Antirrhinum majus L.	Scrophulariaceae	C	Mediteranean	1
Apium graveolens subsp. *graveolens* L.	Apiaceace	C	Atlantic-mediteranean	1
Artemisia annua L.	Asteraceae	I	Temperate Asia - SE Europe	6
Atriplex hortensis L.	Chenopodiaceae	C	Asia	2
Avena sativa L.	Poaceae	C	Asia Minor	3
Bassia scoparia (L.) A.J. Scott	Chenopodiaceae	I	Asia and Eastern Europe	6
Bidens frondosa L.	Asteraceae	I	North America	1
Brassica rapa subsp. *oleifera* DC.	Brassicaceae	C	Mediteranean	5
Brassica rapa subsp. *sylvestris* (L.) Janchen	Brassicaceae	N	Atlantic-mediteranean	1
Bromus madritensis L.	Poaceae	C	Mediteranean	4

Bromus rigidus Roth	Poaceae	C	Mediteranean	1
Bromus willdenowii Kunth	Poaceae	C	South America	3
Calendula officinalis L.	Asteraceae	C	Mediteranean	4
Catalpa bignonioides Walter	Bignoniaceae	C	North America	1
Cercis siliquastrum L.	Caesalpiniaceae	C	Mediteranean	1
Chamomilla suaveolens (Pursh) Rydb.	Asteraceae	N	N-E Asia and North America	1
Chenopodium ambrosioides L.	Chenopodiaceae	I	Tropical America	1
Chenopodium botrys L.	Chenopodiaceae	C	Southern Europe and Western Asia	2
Chloris barbata Sw.	Poaceae	C	Tropical America	1
Citrullus lanatus (Thunb.) Mansf.	Cucurbitaceae	C	Western Kalahari	3
Cladium mariscus subsp. *martii* (Roem. et Schult.) Soó	Cyperaceae	N	Mediteranean-Central Asia	1
Commelina communis L.	Commelinaceae	N	Temperate Asia	1
Conyza canadensis (L.) Cronquist	Asteraceae	I	North America	6
Conyza sumatrensis (Retz.) E. Walker	Asteraceae	C	South America	3
Cucurbita pepo L.	Cucurbitaceae	C	North America	1
Cuscuta campestris Yunck.	Convolvulaceae	I	North America	6
Cuscuta suaveolens Ser.	Convolvulaceae	N	South America	
Cydonia oblonga Mill.	Rosaceae	C	SW Asia	1
Datura innoxia Mill.	Solanaceae	C	Southern and Central America	2
Datura stramonium L.	Solanaceae	I	Unknown	5
Echinocystis lobata (Michx.) Torr. & A. Gray.	Cucurbitaceae	I	North America	1
Elaeagnus angustifolia L.	Elaeagnaceae	I	Asia	6
Eleusine indica (L.) Gaertn.	Poaceae	I	Tropical and subtropical Asia	5
Erigeron annuus (L.) Pers.	Asteraceae	I	North America	5
Euonymus japonicus Thunb.	Celastraceae	C	Asia	1
Euphorbia maculata L.	Euphorbiaceae	I	North America	4
Fallopia aubertii (L. Henry) Holub	Polygonaceae	C	Eastern Asia	1
Ficus carica L.	Moraceae	C	SV Asia	1
Foeniculum vulgare Mill.	Apiaceae	N	Mediteranean and western Asia	1

Fragaria × *ananassa* Duchesne	Rosaceae	C	Hybrid	2
Fraxinus americana L.	Oleaceae	I	North America	2
Fraxinus pennsylvanica Marshall	Oleaceae	I	North America	3
Galinsoga parviflora Cav.	Asteraceae	I	South America	3
Gleditsia triacanthos L.	Caesalpiniaceae	I	North America	4
Helianthus annuus L.	Asteraceae	C	North and Central America	6
Helianthus tuberosus L.	Asteraceae	I	North America	3
Hemerocallis fulva (L.) L.	Liliaceae	C	Eastern Asia	1
Hibiscus syriacus L.	Malvaceae	C	Eastern and Southern Asia	2
Hordeum distichon L.	Poaceae	C	Eastern Asia	2
Hordeum marinum Huds.	Poaceae	N	Atlantic-mediteranean	1
Hordeum murinum subsp. *murinum* L.	Poaceae	C	Atlantic-mediteranean	5
Hordeum vulgare L.	Poaceae	C	Middle East	4
Impatiens balsamina L.	Balsaminaceae	C	South-eastern Asia	1
Ipomoea hederacea Jacq.	Convolvulaceae	N	Tropical America	2
Ipomoea lacunosa L.	Convolvulaceae	N	North America	2
Ipomoea purpurea (L.) Roth	Convolvulaceae	C	Tropical America	4
Iris germanica L.	Iridaceae	N	East-mediteranean	1
Iva xanthifolia Nutt.	Asteraceae	I	North America	5
Juniperus virginiana L.	Cupressaceae	C	North America	2
Koelreuteria paniculata Laxm.	Sapindaceae	C	Eastern Asia	2
Lemna minuta Kunth	Lemnaceae	I	North America	2
Lepidium virginicum L.	Brassicaceae	I	North America	3
Lonicera japonica Thunb.	Caprifoliaceae	C	Eastern Asia	2
Lycium barbarum L.	Solanaceae	C	Eastern Asia (China)	2
Lycopersicon esculentum L.	Solanaceae	C	Southern and Central America	3
Maclura pomifera (Raf.) Schneid.	Moraceae	C	North America	1
Malus domestica Borkh.	Rosaceae	C	Hybrid	4
Medicago falcata L.	Fabaceae	N	Eurasia	2
Medicago sativa subsp. *sativa* L.	Fabaceae	N	Central and Western Asia	1

Mentha × piperita L.	Lamiaceae	N	Hybrid	2
Mentha spicata L.	Lamiaceae	N	Hybrid	1
Mirabilis jalapa L.	Nyctaginaceae	C	Mexic	1
Morus alba L.	Moraceae	I	Eastern Asia (China)	4
Nicotiana alata Link & Otto	Solanaceae	C	South America	2
Oenothera biennis L.	Onagraceae	N	North America	1
Oxalis corniculata L.	Oxalidaceae	I	Southern Europe/Eastern Asia	3
Oxalis stricta L.	Oxalidaceae	I	North America	3
Panicum capillare L.	Poaceae	I	North America	1
Panicum dichotomiflorum Michx.	Poaceae	N	North America	2
Parthenocissus inserta (Kerner) Fritsch	Vitaceae	I	North America	5
Parthenocissus tricuspidata (Siebold & Zucc.) Planch.	Vitaceae	N	Japonia and China	2
Petroselinum crispum (Mill.) Fuss	Apiaceae	N	East Mediteranean	1
Petunia × atkinsiana D.Don	Solanaceae	C	Hybrid	1
Phalaris canariensis L.	Poaceae	C	Mediteranean	1
Phytolacca americana L.	Phytolaccaceae	I	North America	1
Prunus armeniaca L.	Rosaceae	C	Central Asia	4
Prunus cerasus L.	Rosaceae	C	Asia Minor and Caucasus	1
Prunus persica (L.) Batsch	Rosaceae	C	Tibet, W China	3
Raphanus sativus L.	Brassicaceae	C	Probably hybrid	2
Robinia pseudacacia L.	Fabaceae	I	North America	6
Salvia splendens Sellow ex Schult.	Lamiaceae	C	South America	1
Satureja hortensis L.	Lamiaceae	C	Mediteranean	1
Setaria faberi Herrm.	Poaceae	C	Eastern Asia	2
Solanum carolinense L.	Solanaceae	N	North America	1
Solanum tuberosum L.	Solanaceae	C	South America	1
Sophora japonica L.	Fabaceae	C	Eastern Asia	4
Sorbaria sorbifolia (L.) A. Braun	Rosaceae	C	Asia	1
Sorghum bicolor (L.) Moench.	Poaceae	C	South-saharian Africa	1
Sorghum halepense (L.) Pers.	Poaceae	I	North Africa , Asia Minor	6
Tagetes patula L.	Asteraceae	C	Mexic	1

Tecoma radicans (L.) Juss.	Bignoniaceae	C	North America	3
Thuja occidentalis L.	Cupressaceae	C	North America	1
Thuja orientalis L.	Cupressaceae	C	Eastern Asia	2
Trigonella caerulea (L.) Ser.	Fabaceae	N	Mediteranean	1
Triticum aestivum L.	Poaceae	C	Middle East	5
Ulmus pumila L.	Ulmaceae	I	Central and esatern Asia	3
Veronica persica Poiret	Scrophulariaceae	I	South-western Asia	3
Viola × *wittrockiana* Gams	Violaceae	C	Hybrid	1
Vitis vinifera L.	Vitaceae	N	SW Asia and Mediteranean	6
Xanthium orientale subsp. *italicum* (Moretti) Greuter	Asteraceae	I	America	6
Xanthium spinosum L.	Asteraceae	I	South America	3
Zea mays L.	Poaceae	C	Central America	4

Invasive status: C – casual; N – naturalized; I – invasive.
Persistance was estimated as the number of years during the inventory period that the species was present, maximum number is 6 years.

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 19-30
DOI: 10.1515/ahbb-2016-0002

GROWTH PERFORMANCE AND BIOCHEMICAL ANALYSIS OF *LYNGBYA* SP. BDU 90901 UNDER DIFFERENT NITRATE CONCENTRATIONS

VEENA Dalavai[1], PARAMAGEETHAM Chinthala[1*], PRASADA BABU Gundala[1], JAYA KUMAR Kannali[1], CHARAN TEJA Pindi[1], SWARNA KUMARI Gajula[1]

Abstract: The present research work was carried out for assessing the optimum culture conditions for the growth and chemical constituents of *Lyngbya* sp. BDU 90901. The blue green bacteria belonging to Cyanobacteria (*Lyngbya* sp. BDU 90901) was obtained from NFMC, Bharatidasan University, exposed to different nutrient concentrations of nitrogen to claim the biologically effect of proteins, glucose and pigmentation. Growth medium of ASN III was used for vegetative control growth, while experimental growth was performed under different nitrate concentrations. The applied concentrations were 0, ½, 1, 1½ and 2 folds of nitrate concentrations of original ASNIII medium. The metabolites considered for biochemical analysis were pigmentation, protein and glucose content. Optical density was considered for growth parameter and so deliberated from 0^{th} day to 10^{th} day and intervals of 5 days up till 30^{th} day. Under different nitrate concentrations, on 15^{th} day, the rise in chlorophyll-a and carotenes was noticed in 1 and 2 folds respectively, the increment in protein content was 1 fold, the glucose content was high in ½ and 1 fold on the 20^{th} day.

Key words: Marine *Cyanobacteria*, nitrate availability, glucose, proteins and pigment analysis.

Received 18 July 2016 *Accepted 16 October 2016*

Introduction

Cyanobacteria is the major group of bacteria that occurs throughout the world. They are also known as blue-green algae. These photosynthetic prokaryotes are found in almost aquatic and terrestrial environment (Castenholz & Waterbury 1989).They show specify growth pattern in a specific environment and therefore the distribution, ecology, periodicity, qualitative and quantitative occurrence of *Cyanobacteria* differ widely. They are pioneer oxygen prototroph on earth whose distribution around the world is surpassed only by bacteria. Their diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colorless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkylophilic, planktonic to barophilic, fresh water to marine including hyper saline (Yoo et al. 1995, Broady 1996, Thajuddin & Subramanian 2005). However their abilities to survive under adverse conditions by forming resting spores, opportunistically colonizing microhabitats and surviving under conditions of high UV-flux through production of UV - absorbing

[1] Department of Microbiology, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.
[*] Correspondence: paramageetham@yahoo.com

pigments, has made them one of the most successful life forms on Earth. It can flourish well either in nutrient rich and warm water or at times in water with apparently low nutrient concentrations, subjected to high temperature and bright light conditions (Bhatnagar 2008). *Cyanobacteria* offer a greatest opportunity as these are considered to be one of the potential organisms useful to mankind in many ways. Blue green bacteria (BGA) have simple metabolic requirement and occupy a wide range of ecological niche. In global programs, the focus has been identifying their antifungal, antiviral, antimitotic, antihelmienthic, anticoagulant, haemagglutinating and toxic metabolites (Kumar et al. 2003, Thajuddin & Subramanian 2005, Ali et al. 2008). The extensively utilized pigments in bioindustry, are the phycobiliproteins, which account for about 20% of total dry weight of many *Cyanobacteria* (Prasanna et al. 2007). Carotenoids are the most common, naturally occurring terpenoid pigments. They carry out important functions in photosynthesis, nutrition and protection against oxidative damage. Most common carotenoids in cyanobacteria are β-carotene, zeaxanthin, ketocarotenoid, echinenone and myxoxanthophyll (Britton et al. 2008). *Cyanobacteria* that produce numerous chemicals including nitrogen containing compounds, polyketides, lipopeptides, cyclic peptides and many others (Shimizu 2003). Several strains of *Cyanobacteria* were found to accumulate polyhydroxyalkanoates, which can be used as a substitute for non biodegradable petrochemical- based plastics. Recent studies showed that oil polluted sites are rich in cyanobacterial consortia capable of degrading oil components (Kumar et al. 2009, Thenmozhi et al. 2011). *Cyanobacteria* are an extremely diverse group of gram negative prokaryotes showing diversity in physiology, morphology, developmental characteristics and habitats (Whitten et al. 2000). *Cyanobacteria* photosynthesis provides oxygen, a key electron acceptor to the pollutant degrading mechanism (Subashchandrabose et al. 2011). *Cyanobacteria* show effective bioremediation of metals (Kumar et al. 2011, Rajeshwari et al. 2011) aromatic compounds (Kumar et al. 2009) organic or recalcitrant pollutants, achieving enhanced rates of degradation and ensuring better survival. Most *Cyanobacteria* are obligate photoautotrophs, but some species can grow as heterotrophs in the dark at the expense of glucose, fructose or sucrose. Under anaerobic conditions, some species can perform lactate fermentation (Oren & Shilo 1979). Nitrogen fixation occurs both in heterocystous *Cyanobacteria* and in some non-heterocystous *Cyanobacteria*. To avoid contact of nitrogenase with oxygen (and then its permanent inactivation) these latter *Cyanobacteria* adopt a temporal separation between the photosynthetic and the nitrogen fixation processes (Bergmann et al. 1997). Increased respiration rates allow to control the oxygen concentration inside the cell, due to diffusion, necessary to carry out cell metabolism. In heterocystous forms, the nitrogen fixation process is spatially separated from the oxygenic photosynthesis. Nitrogen fixation is carried out in specialized cells, the heterocysts (Adams & Duggan 1999). These have many characteristics that allow to reduce diffusion of oxygen, such as a thick cell wall surrounded by a complex external envelope and a reorganization of the photosynthetic apparatus: lack of PS II to avoid internal oxygen production, presence of PS I to obtain ATP through cyclic photophosphorylation. Reducing power is obtained from vegetative cells in the form of sugars. Molecular nitrogen is fixed into ammonia and immediately converted to organic form, usually as glutamine. As nitrogen fixation is a very energy - consuming process,

nitrogenase is produced and heterocysts are differentiated only in the absence of combined nitrogen in the environment surrounding the cell.

Material and methods

The marine cyanobacterial culture (*Lyngbya* sp. BDU 90901) was procured from National Facility of Marine Cyanobacteria (NFMC), Bharatidasan University, Tiruchirapalli, Tamil Nadu. The pure cultures were maintained on ASNIII medium (Table 1) at 24±2°C under a light intensity of 1500 lux and light and dark cycles of 16:8 hrs. Then the cultures were inoculated in ASNIII medium under different nitrate concentrations 0N (0g/L), ½N (0.75g/L), 1N (1.5g/L), 1½N (2.25g/L) and 2N (3.0g/L) medium at 24±2°C under a light intensity of 1500 lux and light and dark cycles.

Table 1 Composition of ASNIII Medium (g/l)

S.No.	Chemical Component	g/L
1	$NaCl_2$	2.5
2	$MgCl_2.6H_2O$	2
3	KCl	0.5
4	**$NaNO_3$** *	-
5	$K_2HPO_4.3H_2O$	0.02
6	$MgSO_4.H_2O$	3.5
7	$CaCl_2.2H_2O$	0.5
8	Citric acid	0.003
9	Ferric ammonium citrate	0.003
10	EDTA(disodium salt)	0.00055
11	$NaNO_3$	0.02
12	Trace metal mix	1ml
13	Distilled water	1000ml
14	pH	7.5
	Trace metal mix (g/l):-	
1	H_3BO_3	2.86
2	$MnCl_2.4H_2O$	1.81
3	$ZnSO_4.7H_2O$	0.222
4	$Na_2MoO4.2H_2O$	0.39
5	$CuSO_4.5H_2O$	0.079
6	$Co(NO_3)_2.6H_2O$	0.0494

Note - * mark indicates **$NaNO_3$** was added in different concentrations in the ASN III medium.

Estimation of Chlorophyll-a and Carotenoids (Sinetova et al. 2012). A volume of 1ml of cyanobacterial culture was taken in centrifuge tubes. Cells were centrifuged at 15000rpm at laboratory temperature for 7 minutes and thoroughly supernatant was discarded. 1ml of methanol was added and the sample was homogenized by vortexing and the samples were incubated at 4°C for 7 minutes. The samples were centrifuged at 15000 rpm for 7 minutes the pellet was bluish purple colour. Triplicates were maintained for calculations of average and standard deviations. The pigment concentration was measured by using methanol as blank by spectrophotometer at 420nm, 665nm and 720nm.

The concentration of chlorophyll-a and carotenoids was calculated by using the formula,

Chl a [µg/ml] = 12.9447 (A_{665} - A_{720})

Carotenoids [µg/ml] = [1000 (A_{470} - A_{720}) - 2.86 (Chl a [µg/ml] / 221

Estimation of glucose content (Plumner 1987). A volume of 1ml of cyanobacterial culture was taken in a test tube, 2ml of distilled water and 1ml of dinitrosalicyclic acid reagent was added. The test tubes were boiled for 5 minutes in hot water bath. The mixture was cooled to room temperature and the level of extinction was measured at 540nm. The total glucose content was calculated using a standard curve established with a glucose solution.

Estimation of total soluble protein content (Lowry et al. 1951; Herbert et al. 1971). Preparation of reagents:

A. 1N sodium hydroxide solution

B. (i) 5% sodium carbonate solution
   (ii) 0.5% copper sulphate solution in 1% sodium potassium tartrate.
   2ml of reagent B (ii) was mixed with 50ml of freshly prepared reagent B (i).

C. 1N Folin - Ceiocalteau reagent

A volume of 0.5 ml of homogenized cyanobacterial suspension was taken in test tubes. 0.5 ml of reagent A was added and boiled for 10minutes and cooled in running tap water. 2.5 ml of reagent B was added in each tubes and incubated at room temperature for 10 minutes. 0.5 ml of regent C was added and the tubes were kept at room temperature for 15 minutes. The intensity of blue colour was read as absorbance at 650nm using appropriate blank. The protein content was estimated using standard calibration curve prepared from bovine serum albumin and expressed in terms of mg/ml.

Results and discussion

The *Lyngbya* sp. BDU90901 is a filamentous nitrogen fixing, marine *Cyanobacteria.* In marine environments living organisms are exposed to nutrient limitation, light intensity and quality, temperature, pH, salinity, draught, pollution etc. Among the nutrients, nitrogen is an essential major element required for the synthesis of various components. Nitrogen is a critical nutrient required for the growth of all organisms. The biological substances such as peptides, proteins, enzymes, chlorophylls, energy transfer molecules (ADP, ATP) and genetic materials such as RNA, DNA contain organic nitrogen (Harisson et al. 1990). Algae play an important role in converting inorganic nitrogen to its organic form through assimilation. Nitrate and nitrite undergoes reduction with the assistance of nitrate reductase and nitrite reductase, respectively. Most *Cyanobacteria* are nitrogen fixers, converting atmospheric nitrogen to ammonia via nitrogenase enzyme (Postgate 1987). The element nitrogen constitutes about 5-10% of the dry weight of a cyanobacterial cell. Nitrogenase reduces nitrogen into ammonia which in turn is metabolized to glutamine in the presence of enzyme glutamine synthetase. Now this glutamine is then transported from heterocyst to the adjacent vegetative cell, where it is converted into glutamate by the enzyme glutamine oxoglutarate aminotransferase. Both glutamine and glutamate, by various transamination reactions from other aminoacids. The fixed nitrogen may be utilized in number of ways like it is assimilated by the *Cyanobacteria* themselves. Soluble

nitrogenous compounds are liberated from healthy cells into the culture medium and after death the nitrogenous compounds are broken down into ammonia, which is eventually converted into nitrate by nitrifying bacteria. During development of heterocyst three DNA excision events take place that allows expression of nitrogen fixing genes. *Cyanobacteria* are versatile tetrapyrrole synthesizers that are able to produce end products representing all major branches of the tetrapyrrole biosynthetic pathway: hemes, chlorophylls, phycobilins and siroheme. *Cyanobacteria* have an elaborated and highly organized system of internal membranes which function in photosynthesis (thylakoids) (Stainer & Cohen – Bazire 1977; Castenholz & Waterbury, 1989). The lipophilic pigments chlorophyll-a (both reaction centers and antenna) and photosynthetic carotenoids are located within the thylakoids, while the hydrophilic antenna pigments are located in the phycobilisomes which are attached to the outside of the thylakoid membranes. Carotenoids comprise the largest class of naturally occurring pigments in organisms. More than 640 carotenoids have been identified to date. Carotenoids in *Cyanobacteria* have two main functions: they serve as light-harvesting pigments in photosynthesis and they protect against photooxidative damage. The reserve carbohydrate is glycogen (Stainer & Cohen – Bazire 1977. Castenholz & Waterbury 1989). *Cyanobacteria* also contain cyanophycin, a nitrogen reserve polymer made of arginine and aspartic acid, polyphosphate granules and carboxysomes, that are a cell reserve of the photosynthesis key enzyme rubisco (ribulose1, 5- phosphate carboxylase). Some *Cyanobacteria* also contain poly beta hydroxyl butyrate granules. Photosynthesis in *Cyanobacteria* uses water as an electron donor and produces oxygen as a byproduct. The marine *Cyanobacteria*, *Lyngbya* sp. BDU 90901 pure cultures were maintained in different nitrate concentrations of ASNIII medium (Table 1), on 0^{th} day (Fig. 1), 5^{th} day (Fig. 2), 10^{th} day (Fig. 3), 15^{th} day (Fig. 4), 20^{th} day (Fig. 5), 25^{th} day (Fig. 6) & 30^{th} day (Fig. 7), revealed that, the increment in protein content was observed in 1 fold on 15^{th} day (Fig. 11), the glucose content was high in ½ and 1 fold on 20^{th} day (Fig.10), the rise in chlorophyll-a (Fig. 8) and carotenes (Fig. 9) was noticed in 1 and 2 folds on 15^{th} day respectively. Nitrate is probably the most abundant source of combined nitrogen for cyanobacterial nutrition. The assimilation of nitrate by *Cyanobacteria* involves nitrate uptake and reduction of intracellular nitrate via nitrite to ammonium, which is the N form incorporated into organic compounds. Nitrite, which can also fulfill the N requirement of *Cyanobacteria*, is taken up into the cell and then reduced to the level of ammonium. Many *Cyanobacteria* are able to grow at the expense of atmospheric nitrogen under aerobic conditions and many more are able to perform nitrogen fixation when anaerobic conditions are provided experimentally. Given the widespread distribution in nature of these microorganisms, it is believed that *Cyanobacteria* contribute significantly to the process of biological nitrogen fixation and thus participate in restoring to the soil combined- nitrogen lost through denitrification.

Conclusions

The *Cyanobacteria* mainly use inorganic compounds like nitrate, ammonium and dinitrogen to fulfill their N requirements, but urea and other organic sources of N, such as aminoacids, can also be assimilated by some *Cyanobacteria*. The availability of nitrogen is a key factor in regulating survival of *Cyanobacteria.* The present work concluded that the *Lyngbya* sp. *BDU* 90901grown under different nitrate concentrations,

showed an increment in protein, glucose, chlorophyll-a and carotenes mainly in ½, 1 and 2 folds of nitrate than that of 0 fold. So it is easy to perceive that the growth and rise in biochemical components was observed in the presence of nitrate when compared to that of the absence of nitrate. Thus this study indicates that the nitrogen is a vital element for the growth of *Lyngbya* even it fixes nitrogen.

Acknowledgments. This research was financially supported by the Department of Science and Technology (DST Inspire program), New Delhi, India.

References

Adams, D.G. & Duggan, P.S. (1999). Heterocyst and akinete differentiation in cyanobacteria. Tansley review No. 107. *New Phytologist*, 144, 3-33.

Ali, D.M., Kumar, V.T. & Thajuddin, N. (2008). Screening of some selected hypersaline cyanobacterial isolates for biochemical and antibacterial activity. *Indian Hydrobiology*, 11(2), 241 – 246.

Bergmann, B., Gallon, J.R., Rai, A.N. & Stal, L.J. (1997). Nitrogen fixation by non heterocystous cyanobacteria. *FEMS Microbiol. Rev.*, 19, 139-185.

Bhatnagar, A., Makandar, M.B., Garg, M.K. & Bhatnagar, B. (2008)**.** Community structure and diversity of cyanobacteria and green algae in the soils of Thar Desert (India). *J Arid Environ*, 72, 73-83.

Britton-Simmons, K.H. & Abbott, K.C. (2008). Short- and long-term effects of disturbance and propagule pressure on biological invasion. *Journal of Ecology*, 96, 68-77.

Broady, P.A., Garrick, .R & Anderson, G.M. (1996). Diversity, distribution and dispersal of Antartic terrestrial algae. *Biodiversity Conserv.*, 5, 1307-1335.

Castenholz, R.W., & Waterbury, J.B. (1989). Oxygenic photosynthetic bacteria, group É. Cyanobacteria pp.1710–1728.. In Staley, J. N. et al. (eds.). *Bergey's Manual of Systematic Bacteriology*. Baltimore: Williams and Wilkins Co.

Harisson, P.J., Thompson, P.A. & Calderwood, G.S. (1990). Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *Journal of Applied Phycology*, 2(1), 45-56.

Heimsath, A.M. & Dietrich, W.E. (1995). Process-based model linking pocket gopher (Thomomys bottae) activity to sediment transport and soil thickness. *Geology*, 33(11), 917-920.

Herbert, D., Phipps, P.J. & Strange, R.E. (1971). Methods in Microbiology, vol. 5B, pp. 284-291. NewYork: Academic Press.

Koksharova, O.A., Klint, J. & Rasmussen, U. (2007). Comparative proteomics of cell division mutants and wild-type of *Synechococcus* sp. strain PCC 7942. *Micobiology*, 153, 2505– 2517.

Kumar, K., Lakshmanan, A. & Kannaiyan, S. (2003). Bioregulatory and therapeutic effect of bluegreen algae., *Indian Journal of Microbiology*, 43(1), 9–16.

Kumar, M.S., Muralitharan, G. & Thajuddin, N. (2009). Screening of a hypersaline cyanobacterium, Phormidium tenue, for the degradation of aromatic hydrocarbons: naphthalene and anthracene. *Biotechnol. Lett.*, 31, 1863–1866.

Kumar, M.S., Rajeshwari, K., Johnson, S., Thajuddin, N. & Gunasekaran, M. (2011). Removal of Pb (II) by Immobilized and Free Filaments of Marine *Oscillatoria*

sp. NTMS01 and *Phormidium* sp. NTMS02. *Bull. Environ. Contam. Toxicol.*, 87, 254-259.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* 193, 265-275.

Oren, A. & Shilo, M. (1979). Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica:* sulphur respiration and lactate fermentation. *Archives of Microbiology*, 122, 77-84.

Plummer, P. (1987). *Glycogen determination in animal tissues. An Introduction to Practical Biochemistry*. 3rd edn. Maidenhead: McGraw Hill Book, 332 pp.

Postgate, J. (1987). *Nitrogen fixation.* London: Edward Arnold Publishing, 77pp.

Prasanna, R., Sood, A., Suresh, A., Nayak, S. & Kaushik, B.D. (2007). *Acta Botanica Hungarica*, 49(1–2), 131–156.

Rajeshwari, K., Kumar, M.S. & Thajuddin, N. (2011). Adsorption isotherms for Cr (VI) by two immobilized marine cyanobacteria. *Ann Microbiol,* doi: 10.1007/s13213-011-0252-3.

Shimizu, Y. 2003**.** Microalgal metabolites. *Current Opinion in Microbiology,* 6, 236–243.

Sinetova, M. A., Cerveny, J., Zavrel, T. & Nedbal, L. (2012). On the dynamics and constraints of batch culture growth of the cyanobacterium *Cyanotheca* sp. ATCC 51142. *J. Biotechnol.*, 162, 148-155. doi: 10.1016/j.jbiotech.2012.04.009.

Stainer, R.Y. & Cohen–Bazire, G. (1977). Phototrophic prokaryotes: the cyanobacteria . *Annual reviews of Micrbiology*, 31, 225-234.

Subashchandrabose, S.R., Ramakrishnan, B., Megharaj, M., Venkateswarlu, K. & Naidu, R. (2011). Consortia of cyanobacteria / microalgae and bacteria: Biotechnological potential. Biotechnology Advances. doi: 10.1016/*j.biotechadv*. 2011.07.009.

Thajuddin, N. & Subramanian, G. (2005). Cyanobacterial biodiversity and potential applications in biotechnology. *Current Science,* 89(1), 47 – 57.

Thenmozhi, R., Nagasathya, A. & Thajuddin, N. (2011). Studies on biodegradation of used engine oil by consortium cultures. *Advances in Environmental Biology*, 5(6), 1051 – 1057.

Whitten, B.A. & Potts, M. (2000). *The ecology of cyanobacteria*. Dordrecht: Kluwer Academic Publishers.

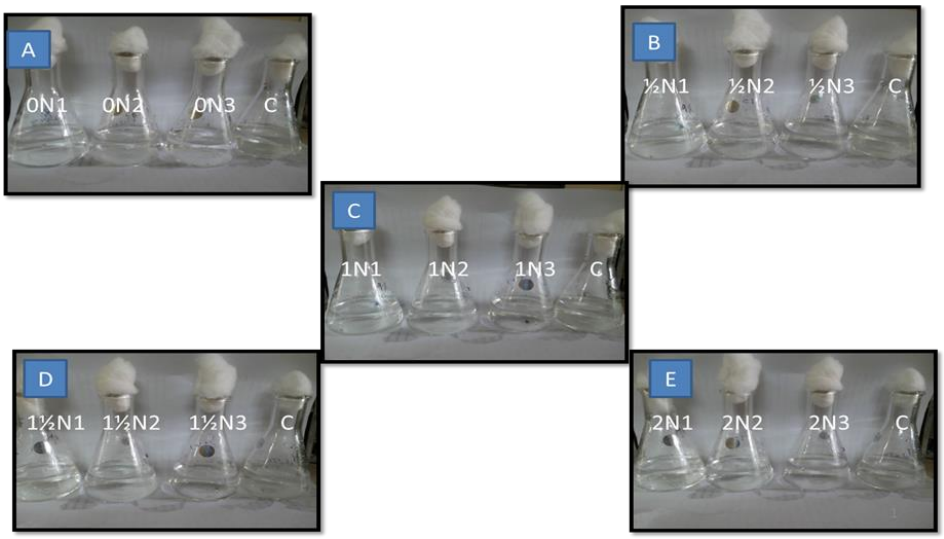

Fig. 1. Growth of *Lyngbya* sp. BDU90901 under different nitrogen concentrations at **0th day culture**. **A**- 0N, **B**-½N, **C**- 1N, **D**-1½N and **E**-2N respectively.

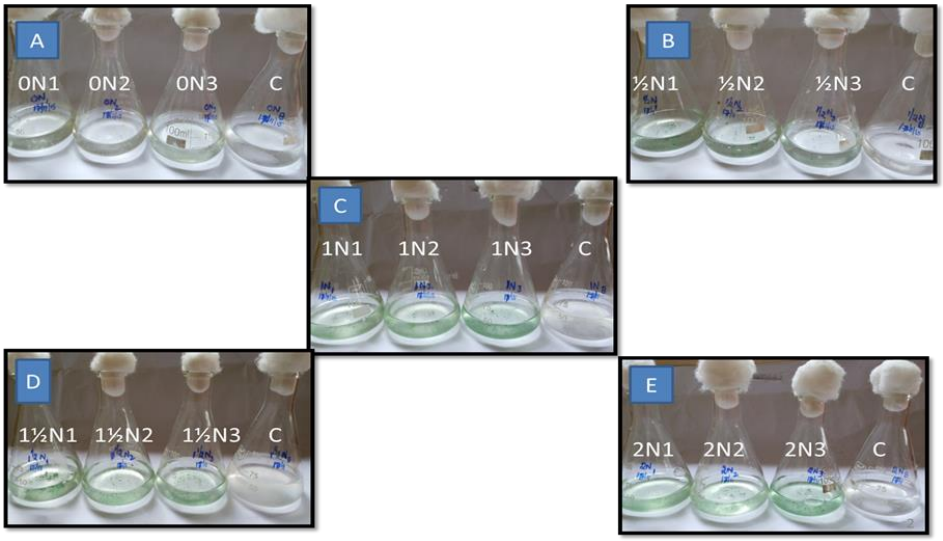

Fig. 2. Growth of *Lyngbya sp.* BDU90901 under different nitrogen concentrations at **5th day culture. A**- 0N, **B**-½N, **C**- 1N, **D**-1½N and **E**-2N respectively.

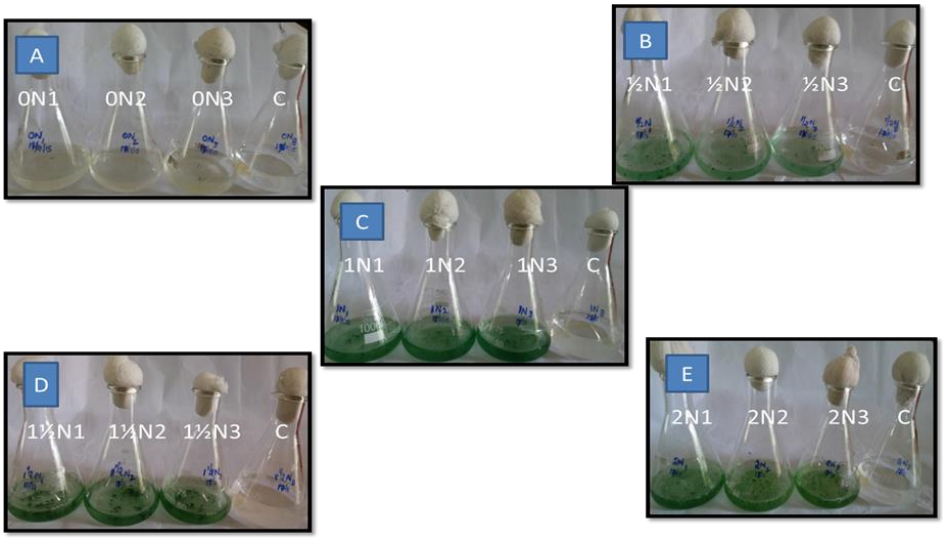

Fig. 3. Growth of *Lyngbya* sp. BDU90901 under different nitrogen concentrations at **10th day culture. A**- 0N, **B**-½N, **C**- 1N, **D**-1½N and **E**-2N respectively.

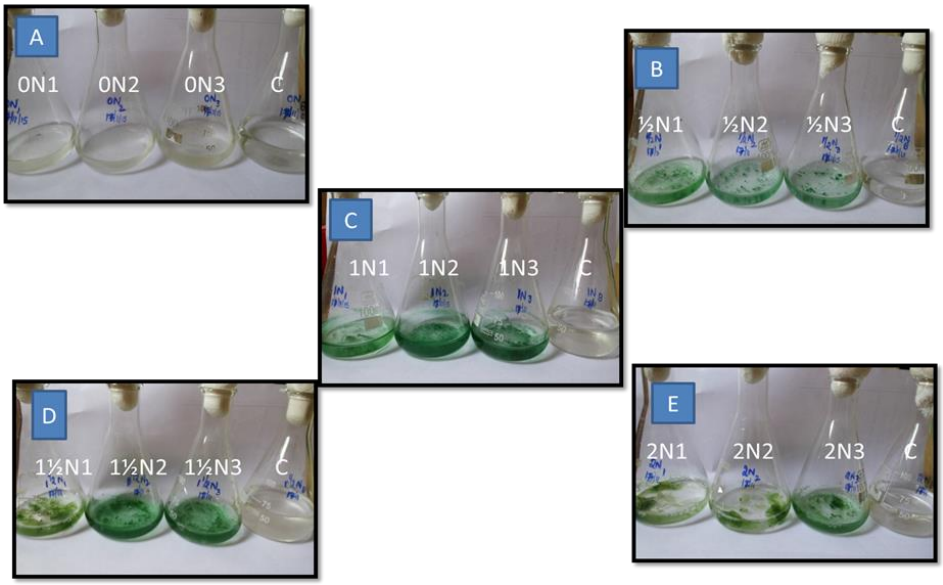

Fig. 4. Growth of *Lyngbya* sp. BDU90901 under different nitrate concentrations at **15th day culture. A**- 0N, **B**-½N, **C**- 1N, **D**-1½N and **E**-2N respectively.

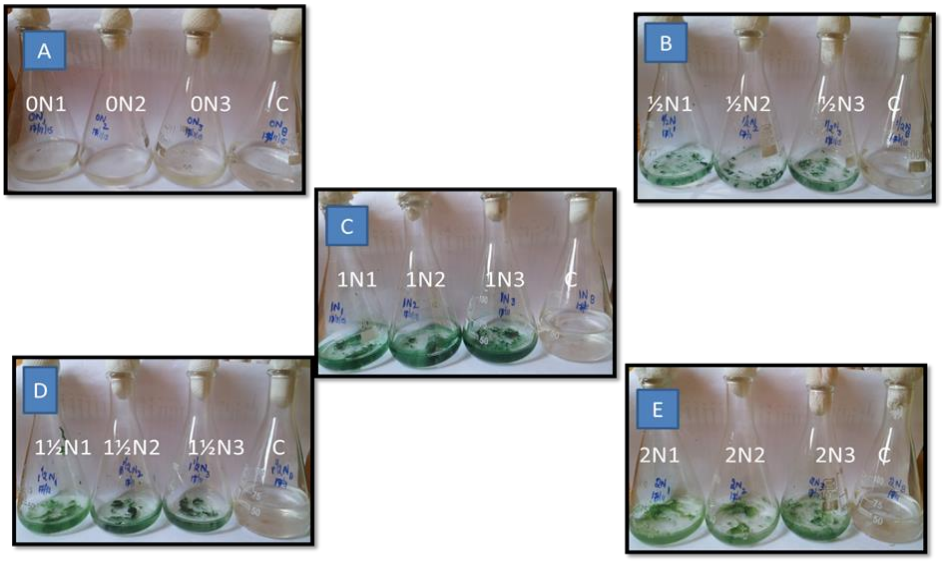

Fig. 5. Growth of *Lyngbya* sp. BDU90901 under different nitrate concentrations at **20th day culture. A**- 0N, **B**-½N, **C**- 1N, **D**-1½N and **E**-2N respectively.

Fig. 6. Growth of *Lyngbya* sp. BDU90901 under different nitrate concentrations at **25th day culture. A**- 0N, **B**-½N, **C**- 1N, **D**-1½N and **E**-2N respectively.

Fig. 7. Growth of *Lyngbya* sp. BDU90901 under different nitrate concentrations at **30th day culture. A**- 0N, **B**-½N, **C**- 1N, **D**-1½N and **E**-2N respectively.

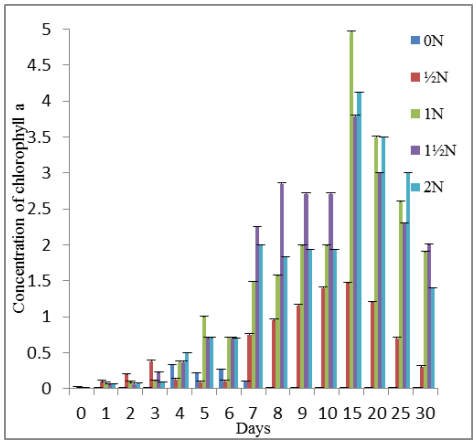

Fig. 8. Estimation of chlorophyll –a from *Lyngbya* sp. BDU 90901 under different nitrate concentrations for 30 days of incubation.

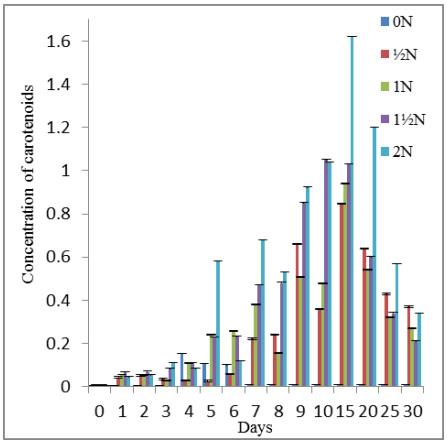

Fig. 9. Estimation of carotenoids from *Lyngbya* sp. BDU 90901 under different nitrate concentrations for 30 days of incubation.

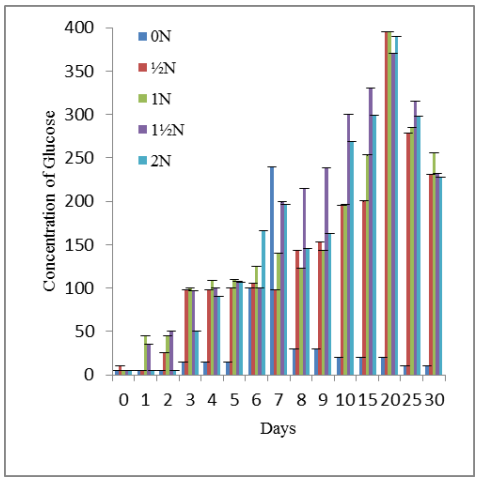

Fig. 10. Estimation of glucose from *Lyngbya* sp. BDU 90901 under different nitrate concentrations for 30 days of incubation.

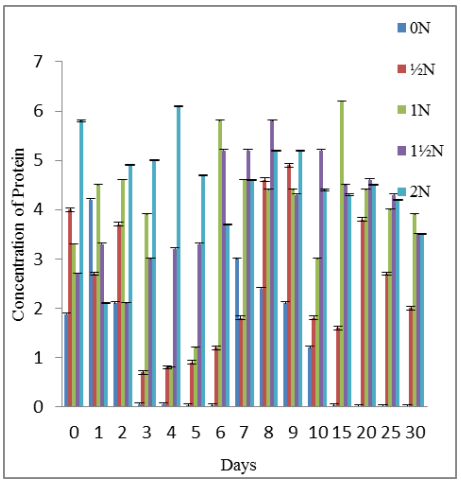

Fig. 11. Estimation of protein from *Lyngbya* sp. BDU 90901 under different nitrate concentrations for 30 days of incubation.

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 31-40
DOI: 10.1515/ahbb-2016-0003

OPHRYS APIFERA (ORCHIDACEAE) IN TRANSYLVANIAN FLORA, ROMANIA

SZATMARI Paul-Marian[1*]

Abstract: This article presents new floristics data regarding the distribution of *Ophrys apifera* in Transylvania, Romania. This new data is the result of floristics studies of Sălaj County flora in northwest Romania. The newly found populations of this species of orchid in Sălaj County are at the known north limit of the species in Romania, and potentially the only present populations of this species in Transylvania, as the present existence of the other two populations previously mentioned in southern Transylvania is doubtful. The paper also considers the general distribution of *Ophrys apifera* in Romania.
Key words: *Ophrys apifera*, rare species, Transylvania, Romania, distribution.

Received 05 October 2016 *Accepted 11 November 2016*

Introduction

Ophrys apifera Huds. is a rare species of orchids with flowers that mimic the shape of a bee in order to attract pollinators, through pollination by deception. The species belongs to *Ophrys fuciflora* complex, section *Euophrys*, family Orchidaceae, order Asparagales (Delforge 2005).

Ophrys apifera Huds. is a perennial species, having spherical tubercules. Stem is straight, cylindrical, reaching 15-50 cm (occasionally 70). It has 2-4 lower leaves oblong-lanceolate, green, and 2-3 upper leaves that surround the stem. The elongated inflorescence has 2-7 (11) many-colored flowers, pinkish, pinkish-violet, or greennish. The lateral, inner tepals are velvety-hairy, and the labellum is clearly three-lobed. The median lobe of the labellum presenting a glabrous appendix. The lateral labellum lobes are without conical appendices. The labellum is reddish-brown with diverse yellow patterns. Floral bracts are linear-lanceolate. Sepals are wide ovate lanceolate, the dorsal sepal is concave with a forward bent tip. Fruits are capsules. Flowering time is May-June (Soó 1959, Nyárádi & Beldie 1972, Ciocârlan 2009, Negrean & Dihoru 2009). As most orchids, *Ophrys apifera* Huds. does not spread easily considering their minute seeds and the endotrophic mychorrizae symbiosis necessary for seed germination, orchids being depended of specific fungi species for seed germination (Negrean & Dihoru 2009).

Ophrys apifera Huds. is critically endagered in Romania, its populations being drastically affected by habitat loss due to human activities (Panțu 1915, Dihoru & Negrean 2009). It is listed on all the red lists, and labelled as rare (R) (Oltean et al.

[1] Biological Research Center Jibou, "Vasile Fati" Botanical Garden Jibou, Nr.14, Parcului Street, 455200 Jibou, Sălaj County, Romania.
[*] Correspondence: paul_marian87s@yahoo.com

1994: 34), critically endangered (CR) (Negrean & Dihoru 2009) and endangered (E) (Boşcaiu et al. 1994, Dihoru & Dihoru 1994, Negrean 2001).

The discovery of new populations of this species in an area where they were not previously known to inhabit is an important step in the species conservation. Until this inventory *Ophrys* species were not known to inhabit Sălaj County. The nearest populations are *Ophrys sphegodes* Mill. on Plopiș Mountains (Șes Mountain) in Bihor County, at the border with Sălaj County (Rákosy 2009) where this species was recorded for the first time in Romanian flora (Coldea 1968). Recently, Șes Mountain area was throughly examined on Bihor County side (Hurdu et al. 2015), as well as Sălaj County side, these studies led to the discovery of the new populations presented here. The thorough examination of the Bihor side of Șes Mountain led to discovering important populations of 27 species of orchids, amongst which two populations in the *Ophrys* genus: *O. sphegodes* Mill. and *O. scolopax* subsp. *cornuta* (Steven) E.G.Camus. Throughout Central Europe, Romania represents an important location regarding the number of orchid species, new species having been discovered recently in Transylvania and Banat regions – *Epipactis albensis* Nováková & Rydlo in Turda River Gorge - Cheile Turzii (Molnár & Sramkó 2012) and *Epipactis greuteri* H.Baumann & Künkele in National Park Semenic-Cheile Carașului (Ardelean 2011).

The general distribution of *Ophrys apifera* Huds. is relatively wide, ranging throughout the southern, central and western Europe, Asia Minor, Caucasus, and North Africa (Tutin et al. 1964-1980, Nyárádi & Beldie 1972, Govaerts et al. 2016). An Atlantic-mediterranean species (Sanda et al. 1983, Ciocârlan 2009), it was recently discovered in Poland (Osiadacz & Kręciała 2014) and western Urkraine, in Ivano-Frankivsk region (Danylyk & Borsukevych 2011). In Romania it is mostly present in the southern foothills of the Southern Carpathians in Dâmbovița and Prahova counties, as well as in the southwest area of Banat region, in Mehedinți County and Danube River Gorge. Recently two individuals were also discovered in Buzău County (Anastasiu 2015).

The authors of the Red List in Romania mention the necessity of establishing special reservations for all *Ophrys* species, in particular the ones with large, strong populations that would include the ones in Sălaj County. Throughout Central and West Europe efforts are made to protect these orchid species (Dihoru & Negrean 2009). Throughout Carphatians area *Ophrys apifera* Huds. is considered endangered (Witkowski et al. 2003). It is protected in the Czech Republic, Slovakia, Hungary (Witkowski et al. 2003, Molnár 2011) and listed on the red lists in Ukraine (Didukh 2009), Bulgaria (Tashev et al. 2006), Czech Republic & Slovakia (Čeřovský et al. 1999), and it is proposed to be protected in Poland. At the same time it is intended to expand the reserve to include newly discovered individuals of this species in Poland (Osiadacz & Kręciała 2014), as well as for the new populations in Bulgaria (Tashev et al. 2006). Worldwide, according to the IUCN the species is not endangered (”least concern”) (Bilz et al. 2011). At ”Vasile Fati” Botanical Garden in Jibou (Sălaj County) efforts are underway to cultivate this species in the habitats specially designed in the ”Romanian Flora” sector, just as it was cultured another rare species of the same genus (*Ophrys scolopax* subsp. *cornuta* (Steven) E.G.Camus) in „Dimitrie Brândză” Botanical Garden in Bucharest (Panțu & Procopianu-Procopovici 1901).

Material and methods

Species identification was accomplished with the use of Romanian Flora taxonomic keys (Ciocârlan 2009, Sârbu et al. 2013). The majority of the nearby species were identified on site, the few that could not be identified in the field, were collected, preserved and the species identification was determined in the lab. The scientific nomenclature followed "The Plant List" site (2013). One specimen from each site was collected and preserved for each of the herbaria in Bucharest, Cluj-Napoca in Cluj County and Carei in Satu Mare County.

Results and discussion

The new *Ophrys apifera* Huds. populations (Fig. 1) were discovered on May 20th, 2014, northwest of Ugruțiu village, located in southeast Sălaj County, by the neighbouring Cluj County. At this location *Ophrys apifera* Huds. plants inhabit steep hills with xerophytic vegetation in Ugruțiu Valley heading towards Hida village, at about 329 m elevation. Main coordinates of this new population are 47°01'09.20"N and 23°21'42.15"E. On May 23rd , 2014, a second population was discovered about 200 m up from the bottom of the hill, at about 309 m elevation, located at 47°01'08.83"N and 23°21'35.76"E. On June 9th, 2015, another population was located at about 300 m from the previous two populations, at 338 m elevation, at 47°01'08.54"N and 23°21'49.88"E (all coordinates from GoogleEarth). We conclude that the species is well established at this location and the populations are stable. All three above mentioned populations were represented by 20-25 individuals in full bloom at the time they were located. Most of the individuals had about 5-7 flowers (Fig. 1).

Fig. 1. Ophrys apifera Huds. in Sălaj County, Romania.

Ophrys apifera Huds. is a heliophile species, inhabiting hilly areas, on dry, sandy and nitrogen poor soils (Negrean & Dihoru 2009), occasionally in several areas of Europe it can be found up to 1800 m altitude, probably being one of the best adapted species of the genus *Ophrys* to higher elevations. Being adapted to high altitude could be due to their high concentration of flavonoids in the sepals and labellum, which would indicate the need of stronger protection against UV-B radiation (Karioti et al. 2008). The Ugruțiu habitat fits perfectly with the description given by Nyárádi & Beldie (1972): "sunny slopes, scrub, in the hilly regions on limestone soils". All the steppe slopes in the Ugruțiu area are mainly on chalky, calcareous limestone, sometimes with gypsum patches that accomodate a specific flora. *Ophrys apifera* Huds. populations were exclusively found on grassy pastures, with short grasses, sunny exposure to partial shade, sometimes at the edge of shruby areas.

From a floristic point of view the steep slopes in Ugruțiu area are amongst the most species rich throughout Sălaj County, the majority of species found here are of great phytogeographical importance with numerous steppe microelements that make the transition with the steppe pastures in Cluj County. The number of species on these slopes reach more than 200 taxa. The dominant species are: *Festuca rupicola* Heuff., *Festuca valesiaca* Schleich. ex Gaudin, *Bothriochloa ischaemum* (L.) Keng, *Brachypodium pinnatum* (L.) P. Beauv. and *Stipa pennata* L., similar to the other associations where *Ophrys apifera* Huds. was found before: *Festucion rupicolae* (Sanda et al. 1983); *Festuco-Brometalia* (Tashev et al. 2006). Amongst the species of great importance found in Ugruțiu area, we include: *Adonis vernalis* L., *Allium albidum* Fisch. ex M.Bieb., *Astragalus austriacus* Jacq., *Astragalus monspessulanus* L., *Astragalus onobrychis* L., *Cleistogenes serotina* (L.) Keng, *Jurinea transsilvanica* (Spreng.) Simonk., *Jurinea mollis* (L.) Rchb., *Linum flavum* L., *Linum tenuifolium* L., *Onosma pseudoarenaria* Schur, *Plantago argentea* Chaix, *Pulsatilla montana* (Hoppe) Rchb. subsp. *dacica* Rummelsp., *Salvia nutans* L., *Klasea radiata* (Waldst. & Kit.) Á.Löve & D.Löve, *Stipa pennata* L., *Stipa pulcherrima* K.Koch, *Stipa capillata* L., *Stipa tirsa* Steven, *Anacamptis coriophora* subsp. *fragrans* (Pollini) R.M. Bateman, Pridgeon & M.W. Chase, *Cephalaria radiata* Griseb. & Schenk, *Carex humilis* Leyss., *Dictamnus albus* L. etc. Some of these species are present in Sălaj County only in Ugruțiu area, or neighbouring hills within 5 km proximity. Some of these species include: *Ajuga laxmannii* (Murray) Benth., *Carduus hamulosus* Ehrh., *Oxytropis pilosa* (L.) DC., *Phlomoides tuberosa* (L.) Moench, *Allium fuscum* Waldst. & Kit..

On June 1th, 2016 we found other four populations of *Ophrys apifera* Huds. at the northernmost latitude, which is a remarkable aspect of concerning the spread of the species in Romania. All populations were found in Sălaj County. The northernmost populations are near Jibou city, where these populations also grow on limestone (47°15'16.38"N and 23°14'11.62"E, 221 m altitude), accompanied by large populations of other orchid species like: *Orchis purpurea* Huds. and *Neotinea ustulata* (L.) R.M.Bateman, Pridgeon & M.W.Chase. A second larger population (30 individuals) is at the edge of the forest in the southern part of the city (47°15'12.81"N and 23°13'48.81"E, 271 m altitude).

A third population was discovered in an area extremely rich in terms of flora – the Ortelec limestone hills in the immediate vicinity of the city Zalău (47°12'39.17"N and 23°07'57.01"E, 289 m altitude). These limestone slopes are bordered by a forest of

downy oak (*Quercus pubescens* Willd.), a species that is found here also at the northern limit of his area. In addition to numerous rare species of the typical thermophilic limestone areas, we can also find other species of orchids, of which stands out: *Anacamptis pyramidalis* (L.) Rich., *Orchis militaris* L., *Anacamptis morio* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *Neotinea ustulata* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *Cephalanthera damasonium* (Mill.) Druce, *Epipactis microphylla* (Ehrh.) Sw., *Neottia nidus-avis* (L.) Rich..

The fourth population is at the Meseş Mountains foothills in the village Ciumărna. The few individuals were encountered at the edge of the woods, also together with other orchid species that are typical for grasslands (*Anacamptis coriophora* subsp. *fragrans* (Pollini) R.M.Bateman, Pridgeon & M.W.Chase, *Anacamptis pyramidalis* (L.) Rich.) or forests (*Neottia nidus-avis* (L.) Rich., *Epipactis helleborine* (L.) Crantz, *Platanthera bifolia* (L.) Rich., *Cephalanthera damasonium* (Mill.) Druce), and coming into contact in this place. A few individuals of Lady's Slipper Orchid (*Cypripedium calceolus* L.) were also found at the same place with *Ophrys apifera* Huds..

The chorologie of *Ophrys apifera* Huds. accordind to some new publications, the Red Book (Dihoru & Negrean 2009) and Flora Republicii Socialiste România (Nyárádi & Beldie 1972) includes the following locations:

- Dâmbovița County: Mănăstirea Viforâta on Valea lui Enache, 10.VI.1910, G. Grințescu [BUCA 39.857 etc.], Valea Cocoșatului, 5.VI.1909, alt. 300 m, G. Grințescu [BUCA 39.856 etc.] (Negrean & Dihoru 2009); Doicești on Coasta Dealului, alt. 350 m, 12.VI.1916, G. Grințescu [BUCA 39.855 etc.]; the lakes at the quarry, 400 m, 12.VI.1916, G. Grințescu [BUCA 39.855 etc.]; Ocnița (Negrean & Dihoru 2009); Târgoviște (Nyárádi & Beldie 1972); Dâmboviţa County, on grassy slopes on southern foothills of Southern Carpathians (Dumitru 1980, Dumitru & Săvescu 2011). Recently mybiosis.info site added new pictures of a new population in Vârfuri area, at 500 m altitudine (Stoichiță 2014).
- Prahova County: Ocnița-Filipești, on Coasta Dealului, 350 m, 15.VI.1916, G. Grințescu [BUCA 124.585 etc.]; Hârșa and Plopu, 350 m, 15.VI.1916, G. Grințescu [BUCA 39.853 etc.] (Negrean & Dihoru 2009); West Tinosu, Lunca Prahovei, 6.VI.1971, G. Negrean [HGN] (Negrean & Dihoru 2009); Breaza, Scăieni (Nyárádi & Beldie 1972).
- Buzău County: Cocârceni (hayfields), 45.363671°N, 26.486291°E, cca. 440 m alt., June, 1, 2014 (Anastasiu 2015).
- Mehedinți County: Svinița-Tricule (Ștefureac 1971); Bala (Comănești, on the Pietricica Hill), at grove edges, 350 m, FQ-47, 13.06.2003, coll. I. Costache (CRA 18929) (Vladimirov et al. 2006). Mybiosis.info site published pictures from Banat region of Mehedinți County on Danube River Gorge area, the species being confirmed to exist in this region as well (Ardelean 2009).
- Gorj County: Slivilești (pers. comm.).
- Hunedoara County: Geoagiu-Băi, cca. 500 m northwes from the spa resort, alt. 430 m, 12.VI. 1966, G. Pap [CLA] (Pázmány 1966, Pázmány & Pap 1966).
- Sibiu County: Poplaca (Schur 1866), this presence being contested by other authors (Simonkai 1886, Panțu 1934, Rösler 2003). In *Cormoflora județului Sibiu* (Drăgulescu 2010) the presence of this species in Sibiu County is also doubted.

- Sălaj County: Ugruțiu, 47°01'09.20" N, 23°21'42.15" E, ca 329 m, 20.V.2014; 47°01'08.83" N, 23°21'35.76" E, ca 309 m, 23.V.2014; 47°01'08.54" N, 23°21'49.88" E, ca 338 m, 9.VI.2015; Jibou, 47°15'16.38" N, 23°14'11.62" E, ca 221 m; 1.VI.2016; 47°15'12.81" N, 23°13'48.81" E, ca 271 m, 1.VI.2016; Ortelec near Zalău, 47°12'39.17" N, 23°07'57.01" E, ca 289 m, 1.VI.2016; Ciumărna, Meseș Mountains, 1.VI.2016.

The map in Fig. 2 shows *Ophrys apifera* Huds. populations mainly on the foothills of the Southern Carpathians and in the Danube River Gorge area, while in Transylvania the populations are isolated at great distances in Hunedoara County, Sibiu County and now Sălaj County.

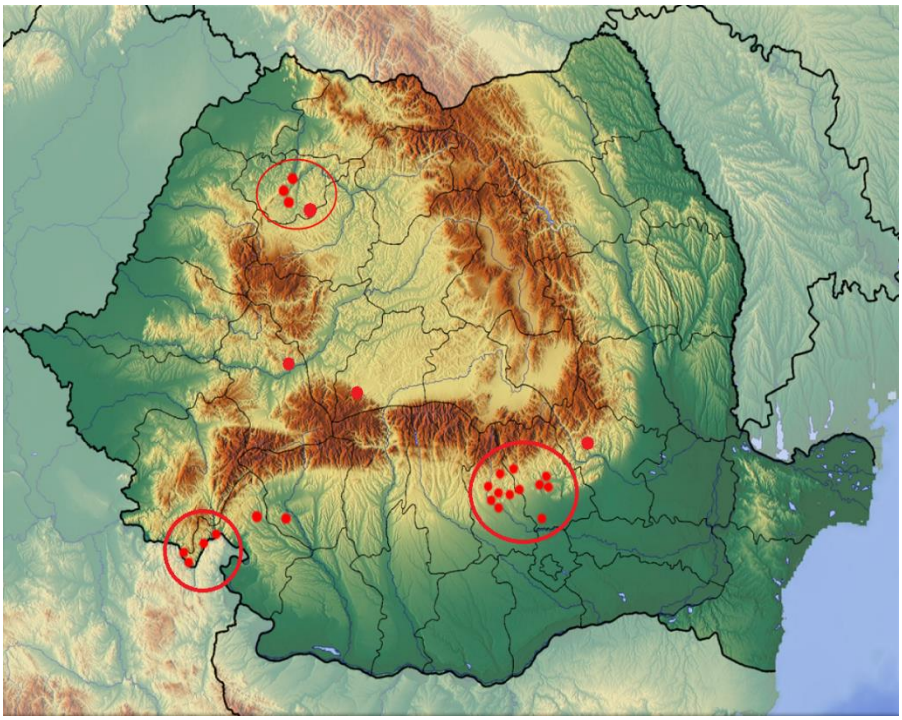

Fig. 2. Ophrys apifera Huds. distribution in Romania

Conclusions

As mentioned above data regarding *Ophrys apifera* Huds. distribution in Romania is old, and it is possible that some of the previously mentioned populations to have dissapeared. Also, the presence of this species in Transylvania was contested by older studies (Nyárádi & Beldie 1972), to be discovered recently in Sălaj County, at a considerable distance from other populations in Romania, which would suggest that additional populations might exist in other areas with similar habitat.

The newly discovered populations must be protected as soon as possible, especially from the ever increasing sheep populations in the region, sheep herds are

ocassionaly allowed to graze on the above mentioned locations. We have observed numerous *Ophrys apifera* Huds. individuals that were trampled and destroyed by the passing sheep herds through the Ugruțiu Valley area.

The Sălaj region was one of the least studied areas in Romania, but after the recent researches it is observed that possesses a remarkable biodiversity, being a contact area between species coming from all four cardinal points. The most remarkable is that here seems to be the northern limit for most thermophilic species that come from the south, the vast limestone areas creating a favorable microclimate for the northernmost outpost of these species in Transylvania.

Aknowledgements. Our field studies were supported by "Vasile Goldiș" West University in Satu Mare and the Center of Biological Research in Jibou (Jibou Botanical Garden), that provided the means of transportation and to which we are expressing our gratitude. Special thanks go to Professor Gavril Negrean for his numerous suggestions and the vast bibliography that he shared with me. I also want to express my gratitude to both Carol Karácsonyi and Gavril Negrean, whom I joined on field studies, that led to important new data regarding Sălaj County flora, as well as to Adorian and Corina Ardelean, mybiosis.info site administrators for their willingness in sharing their data.

References

Anastasiu, P. (2015). New chorological data for rare vascular plants from Romania. *Acta Horti Bot. Bucurest.*, 42, 57-62.

Ardelean, C. (2009). *Ophrys apifera* in mybiosis.info. 2016 Nature Object Digital Repository [2009MAY06]. http://mybiosis.org.

Ardelean, C. (2011). *Epipactis greuteri (Orchidaceae)* a new species for Romanian flora. *Journal Europaischer Orchideen*, 43 (3), 527-534.

Bilz, M., Kell, S.P., Maxted, N. & Lansdown, R.V. (2011). *European Red List of Vascular Plants*. Luxembourg: Publications Office of the European Union.

Boşcaiu, N., Coldea, G. & Horeanu, C. (1994). Lista Roşie a plantelor vasculare dispărute, periclitate, vulnerabile și rare din flora României. *Ocrot. Nat.*, 38 (1), 45-56.

Čeřovský, J., Feráková, V., Holub, J., Maglocký, Š. & Procházka, F. (1999). *Červená kniha ohrožených a vzácných druhů rostlin a živočichů ČR a SR.* Bratislava, 5: 453 pp. Vyšší rostliny. Príoda a s.

Ciocârlan, V. (2009). *Flora ilustrată a României*. Bucureşti: Edit. Ceres.

Coldea, G. (1968). *Ophrys sphegodes* Mill. în flora României. *Studii și cercetări biologice. Seria Botanică*. București, 20(2), 137-140.

Danylyk, I.M. & Borsukevych, L.M. (2011). A new find of *Ophrys apifera* Huds. (*Orchidaceae*) in Ukraine. *Ukr. Botan. Journ*., 68(1), 58-63.

Delforge, P. (2005). *Guide des Orchidées d'Europe, d'Afrique du Nord et du Proche-Orient.* Paris: Delachaux et Niestlé. 640 pp.

Didukh, Y.P. (ed.) (2009). *Červona knyga Ukrainy*. Roslynnyi svit. Kyiv: Globalkonsaltyng. 912 pp.

Dihoru, G. & Dihoru, A. (1994). Plante rare, periclitate şi endemice în Flora României - Lista roşie ● Red list of rare, threatened and endemic plants from Flora of Romania. *Acta Bot. Horti Bucurest*., /1993-1994/, 173-197.

Dihoru, G. & Negrean, G. (2009). *Cartea roşie a plantelor vasculare din România*. București: Edit. Academiei Române.

Drăgulescu, C. (2010). *Cormoflora judeţului Sibiu*. Ediţia a doua revizuită. Sibiu: Edit. Universităţii „Lucian Blaga" Sibiu. 831 pp.

Dumitru, M.D. (1980). *Cercetări asupra florei şi vegetaţiei Subcarpaţilor dintre Ialomiţa şi Dîmboviţa*. Unpublished doctoral dissertation. Bucureşti: Universitatea din Bucureşti, Facultatea de Biologie.

Dumitru, M. & Săvescu, C.M. (2011). *Flora şi vegetaţia judeţului Dâmboviţa* (Conspectul speciilor din flora spontană şi al asociaţiilor vegetale). Târgovişte: Edit. Transversal.

Govaerts, R., Bernet, P., Kratochvil, K., Gerlach, G., Carr, G., Alrich, P., Pridgeon, A.M., Pfahl, J., Campacci, M.A., Holland Baptista, D., Tigges, H., Shaw, J., Cribb, P., George, A., Kreuz, K. & Wood, J. (2016). World Checklist of Orchidaceae. Facilitated by the Royal Botanic Gardens, Kew. Retrieved October 5, 2016, from: http://apps.kew.org/wcsp/.

Hurdu, B.-I., Roman, A., Turtureanu, P.D., Ursu, T.M., Pușcaș, M., Fărcaș, S., Stoica, I.A., Coldea, G. (2015). Orhidaceele din Munții Plopișului (Munții Apuseni). Diversitate floristică, importanță fitogeografică și valoare conservativă. Sesiunea de comunicări științifice a Grădinii Botanice "D. Brândză" București, Ediția a XXI-a. 7 Noiembrie 2015.

Karioti, A., Kitsaki, C.K., Zygouraki, S., Ziobora, M., Djeddi, S., Skaltsa, H. & Liakopoulos, G. (2008). Occurence of flavonoids in *Ophrys* (Orchidaceae) flower parts. *Flora*, 203 (7), 602-609.

Molnár, V.A. (Ed.). (2011). *Magyarország orchideáinak atlasza ● Atlas der Orchideen Ungarns*. Budapest: Kossuth Kiadó.

Molnár, A. & Sramkó, G. (2012). *Epipactis albensis* (Orchidaceae): a new species in the flora of Romania. *Biologia, Sect. Bot*., 67(5), 883-888.

Negrean, G. (2001). Lista roşie a plantelor din România existente în pajişti (inclusiv endemite şi subendemite). Pp. 30-58. In G.Coldea, G. Negrean, I. Sârbu & A. Sârbu (2001). *Ghid pentru identificarea şi inventarierea pajiştilor seminaturale din România*. Bucureşti: Edit. Alo, Bucureşti!.

Negrean, G. & Dihoru, G. (2009). *Ophrys apifera* Huds. subsp. *apifera*. pp. 368-369. In Dihoru, G. & Negrean, G. (2009). *Cartea roşie a plantelor vasculare din România*. Bucureşti: Edit. Acad. Române.

Nyárády, A. & Beldie, A. (1972). *Ophrys* L.. In T. Săvulescu (ed.), *Flora României*, Vol. 12. (Pp. 655-662). Bucureşti: Edit. Academiei Române.

Oltean, M., Negrean, G., Popescu, A., Roman, N., Dihoru, G., Sanda, V. & Mihăilescu, S. (1994). *Lista roşie a plantelor superioare din România*. In M. Oltean (coord.), *Studii, sinteze, documentaţii de ecologie*, Acad. Română, Institutul de Biologie, 1, 1-52.

Osiadacz, B. & Kręciała, M. (2014). *Ophrys apifera* Huds. (Orchidaceae), a new orchid species to the flora of Poland. *Biodiv. Res. Conserv*., 36, 11-16.

Panţu, Z.C. & Procopianu-Procopovici, A. (1901). *Ophrys cornuta* Stev. forma *Banatica* Rchb. Monografia unei plante indigene foarte rare. *Bul. Soc. Naturaliştilor România,* 2, 14-19.

Panţu, Z.C. (1915). Orchidaceele din România. București: *Libr. Socec & Comp*., 1-12, 1-228.

Panţu, Z.C. (1934). Contribuţiuni nouă la Orchidaceele din România. *Mem. Secţ. Şti*. Academia Română, 2 (3), 15-46.

Pázmány, D. (1966). Însemnări floristice. II. *Notulae Bot. Horti Agrobot. Clujensis*, 53-55.

Pázmány, D. & Pap, G. (1966). Răspândirea speciei *Ophrys apifera* Huds. în România. *Notulae Bot. Horti Agrobot. Clujensis*, 67-69.

Rákosy, D. (2009). Observations on a population of *Ophrys sphegodes* Mill. in Romania ● Observaţii asupra unei populaţii de *Ophrys sphegodes* Mill. din România. *Contr. Bot*., Cluj-Napoca, 44, 31–36.

Rösler, R. (2003). Zur Chorologie der Orhideen Rumäniens. *Journal Eiuropäischer Orchideen* (*Stuttgart*), 35 (2), 241-306.

Sanda, V., Popescu, A., Doltu, M.I. & Doniţă N. (1983). Caracterizarea ecologică şi fitocenologică a speciilor spontane din flora României, *Stud. Comunic., Şt. Nat.,* Muz. Brukenthal Sibiu, Suplim., 25, 1-126.

Sârbu, I., Ştefan, N. & Oprea, A. (2013). *Plante vasculare din România*. Bucureşti: Edit. VictorBVictor.

Schur, P.J.F. (1866). *Enumeratio Plantarum Transsilvaniae, exhibens: stirpes phanerogamas sponte crescentes atque frequentius cultas, cryptogamas vasculares, Characeas etiam muscos hepaticasque*. Vindobonae: G. Braumüller, 1-8, 984.

Simonkai, L. (1887 /"1886"/). *Erdély edényes Flórájának helyesbített Foglalata*. (*Enumeratio Florae transsilvanicae vesculosæ critica*). Ex mandato Societas Scientiarum Naturalium Regiæ hungaricæ. Budapest: Kiadja a Magyar Természettudományi Társulat. Franklin-Társulat könyvsajtója, 1-49, 1-678.

Soó, R. (1959). *Ophrys*-Studien. *Acta Bot. Hung.*, 5 (3-4), 437-471.

Soó, R. (1971). Species and subspecies of the Genus *Ophrys*. *Acta Bot. Acad. Sci. Hung.*, 1970, 16(3-4), 373-392.

Soó, R. de (1980). *Ophrys* L. Pp. 344-349. In Tutin, T.G. & al. (eds). *Flora Europaea*. Vol. 5. Alismataceae to Orchidaceae (Monocotyledones). Cambridge: Cambridge University Press.

Stoichiță, H. (2014). *Ophrys apifera* in mybiosis.info. 2016 Nature Object Digital Repository [2014JUN06]. http://mybiosis.org.

Ștefureac, T. (1971). Considerații generale asupra caracterului florei din ținutul Maramureșului. *Comun. Bot. A VII-a Consf. Națion. Geobot.* (Satu Mare, Maramureș, 17-26 VII 1969), 95-123. Soc. Ști. Biol. Rom.

Tashev, A., Vitkova, A. & Russakova, V. (2006). Distribution of *Ophrys apifera* Huds. (*Orchidaceae*) in Bulgaria. *Fl. Medit*., 16, 247-252.

The Plant List (2013). Version 1.1. Published on the Internet. Retrieved October 5, 2016, from: http://www.theplantlist.org/.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. & Webb D.A. (eds.) (1964–1980). *Flora Europaea.* Vols. 1–5. Cambridge: Cambridge University Press.

Vladimirov, V., Dihoru, G. & Tan, K. (2006). New floristic records in the Balkans. *Phytologia Balcanica*, 12(3), 422-423.

Witkowski, Z. J., Król, W., Solarz, W., Kukuła, K., Okarma, H., Pawłowski, J., Perzanowski, K., Ruzicka, T., Sandor, J., Stanova, V., Tasenkevich, L. & Vlasin, M. (2003). *Carpathian List of Endangered Species*. WWF and Institute of Nature Conservation, Vienna-Krakow: Polish Academy of Sciences.

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 41-55
DOI: 10.1515/ahbb-2016-0004

MONITORING INVASIVE WOOLLY CUPGRASS *ERIOCHLOA VILLOSA* IN THE PIR VILLAGE AREA, SATU MARE COUNTY, ROMANIA, AND ITS IMPACT ON SEGETAL FLORA

SZATMARI Paul-Marian[1*]

Abstract: The focus of this paper is the monitoring a new invasive species in Romania (*Eriochloa villosa*), in particular in the surroundings of Pir village, Satu Mare County, the impact of this species on local segetal flora and new adaptations of this species in a variety of habitats. Monitoring the changes of the plant associations in which this species is encountered suggests that associations and subassociations new for Romania have formed, which are still in primary stages of developement. This paper presents novel data regarding future evolution of these associations.
Key words: *Eriochloa villosa*, invasive species, monitoring, impact, Romania.

Received 07 October 2016 *Accepted 11 November 2016*

Introduction

Monitoring and updating data regarding the spread of the woolly cupgrass *Eriochloa villosa* (Thunb.) Kunth around the world is increasingly becoming a priority. This invasive species is responsible for decreasing crop production in several areas around the world, in particular North America; raising new issues regarding the chemical warfare against agricultural weeds. It can also become a carrier of pathogenic fungi damaging to grain crops (http://www.inspection.gc.ca).

Eriochloa villosa (Thunb.) Kunth is native to the temperate areas of Asia, from Caucasus, Iran, West Siberia and East Russia, to the Far East (China, Japan, Taiwan, Koreea, and Vietnam) (Tsvelev 1984). It was first reported as an adventive species in North America (in the United States) since 1940 (Clayton et al. 2008), affecting crops in over 13 American states. Since 1987 it was reported in South America in Suriname (Paramaribo region) (***). It was detected in Canada since 2000 (Allison & Darbyshire 2001), and later in Costa Rica and New Zealand (http://www.inspection.gc.ca). In Europe it was mentioned in Ukraine (Clayton 1980) and France (Riviere et al. 1992) without major agricultural impacts. Later its presence was also mentioned in Romania (Ciocârlan & Sike 2006) and Hungary (Partosfalvi et al. 2008). In Hungary *Eriochloa villosa* is spreading in the northern region (Balogh & Novák 2014). Recently it was observed also in the eastern part near Debrecen (Szilágyi et al. 2015) and on the south-western border (Bartha et al. 2015).

[1] Biological Research Center Jibou, ”Vasile Fati” Botanical Garden Jibou, Nr.14, Parcului Street, 455200 Jibou, Sălaj County, Romania
[*] Correspondence: paul_marian87s@yahoo.com

In Romania it was first noticed and mentioned in Satu Mare County at Livada (Ciocârlan & Sike 2006) and Timiș County also in 2006, at several locations (Fărcășescu et al. 2008). Later was also spotted in Arad County, also at several locations (Ardelean et al. 2009), while Carol Karácsonyi mentions its presence at new locations in Satu Mare County around Pir village as well as in Bihor County at Reghea (Karácsonyi 2011). Both locations are part of Tășnadului Hills area, an area where the species spreads rapidly and in 2014 reached Acâș and Supuru de Jos (from Satu Mare County, arriving at the border of Sălaj County).

Figure 1 presents a map with the places in Romania where this invasive species was noted in the Western Romanian Plain and Hills (Ciocârlan & Sike 2006, Fărcăşescu et al. 2008, Ardelean et al. 2009, Negrean 2011, Karácsonyi 2011, Dărăban et al. 2012, Negrean 2012). It is clear that this species is spreading rapidly in the western counties of Romania: Timiș, Caraș-Severin, Arad, Satu Mare, Bihor and even Maramureș at Săbișa (Negrean 2012) and Sălaj in the last years at Someș-Odorhei (2015), Crasna, Muncel and Bocșa (2016). The presence of this species is probably much more extensive throughout these counties (the data presented here include monitoring up to 2016). In the future we anticipate that it will be noted in Serbia, as it was already found in at least five locations on the border of Romania with Serbia. In the fall of 2015 it was discovered in Sălaj County, the region thus becoming the entry gate towards Transylvania, following Someș river valley to the North and the hills Dealurile Tășnadului – Dealurile Silvaniei to the east and south of the County.

Eriochloa villosa (Thunb.) Kunth belongs to grasses family (Poaceae), tribe Paniceae (Ciocârlan 2009). It is an annual species but in midler climates it can become perennial, ranging in height from 0.3 to 1 (2) m (http://www.inspection.gc.ca), in Romania reaching 60 – 100 cm (Ciocârlan & Sike 2006). Due to its great seed production it can easily spread to new areas. Amongsts the countries were it was encountered it was mentioned mainly in maize, sunflower, soy and rice fields as well as in fallow arable fields. In september 2016 the species was observed infesting a sorghum [*Sorghum bicolor* (L.) Moench] field near Acâș village (Satu Mare County).

This adventive species was monitored for three years (2010 – 2012), in Pir village, enough time to conclude that *Eriochloa villosa* can no longer be ignored where it is found, once established it becomes a difficult to control agricultural weed. Its ability and speed of spreading is similar to that of the annual ragweed *Ambrosia artemisiifolia* L. another species that poses great problems in Romania and throughout Europe. Pir village is located in the south-east of Satu Mare County where the hills Dealurile Tășnadului and the plain Câmpia (Valea) Ierului meet. The habitats in the area are very diverse ranging from marshes, to tree groves, forests and xerophytic pastures.

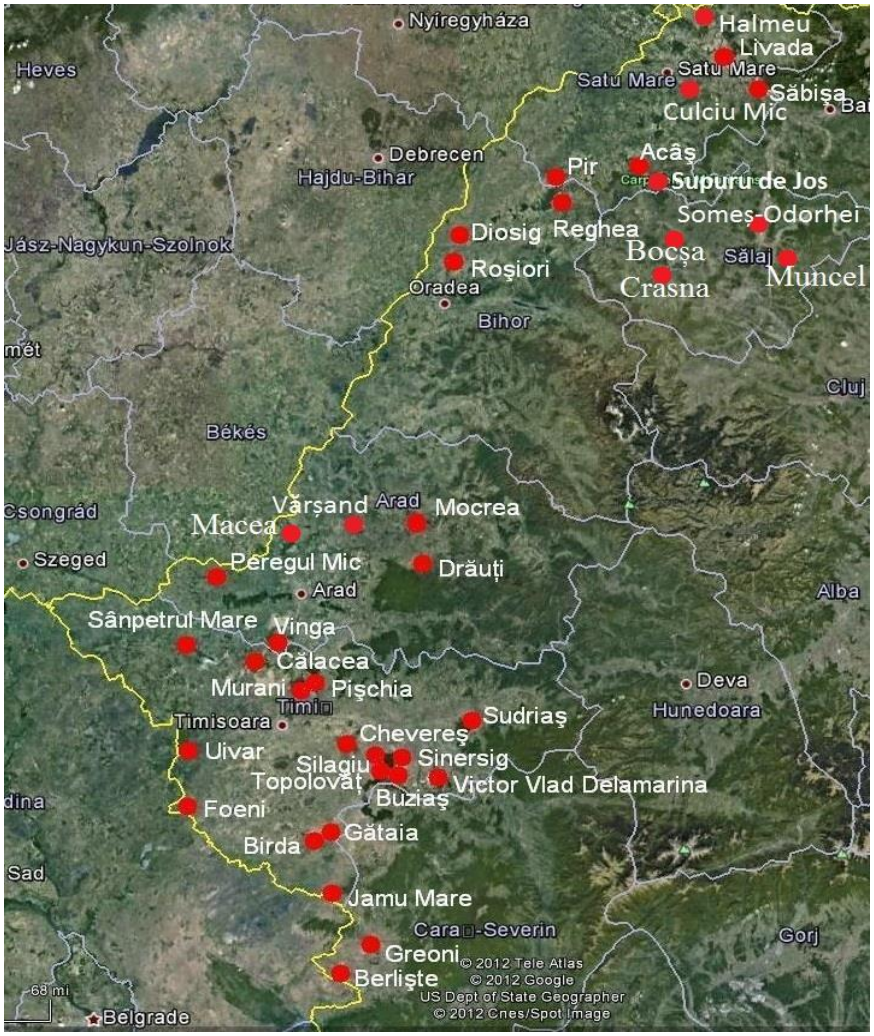

Fig. 1. The spread of *Eriochloa villosa* in western Romania (2016).

Material and methods

Monitoring of this species was done mainly through field observations, mapping the areas where it was encountered. Phytosociological (phytocoenological) sampling plots (relevé) method was also used to determine the abundance of individual plants and the other dominant species present at the same locations. Species identification was performed with the use of taxonomic key *Flora ilustrată a României* (Ciocârlan 2009), and the collected specimens were added to the personal herbarium and the herbarium of the botanical garden in Cluj-Napoca.

Results and discussion

Eriochloa villosa (Thunb.) Kunth (Fig. 2) was first observed in the area of Pir village by Carol Karácsonyi, during floristics research in the hilly region of Dealurile Tășnadului area, in 2009, who together with Gavril Negrean noticed a new association in a maize field that had dominating species *Setaria pumila* (Poir.) Roem. & Schult. and *Eriochloa villosa* (Thunb.) Kunth, accompanied especially by *Digitaria sanguinalis* (L.) Scop., *Setaria verticillata* (L.) P.Beauv. and *Oxalis stricta* L. (Karácsonyi 2011).

Fig. 2. Eriochloa villosa in the field (dominating with *Setaria pumila* on the right).

This is the first location from which we started the monitoring of this species. The maize field entirely infested with *Eriochloa villosa* is located in between a damp area and Pir Forest. This habitat fits perfectly with the ecological requirements of this species which prefers damp areas (http://www.inspection.gc.ca), and also posesing a very rapid adaptability. It is very interesting to note that this species is not expanding to the natural (uncultivated) habitats around the cultivated fields. The appearance of this species in this area is probably due to the agricultural activities that brought maize seed stocks infested with *Eriochloa villosa* seeds.

According to the field observation around Pir village area, it was noted that *Eriochloa villosa* follows the same seasonal patterns as noted in areas of North America, flowering around middle July, early August, sometimes later (http://www.inspection.gc.ca). The height of plants varies from 50-60 cm tall at the edges of fields or roads to 90-100 cm tall for the specimens towards the center of the fields or in highly damp areas. This species it is not well equiped for periods of long draught as it happened in 2012, an exceptionally dry year for the region when it did not rain in Pir village area for four months. Plants located in areas which dried earlier such as hill tops or asphalted road margins did not make it.

Figures 3, 4 and 5 present the rapid expansion of this species around Pir village. In 2010 this species was only found on a circa 200 m^2 portion of the maize field in front of Pir Forest, the next year 2011 it was noted at 2 km distance from the initial point. In 2012 the species became common almost on the whole southern part of Pir village, areas that are part of Dealurile Tășnadului hills area. In the fall of 2013 the species was already observed in Câmpia Ierului plains located only about 4 km away, where the plain meets the hills. The delay of expansion in the northern marshy area of the Pir village in Câmpia Ierului plain supports the observations in Arad County, where the species was noted in the thermophilous locations (Ardelean et al. 2009), the thermic gradient affecting its expansion, seemingly being more thermophilous. It can be noted that according to the composition of the local vegetation (xerophytic pastures, *Quercus cerris* L. forests) the hills in the southern part of the Pir village have a slightly milder climate and warmer that the northern part. Probably Câmpia Ierului wetlands that are the northern border of the village have a cooler microclimate, with the exceptions of summers.

In the Pir village area *Eriochloa villosa* spread in almost all cultivated fields located on hills. It was found mainly in the maize fields, but also in sunflower fields, in which for now only at the edges, and it is expected that in the following years to be slowly invaded as well. Interestingly it does not grow in the wheat fields, not even on the edges, probably due to its later fruit ripening during August, when wheat fields are already cropped.

Besides cultivated fields *Eriochloa villosa* is rapidly spreading along routes of transportation, what is interesting is that most of those are far from the damper areas usually prefered by *Eriochloa villosa*, but which can survive even amongst gravel of country roads. It can be noted that roads can become a route or a vector of spreading to new locations. Thus the road connecting Pir village to Chereușa to the east has numerous *Eriochloa villosa*, and probably already made it to the nearby village. The same was noted on the road that comes down to the Piru Nou village, from where they can easily spread to Câmpia (Valea) Ierului plain.

Another interesting spreading area of this species is throughout the cemeteries around the village where we found it growing on abandoned resting sites together with species of *Sedum* spp. which are known to prefer less damp areas. These invididuals that survive in harsher conditions, with less water availability, develop numerous lateral branches forming a shorter, compact, and bushier tussock, sometimes even less than 50 cm tall. In areas with optimum conditions *Eriochloa villosa* individuals are less branched, sometimes becoming pendulous. All these observations suggests that this species has great adaptability, easily colonizing disturbed habitats such as cultivated areas and roads, potentially becoming a major problem in the affected areas.

Fig. 3. Eriochloa villosa presence (in red) Pir village area in 2010.

Fig. 4. Eriochloa villosa presence (in red) Pir village area in 2011.

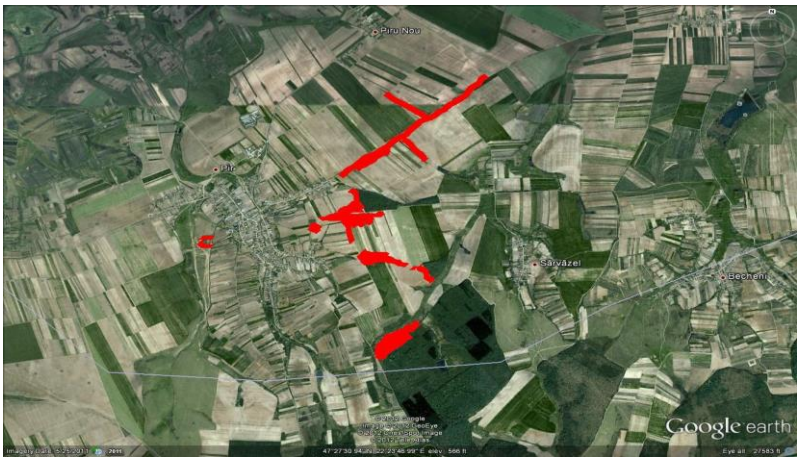

Fig. 5. Eriochloa villosa presence (in red) Pir village area in 2012.

Impact on local segetal flora. The main studies on *Eriochloa villosa* focused on its impact on cultivated fields and how can it be controled effectively to prevent crop losses. In this paper we will focus on its impact on native flora and the modifications on the plant composition where *Eriochloa villosa* settles. This study presents competitions and possible new associations between segetal species, the so called weeds growing on agricultural fields. The plant species growing on natural habitats are not considered here as *Eriochloa villosa* seems not to settle on areas that did not suffer disturbances. Focusing on observations on the impact of local agricultural weeds could offer important clues on the evolution of plant associations under the pressure of an invasive adventive species. The composition of plant associations with *Eriochloa villosa* is largely affected by the soil water availability, an interesing aspect to analyze. Thus, in the area of Pir village there are two distinct plant associations in which *Eriochloa villosa* is dominant. In the areas where *Eriochloa villosa* is only sporadic there are not major changes, at least in this preliminary data.

We analyzed two different habitats in which this species is found, one with damper soil (Table 1) and another with a deeper aquifer where during summer the lack of water affects the vegetation for several weeks (Table 2). The first habitat, from where the expansion of this species started in this region, is located in a valley surrounded by wetland and forest, the second habitat is located on the crest of the hills located in the southeastern area of the village and the road that connects Pir and Chereușa localities.

Table 1 presents three sampling plots from the above mentioned damper area. The first sampling plot (1) shows data that we collected before *Eriochloa villosa* appeared in the area, in 2008. This data is very important as it offers a comparison and the necessary perspective suggesting the rapid succesion of plant composition. It is easily noticeable that the dominant species are *Echinochloa crus-galli* and *Setaria pumila*, that together form *Echinochloo-Setarietum pumilae* association Felfödy 1942 em. Mucina 1993. The characteristic accompaning species are *Hibiscus trionum* L., *Portulaca oleracea* L., *Polygonum lapathifolium* L., *Galinsoga parviflora* Cav., *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L. (Sanda et al. 2008). The sampling plots 2 and 3 show data collected four years later suggesting the dominat role that *Eriochloa villosa* has assumed. It is noticeable that *Echinochloa crus-galli* population is drastically reduced, being transformed from a common species to a less present species. On the other side *Setaria pumila* population increased, possibly under the influence of the great number of *Eriochloa*. Looking at the other species several concerning data should be mentioned. Thus more than half of those species dissapeared from the surveyed maize fields, especially in the area with the greatest abbundance (5) of the invasive species (sampling plot 3). Indeed in some areas there were only *Eriochloa villosa* and *Setaria pumila*, with a random individual of *Xanthium strumarium* or *Echinochloa crus-galli*. In some areas *Eriochloa villosa* forms an almost compact carpet, where other species cannot survive. Cultivated plants are also overcrowded, reducing crop production. The species that continue to be abundant are: *Ambrosia artemisiifolia* L., *Xanthium strumarium* L., *Convolvulus arvensis* L., *Hibiscus trionum* L., *Cirsium arvense* (L.) Scop., and some species of *Polygonum*.

Table 1. Plant abundance-dominance at the damper location (Th – annual therophyte, TH – biannual therophyte, H – hemicryptophyte, G – geophyte, Adv – adventive, Cosm – cosmopolitan, Eua – Eurasiatic, Eur – European, Med – Mediteranean, Cp – circumpolar)

Bioforms	Geoelements	**Sampling plot number***	**1**	**2**	**3**
		Plant cover (%)	70	90	100
		Test area surface (m^2)	50	30	20
Th	Adv	*Eriochloa villosa*	-	4	5
Th	Cosm	*Echinochloa crus-galli*	2	+	+
Th	Cosm	*Setaria pumila*	3	3	4
H	Cosm	*Convolvulus arvensis*	+	+	-
Th	Eua	*Xanthium strumarium*	+	+	+
TH	Eua	*Hibiscus trionum*	+	+	-
Th	Adv	*Ambrosia artemisiifolia*	+	+	+
G	Eua	*Cirsium arvense*	+	+	-
Th	Cosm	*Polygonum aviculare*	+	-	+
Th	Cosm	*Polygonum lapathifolium*	+	+	+
Th	Eua	*Polygonum persicaria*	+	+	+
Th	Eua	*Gypsophila muralis*	+	+	-
G	Cosm	*Cynodon dactylon*	+	+	-
Th	Adv	*Amaranthus powelii*	+	+	-
Th	Adv	*Amaranthus retroflexus*	+	-	-
Th	Adv	*Oxalis fontana*	+	+	-
Th	Med	*Setaria verticillata*	+	+	-
Th	Eua	*Matricaria perforata*	+	-	-
Th	Adv	*Helianthus annuus*	-	+	-
H	Eur	*Rorippa sylvestris*	+	+	-
Th	Cosm	*Digitaria sanguinalis*	+	+	-
Th	Eua	*Fallopia convolvulus*	+	-	-
H	Eua	*Plantago major*	+	-	-
H	Eua	*Lolium perenne*	+	-	-
TH	Eua	*Daucus carota* subsp. *carota*	+	-	-
H	Cp	*Artemisia vulgaris*	+	-	-
Th	Eua	*Bidens tripartita*	+	-	-
Th	Cosm	*Portulaca oleracea*	+	-	-
Th	Cosm	*Capsella bursa-pastoris*	+	-	-
Th	Eua	*Thlaspi arvense*	+	-	-
Th	Adv	*Galinsoga parviflora*	+	-	-
H	Eua	*Sonchus arvensis*	+	-	-
Th	Eur	*Bromus commutatus*	+	-	-
Th	Adv	*Erigeron annuus* subsp. *strigosus*	+	-	-
Th	Eua	*Setaria viridis*	+	-	-
Th	Med	*Euphorbia helioscopia*	+	-	-
Th	Cosm	*Stellaria media*	+	-	-

***Sampling plot 1** – 2008 before *Eriochloa villosa;* **Sampling plot 2** – 2012 *Eriochloa villosa* in one maize field; **Sampling plot 3** – 2012 maximum abundance of dominant species in a second maize field with a damper soil

Following, we are presenting the analysis of the bioforms and geoelements (Sanda et al. 1983) from sampling plots 2 and 3 taken together, plots that together had only 19 species, in order to observe the percentage of the species associated with *Eriochloa villosa*. The bioforms indicate dominance of the annual therophytes (Th) (29 species – 73.68 %). Hemicryptophytes (H – 2 species – 10.52 %), biannual therophytes (TH) – 1 species (5.26 %) and geophytes (G) – 2 species (10.52 %), indicates a reduced presence. This is due especially to the intensive agricultural use of this field, but also due to the loss of competitive edge when faced with more resistant and better adapted species. Geophytes' percentage value indicate the supremacy of cosmopolitan species (Cosm) – 7 species (36.84 %), followed by the adventive (Adv) and Eurasiatic species (Eua) with the same coverage (26.31 % – 5 species each). The European are represented by only one species (10.52 %). The dominance of the cosmopolitan and adventive species together with *Eriochloa villosa* suggests worrisome future problems, as well as for other locations in the world. This is suggested by the fact that *Eriochloa villosa* does not need new adaptations to arrive in other countries, as it is usually found on agricultural monocultures now present worldwide.

In the dry habitat (Table 2) a different floristic composition is noticeable. The dominant species is millet (*Panicum miliaceum* L.), which in this area of Pir village also has only a recent history. This species is considered to be part of the association *Echinochloo-Setarietum pumilae* Felfödy 1942 em. Mucina 1993 (Sanda et al. 2008), but in the tested sampling plots it becomes the dominant species, having a higher abundance compared to *Echinochloa crus-galli* and *Setaria pumila*. It is possible that in this area it is a facies with *Panicum miliaceum.* Another possible phytosociological grouping might be the association *Erigeron canadensis – Panicetum miliacei* Ștefan 1993 (Sanda et al. 2008). The constant number of the species characteristic to the association (*Chenopodium album* L., *Cirsium arvense, Convolvulus arvensis, Setaria pumila, Echinochloa crus-galli*) are supporting this hypothesis. On a different note *Erigeron canadensis* L. (syn. *Conyza canadensis* /L./ Cronquist) is much less represented in this association. Almost all sampling plots analyzed in the area of Pir, *Eriochloa villosa* and *Panicum miliaceum* are found together, alternating in the percentage of abundance-dominance. These two species forming almost compact weedy areas, that suffocate other species and probably lead to reduced crop production of maize and sunflower. It seems like in the drier areas, on the hills and roadsides, *Eriochloa villosa* prefers the association with *Panicum miliaceum*, while in the damper areas with *Setaria pumila*, as it is noticeable in sampling plot 10, located in an area between a gravel road and the water drainage channel from the road.

It is interesing to note that the sampling plots where *Eriochloa villosa* is missing or it is in smaller numbers (sampling plots 6 and 9), *Echinochloa crus-galli* populations are more numerous. The data is preliminary, but the observation suggests that *Eriochloa villosa* and *Echinochloa crus-galli* occupy the same ecological niche, and the competition between these two species is one that *Echinochloa crus-galli*, is losing. Also in these sampling plots it can be noted the increased drop of the number of individuals and abundance of segetal species the so called agricultural weeds, to almost a half, comparative to sampling plots 6 and 9 where *Eriochloa* is absent, but this is also noticeable where millet (*Panicum miliaceum*) is expanding (sampling plots 7, 8, 9) comparative to sampling plot 5 for example. Possible causes could be the unusually

long drought (4 months) in 2012 when the sampling plots where designed, but it can be observed that *Eriochloa villosa* is drasticaly eliminating its competitors. Remarkably *Eriochloa villosa* shows affinity to related species in Paniceae (*Panicum, Setaria, Echinochloa, Digitaria*), which was observed in other parts of the world, where cultures of *Panicum* spp., *Echinochloa esculenta* (A.Braun) H.Scholz etc. (http://www. inspection.gc.ca) are prefered. I also have to mention that populations of Paniceae tribe and other resistent weeds has risen in conjunction with *Eriochloa villosa* arrival, which was also noticed in Hungary (Partosfalvi et al. 2008). Amongts these species notable are *Setaria pumila* and *Panicum miliaceum* whose populations doubled, slighter change in populations of *Digitaria sanguinalis, Chenopodium album* and even less of a change in populations of *Cirsium arvense* and *Echinochloa crus-galli*, the later one which I consider in competition with *Eriochloa*; even though in Hungary numbers of this species has risen as well (Partosfalvi et al. 2008).

Table 2. Plant abundance-dominance at the drier locations (abbreviations the same as in Table 1 + Atl-Med – Atlantic-mediteranean)

Biof orm s	**Geoel ement s**	**Sampling plot number***	**4**	**5**	**6**	**7**	**8**	**9**	**10**
		Plant cover (%)	85	75	60	95	70	60	90
		Testing area surface (m^2)	20	20	10	30	10	10	10
Th	Adv	*Eriochloa villosa*	3	3	-	2	+	-	4
Th	Adv	*Panicum miliaceum*	3	+	3	4	3	4	+
Th	Cosm	*Setaria pumila*	+	+	+	-	-	+	1
Th	Cosm	*Echinochloa crus-galli*	+	-	1	-	-	+	+
Th	Eua	*Xanthium strumarium*	1	+	+	+	1	+	-
Th	Adv	*Ambrosia artemisiifolia*	+	+	+	+	+	+	-
TH	Eua	*Hibiscus trionum*	+	+	-	+	-	+	-
H	Cosm	*Convolvulus arvensis*	+	+	+	+	+	-	-
Th	Cosm	*Polygonum aviculare*	+	+	+	-	1	1	+
G	Eua	*Cirsium arvense*	-	-	+	+	-	+	-
H	Eua	*Lolium perenne*	+	-	1	-	+	-	-
Th	Eua	*Polygonum persicaria*	-	-	-	-	-	+	-
G	Eua	*Elymus repens*	-	-	+	-	-	+	-
Th	Cosm	*Digitaria sanguinalis*	-	+	-	+	+	-	+
Th	Cosm	*Chenopodium album*	+	-	+	-	+	+	-
Th	Adv	*Xanthium italicum*	+	-	-	-	-	-	-
Th	Adv	*Amaranthus powelii*	-	+	-	-	-	-	-
Th	Adv	*Amaranthus retroflexus*	-	+	-	-	-	-	-
Th	Cosm	*Capsella bursa-pastoris*	-	-	+	-	+	-	-
H	Cp	*Artemisia vulgaris*	-	+	-	-	-	-	+
Th	Eua	*Gypsophila muralis*	-	+	-	-	-	-	-
Th	Atl-Med	*Crepis setosa*	+	-	-	-	-	+	-
Th	Eua	*Crepis foetida* subsp. *rhoeadifolia*	-	-	-	-	+	-	+
TH	Eua	*Picris hieracioides*	-	-	+	-	-	-	+
Th	Eua	*Lactuca serriola*	+	-	+	-	-	-	-

Th	Med	*Conium maculatum*	-	-	-	-	+	-	-
Th	Eua	*Consolida regalis*	+	-	+	-	-	-	-
Th	Adv	*Erigeron canadensis*	-	+	+	-	-	-	-
Th	Eua	*Oxalis corniculata*	-	+	-	-	-	-	-
Th	Cosm	*Centaurea cyanus*	+	-	-	-	-	-	-
Th	Eua	*Thlaspi arvense*	-	+	-	-	-	-	-
Th	Eua	*Viola arvensis*	+	+	-	-	-	-	-
H	Eua	*Plantago major*	-	+	-	-	-	-	-
Th	Cp	*Atriplex patula*	-	-	+	-	-	-	-
Th	Med	*Raphanus raphanistrum*	+	-	-	-	-	-	-
Th	Adv	*Brassica rapa* subsp. *oleifera*	+	-	-	-	-	-	-
Th	Med	*Brassica rapa* subsp. *sylvestris*	+	-	-	-	-	-	-

* **Sampling plot 4** – sunflower field on the hills; **Sampling plot 5** – maize field on the hills; **Sampling plot 6** – road side without *Eriochloa villosa*; **Sampling plot 7** – sunflower lot – next to Cionca Forest; **Sampling plot 8** – sunflower lot – roadside; **Sampling plot 9** – maize lot – roadside – without *Eriochloa villosa;* **Sampling plot 10** – area without agricultural crops – roadside

Analyzing the sampling plots it can be noted the constancy of some species in almost all surveyed fields and roadsides. Some were part of previous associations (*Convolvulus arvensis, Polygonum aviculare*, *Hibiscus trionum, Chenopodium album, Cirsium arvense, Digitaria sanguinalis*), others are invasive adventive species (*Xanthium strumarium, Ambrosia artemisiifolia*). In the same time the number of species and individuals within the populations is increasing towards the margins and decreases in the center of the fields. Subspecies of *Brassica* sp., *Plantago major* L., *Cyanus segetum* Hill (syn. *Centaurea cyanus* L.), *Consolida regalis* Gray, *Raphanus raphanistrum* L. or *Picris hieracioides* Sibth. & Sm. appear only at the margins of the cultivated fields. It is important to mention that *Panicum miliaceum* has a similar distribution with deacreasing number of individuals from the field edges to the center, its place being reclaimed by *Setaria pumila.* Similar distribution can be noted by *Eriochloa villosa* in many other places, around the world.

Bioforms and geoelements also indicate interesting percentages. In their calculations I considered data in all sampling plots to observe the impact the two dominant species (*Eriochloa villosa* and *Panicum miliaceum*) have on the surveyed plant communities. In total there are 37 species, this number, larger than in the previous sampling plots, indicates the addition of some species characteristic to roadsides that in most cases did not penetrate to the interior of fields. Thus, throughout the range of the bioforms, the annual therophytes (Th) are predominant (78.37% - 29 species) which is indicative of reduced soil dampness and intense agriculture. They are followed by the hemicryptophytes (H – 10.81 % - with only 4 species), then followed with equal coverage percentage by biannual therophytes (TH) and roadside geophytes (G): each with 2 species – 5,40 %. Amongst the geoelements, predominant are the Eurasiatic (Eua – 40.54 % – 15 species), followed in equal representation by the cosmopolitan (Cosm) and adventives (Adv) geoelements – 21,62 % with 8 species each. Further, with very small representation, are the Mediteranean species (Med – 8.10 % with 3 species), ruderal circumpolar species (Cp – 5.40 % with 2 species) and Atlantic-mediteranean

(Atl-Med – 2.70 % with only one species). If we consider that the majority of the Eurasiatic species are located towards the field margins indicates that the associations are again dominated by the cosmopolitan and adventive species, species that are posing problems throughout the world.

The last table (Table 3 – sampling plot 11) presents the species occupying an area with a high level of disturbance, plowed a number of times and then left fallow. It can be noted that a large portion of the pioneer species are adventive, the other part are native segetal species. The adventive species layer is again dominated by the *Eriochloa villosa* together with *Xanthium strumarium*, while the native segetal species by *Bromus commutatus* Schrad. and less so by *Bromus arvensis* L.. In lower number are the species usually found together with *Eriochloa villosa* such as: *Setaria pumila, Echinochloa crus-galli, Ambrosia artemsiifolia, Convolvulus arvensis, Cirsium arvense, Digitaria sanguinalis, Hibiscus trionum*, these being present on the surveyed fields for many years. It is important to mention that the presence of so many adventive ornamental species is due to the location of the field adjacent to a cemetery. These species have escaped from gardens and are naturalizing in the area for some years (*Eschscholzia californica* Cham., *Portulaca grandiflora* Hook., *Cosmos bipinnatus* Cav., *Tagetes patula* L., *Helianthus tuberosus* L.). According to numerous observations made in the area of the cemetery *Eriochloa villosa* was completely absent in 2009, 2010, 2011, and then it suddently appeared in 2012, with tens of individuals. The speed of colonizing new territories of *Eriochloa villosa* is impressive and worrisome, now reaching the quarantine status together with *Ambrosia artemisiifolia* (Partosfalvi et al. 2008).

Table 3. Species abundance-dominance on a fallow field. (abbreviations are the same as those in Table 1 + Atl-Med – Atlantic-mediteranean, Pont-Med – Pontic-mediteranean, Balc-Pont-Cauc – Balcanic-pontic-caucasian)

		Number of sampling plot*	**11**
		Plant coverage (%)	70
Bioforms	**Geoelements**	Test area surface (m^2)	15
Th	Adv	*Eriochloa villosa*	1
Th	Cosm	*Setaria pumila*	+
Th	Cosm	*Echinochloa crus-galli*	+
H	Cosm	*Convolvulus arvensis*	+
Th	Eua	*Xanthium strumarium*	1
TH	Eua	*Hibiscus trionum*	+
Th	Adv	*Ambrosia artemisiifolia*	+
Th	Adv	*Xanthium italicum*	+
Th	Eur	*Bromus commutatus*	1
Th	Cosm	*Polygonum aviculare*	+
Th	Med	*Setaria verticillata*	+
Th	Adv	*Eschscholzia californica*	+
G	Eua	*Elymus repens*	+
Th	Eua	*Bromus arvensis*	+
G	Eua	*Cirsium arvense*	+
Th	Atl-Med	*Crepis setosa*	+
Th	Med	*Stachys annua*	+
TH	Pont-Med	*Cephalaria transylvanica*	+

Th	Eua	*Lactuca serriola*	+
Th	Eua	*Avena fatua*	+
Th	Cosm	*Digitaria sanguinalis*	+
TH	Eua	*Daucus carota* subsp. *carota*	+
H	Cp	*Artemisia vulgaris*	+
Th	Cosm	*Chenopodium album*	+
Th	Eua	*Medicago lupulina*	+
Th	Eua	*Trifolium arvense*	+
Th	Adv	*Tagetes patula*	+
Th	Med	*Lactuca saligna*	+
Th	Eua	*Consolida regalis* subsp. *paniculata*	+
H	Eua	*Rubus caesius* var. *arvalis*	+
Th	Adv	*Cosmos bipinnatus*	+
H	Eua	*Chodrilla juncea*	+
Th	Adv	*Portulaca grandiflora*	+
TH	Eua	*Echium vulgare*	+
Th	Balc-Pont-Cauc	*Vicia grandiflora*	+
H	Eua	*Cichorium intybus*	+
G	Adv	*Helianthus tuberosus*	+

*** Sampling plot 11** – fallow field near a cemetery [the species abundance in all tables is marked from + (a few individuals) to 5 (the species is dominating the sample)]

Conclusions

According to the field observations, *Eriochloa villosa* associates with species of Paniceae Tribe (Poaceae): *Setaria pumila* and *Panicum miliaceum*, with which easily forms a range of abundance-dominance. *Eriochloa villosa* settles within previous plant associations, changing their floristic composition. We would like to point out two tendencies of new plant associations: *Eriochloa villosa* together with *Setaria pumila* in the damper areas and *Eriochloa villosa* together with *Panicum miliaceum* in drier areas. As more data from other counties throughout the country will be added, further comparisons regarding these associations can be made. Due to the fact that *Panicum miliaceum* is a species escaped from cultivation and almost naturalized in a few areas throughout the country, it won't be found together with *Eriochloa* in these areas, even if they are on the drier side. This suggests that *Panicum miliaceum* association with *Eriochloa villosa* is only a facies around Pir village, the main codominant species together with *Eriochloa villosa* being *Setaria pumila*, as I observed in the sample plots with less *Panicum miliaceum*.

The dominant species in the damper areas are *Eriochloa villosa, Setaria pumila, Echinochloa crus-galli*, and in drier areas: *Eriochloa villosa* and *Panicum miliaceum*, with lower percentages of *Erigeron canadensis, Setaria pumila, Echinochloa crus-galli*. The species characteristics to both types of the above mentioned associations in constant percentages are: *Hibiscus trionum, Convolvulus arvensis, Digitaria sanguinalis, Cirsium arvense, Ambrosia artemisiifolia, Xanthium strumarium*, with *Chenopodium album* especially in the drier areas.

The establishment of the adventive invasive species *Eriochloa villosa* in new habitats, even the ruderal weedy areas or segetal flora causes drastic perturbations in the

plant species associations. Thus some species are multiplying uncontrollably, to the detriment of others whose numbers are drastically reduced or even eliminated. A careful monitoring of *Eriochloa villosa* is of utmost importance due as its very quick adaptation which would lead to an unprecedented decrease of native flora, further followed by decrease of the fauna that depends on it. Still, field observations suggests that *Eriochloa villosa* is not able to establish itself in the undisturbed habitats, which are for now safe. Regarding its propagation speed, amongst the invasive plants in the Pir area, *Eriochloa villosa* occupies second place only after *Ambrosia artemisiifolia*, and followed by *Prunus serotina* Ehrh. and *Robinia pseudoacacia* L..

References

Allison, K. & Darbyshire, S. (2001). Identification of *Eriochloa villosa*. *Seed Technology*, 23(2), 169–172.

Ardelean, A., Karácsonyi, K. & Negrean, G. (2009). *Eriochloa villosa* – A new alien Graminaceae species for Arad County (Romania). *Studia Universitatis "Vasile Goldiş",* Seria Ştiinţele Vieţii, 19 (2), 281-282.

Balogh, Z. & Novák, R. (2014). Adatok az ázsiai gyapjúfü (*Eriochloa villosa*) Borsod-Abaúj-Zemplén Megyei terjedéséhez és az ellene való védekezés lehetőségeihez. NTN 2014, Abstracts 79.

Bartha, D., Király, G., Schmidt, D., Tiborcz, V., Barina, Z., Csiky, J., Jakab, G., Lesku, B., Schmotzer, A., Vidéki, R., Vojtkó, A. & Zólyomi, S. (eds.) (2015). *Magyarország edényes növényfajainak elterjedési atlasza / Distribution atlas of vascular plants of Hungary*. Sopron: Nyugat-magyarországi Egyetem Kiadó / University of West Hungary Press, 329 pp.

Ciocârlan, V. & Sike, M. (2006). *Eriochloa villosa* (Thunb.) Khunt (Poaceae) in the Romanian flora. *Buletinul Grădinii Botanice Iaşi*, 13, 105–107.

Ciocârlan, V. (2009). *Flora ilustrată a României*. Bucureşti: Edit. Ceres.

Clayton, W. D. (1980). *Eriochloa* Kunth. In Tutin, T.G., Heywood, V.H., Burgess, N. A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D. A. (eds.). *Flora Europaea, Vol. 5. Alismataceae to Orchidaceae (Monocotyledones).* Cambridge: Cambridge University Press, 262 pp.

Clayton, W.D., Harman, K.T. & Williamson, H. (2008). *World Grass Species: Descriptions, Identification and Information Retrieval*. Royal Botanic Gardens, Kew.

Dărăban, I.-N., Arsene, G.-G., Turcuş, V., Petrescu, M.-C. & Ardelean, A. (2012). Present-day condition of cormophytes diversity on alkali soils in Vărşand (Arad County, W-Romania). *Studia Universitatis "Vasile Goldiş",* Seria Ştiinţele Vieţii, 22 (3), 435-441.

Fărcăşescu, A.M., Arsene, G.G. & Neacşu, A.G. (2008). *Eriochloa villosa* (Thunb.) Khunt – a new invasive weed in Romania. *Journal of Plant Diseases and Protection*, Special Issue, 21, 333–334.

Karácsonyi, C. (2011). *Flora şi vegetaţia Dealurilor Tăşnadului şi a colinelor marginale*. Arad: Edit. „Vasile Goldiş" University Press.

Negrean, G. (2011). Addenda to „Flora Romaniae" Volumes 1-12. Newly published plants, nomenclature, taxonomy, chorology and commentaries (Part 1). *Kanitzia*, 18: 89–194.

Negrean, G. (2012). Addenda to „Flora Romaniae" Volumes 1-12. Newly published plants, nomenclature, taxonomy, chorology and commentaries (Part 2). *Kanitzia*, 19, 195-233.

Partosfalvi, P., Madarász, J. & Dancza, I. (2008). Az ázsiai gyapjúfű (*Eriochloa villosa* (Thunb.) Kunth) megjelenése Magyarországon. *Növényvédelem*, 44(6), 141-142.

Rivière, G., Guillévic, Y. & Hoarher, J. (1992). Flore et végétation du Massif Armoricain. Supplément pour le Morbihan. *Erica* [Conservatorie Botanique National de Brest], 2, 6–78.

Sanda, V., Popescu, A., Doltu, M.I. & Doniţă, N. (1983). Caracterizarea ecologică şi fitocenologică a speciilor spontane din flora României. *Stud. Comunic. Șt. Nat*., Muz. Brukenthal, Sibiu, 25 (Supl.), 1-126.

Sanda, V., Ollerer, K. & Burescu, P. (2008). *Fitocenozele din România*. Bucureşti: Edit. Ars Docendi, Universitatea din Bucureşti.

Szilágyi, A., Balogh, Z., Dávid, I., Szabó, L. & Radócz, L. (2015). Woolly cupgrass (*Eriochloa villosa* /Thunb./ Kunth), a recently occured invasive weed in Trans-Tisza Region and a trial for control in maize. *Journal of Agricultural Sciences*, 66, 53-57.

Tsvelev, N.N. (1984). *Grasses of the Soviet Union*. New Delhi, India: Amerind Publishing. [English translation published for the Smithsonian Institution and the National Science Foundation, Washington, DC], 1196 pp.

http://www.inspection.gc.ca – Canadian Food Inspection Agency, 2008.

http://www.eppo.int/INVASIVE_PLANTS/observation_list/Eriochloa_villosa.htm

*** Convenţia între Guvernul României şi Guvernul Bulgariei privind colaborarea în domeniul carantinei, Buruieni de carantină, Lista A2, Monitorul Oficial nr. 315 / 11.28.19

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 57-66
DOI: 10.1515/ahbb-2016-0005

ANALYSIS OF FAMILY BORAGINACEAE FROM CARL STUDNICZKA'S HERBARIUM

VLADOVIĆ Dalibor[1*], MITIĆ Božena[2]

Abstract: We analysed family Boraginaceae (order: Borageineen) with total of 115 herbarium sheets. According to the labels, the majority of herborized material was collected in the area of Austria (20 sheets). Most herbarium sheets belong to Flora Dalamtiens collection (28). In reference to the part of Studniczka's herbarium which has already been analysed, there are five collections which are mentioned for the first time. Following botanist or collectors of herbal material are mentioned for the first time, and there are: Bartholomatus, Csató, François, Haertel, Herbich, Menyhárth and Schrenk. Most herbarium sheets were collected by Studniczka himself (70).
Key words: Studniczka's herbarium, Natural History Museum Split, Croatia.

Received 09 November 2016 *Accepted 16 November 2016*

Introduction

As a botanist amateur, Carl Studniczka collected plants while he was traveling (1869-1904) and working as an officer (data are known from herbarium labels: wide surrounding area of Vienna and Wiener Neustadt in Austria, of Split in Croatia, of Celje in Slovenia, of Kotor in Montenegro, of Olomouc and Leitmeritz in the Czech Republic, of Przemysl in Poland and at last the wide surrounding area of Trieste in Italy). The analysis has shown that this herbarium contains samples of the plants which were collected and sent to Studniczka by many botanists or collectors of that time.

Material and methods

In this paper we analysed the herbarium sheet from family Lamiaceae. From the labels of the herbarium sheets first, we copied the Latin name of the genus and the species; then the date, month and year of collection; followed by the collector (**A**-Alioth **Ay**-Ayasse, **B**-Baenitz, **Ba**-Barth, **Bo**-Bordère, **D**-Dieudonné, **E**-Eggert, **F**-Favrat, **Fr**-Frank, **Fy**-Freyn, **G**-Gandoger, **Ga**-Gautier, **Go**-Göth, **Gr**-Graf, **H**-Halácsy, **He**-Heldreich, **Hn**-Henser, **Ho**-Holuby, **Hu**-Huet A., **K**-Keck, **L**-Legrand, **Le**-Letourneux, **R**-Ressmann, **Re**-Reuterman, **Ri**-Richter, **Rv**-Reverchon, **S**-Studniczka, **St**-Strobl, **T**-Théveneau, **To**-Torrepando, **Z**-Zapfl, **Ze**-Zetterstedt) and the affiliation to the particular herbarium collection (FA-Flora Algeriensis exsiccata, FAE-Flora arveniae exsiccata, FB-Flora Böhmen /including Flora von Böhmen/, FC-Flora carniolica, FD-Flora Dalmatiens, FG-Flora Gallica exsiccata, FGr-Flora von Griechenland, FH-Flora

[1] Natural History Museum and Zoo, Kolombatovićevo šetalište 2, 21 000 Split, Croatia.
[2] Botanic Institute of PMF University of Zagreb, Marulićev trg 20/ II, 10 000 Zagreb, Croatia.
[*] Corespondence: dalibor@prirodoslovni.hr

Hungarica, FI-Flora von Istrien, FN-Flora von N. Oesterreich /including Flora von Unt. Oesterreich/, FO-Flora von Obersteiermark, FOl-Flora von Olmütz, FP-Flora von oesterr. Polen /including Flora von Przemysl/, FT-Flora von Triest, FTr-Flora Transsilvaniae, FW-Flora von Wien, FWN-Flora von Wr Neustadt, HA-Eggert, Herbarium Americanum, HE-Baenitz, Herbarium Europaeum, HT-Herbier Thévenau Béziers, P-Plantes de la Loire, PS-Plantae Scandinavicae., S-Societas Helvetica /including: Société Helvétique and Societas Botanica Helvetica/, SV-Société Vogéso-Rhénane, U-unknown). According to The Plant list and USDA plants Database we listed the name of the species in brackets; from the label we copied the numeric sign, name of the place where the plant had been found with altitude data if available and listed the number of herbarium samples in brackets (for example, 2 h.s.).

Results and discussion

List of species

1. *Alkanna tinctoria* Tausch. 13.4.1873. **Fy** U (*A. tinctoria* /L./ Tausch) Ungarn. Triften bei Pest. Sand 115 m (2 h.s.)
2. *Anchusa arvensis* M.Bieb. = *Lycopsis arvensis* L. 7.1881. **S** FB (*A. arvensis* /L./ M.Bieb.) auf sandäckern bei Skalitz nachst Leitmeritz (5 h.s.)
3. *A. italica* Retz 4.6.1872. **S** FN (*A. azurea* Mill.) üm Wien b. d. Marxer linie (1 h.s.)
4. *A. italica* Retz. 31.5.1878. **S** FD (*A. azurea* Mill.) auf Schutthaufen bei der Schieβs stätte nächst Trinita bei Cattaro (1 h.s.)
5. *A. officinalis* L. 5.6.1881. **S** FB (*A. officinalis* L.) wüste Plätze u. Gräben um Theresienstadt (2 h.s.)
6. *A. officinalis* L. 12.6.1885. **S** FP (*A. officinalis* L.) Raine u. Wiesenrdr um Przemysl (2 h.s.)
7. *A. officinalis* L. β *angustifolia* 3.6.1897. **S** FWN (*A. officinalis* L.) Leithasand bei Neudörfel (1 h.s.)
8. *A. variegata* Lehm 4.1875. **S** FD (*A. variegata* /L./ Lehm.) am Marian bei Spalato (3 h.s.)
9. *A. variegata* Lehm 4.1878. **S** FD (*A. variegata* /L./ Lehm.) auf den Abhängen des Vermacz (4 h.s.)
10. *A. variegata* Lehm 4.1879. **S** FD (*A. variegata* /L./ Lehm.) steinige Abhänge s Vermacz bei Cattaro (3 h.s.)
11. *Asperugo procumbens* (L.) 27.5.1878. **Ga** FAE (*A. procumbens* L.) Localité: Clermont-Ferrand Statio: Bords des fumiers au Salins (alluvions), à 380^{m} (2 h.s.)
12. *A. procumbens* L. 6.6.1881. **S** FB (*A. procumbens* L.) Ackerränder bei Kopist nächst Theresienstadt (1 h.s.)
13. *Borago officinalis* L. 11.4.1873. **S** FD (*B. officinalis* L.) wüsten Plätzen nächst einem Hause bei Meligne (3 h.s.)
14. *B. officinalis* L. 21.6.1901. **S** FT (*B. officinalis* L.) werwild. überall Barcola (2 h.s.)
15. *Cerinthe carthusaniorum* Gdgr. 14.7.1874. **G** FG (*C. minor* L. ssp. *auriculata* /Ten./ Domac) No 72 Hab. in rupibus ad Bovinant supra La G^{de} Chartreuse (Isére) 5000' (2 h.s.)
16. *C. minor* L. β *maculata* 6.1875. **S** FD (*C. minor* L. ssp. *auriculata* /Ten./ Domac) ausserhalb der Hptmaner des Castell Clissa gegen die Signaner stresse zu (2 h.s.)

17. *C. minor* L. 22.5.1881. **S** FB (*C. minor* L.) felsige Orte am Radobil bei Leitmeritz (1 h.s.)
18. *C. minor* L. 30.5.1885. **S** FP (*C. minor* L.) an Rainen u. wüsten Orten um Przemysl (2 h.s.)
19. *C. smithiae* Kern. 5.1879. **Gr** U (*C. glabra* Mill. ssp. *smithiae* /A. Kern./Domac) Istria. Scogl. St Marco (2 h.s.)
20. *Cynoglossum morisoni* DC. 12.8.1875. **E** HA (*C. virginianum* L.) Blüffs in Ill. (1 h.s.)
21. *C. officinale* L. 25.5.1872. **S** FW (*C. officinale* L.) Schanze beim Neugebäude (4 h.s.)
22. *C. pictum* Ait. 1875. **S** FD (*C. creticum* Mill.) unfruchtbaren Orten, Rainen, Wegen um Spalato (2 h.s.)
23. *C. pictum* Ait. = *C. creticum* Vill. 6.1880. **S** FD (*C. creticum* Mill.) üm Cattaro (3 h.s.)
24. *C. pictum* Ait. 7.7.1902. **S** FT (*C. creticum* Mill.) am Hügel Pantalone bei Zaule (2 h.s.)
25. *C. virginicum* Pers. **Hn** U (*C. virginianum* L.) New Jersey (1 h.s.)
26. *Echinospermum deflexum* (Wg.) 23.6.1870. **Ze** PS (*Lappula deflexa* /Wahlenb./ Garcke) Norge: Gudbrandsdalon, Ringeboe (5 h.s.)
27. *E. deflexum* Lehm. 24.7.1895. **S** FWN (*L. deflexa* /Wahlenb./ Garcke) aüf dem Mariahilferberg bei Guttenstein (1 h.s.)
28. *E. lappula* Ait. 28.6.1880. **S** FD (*L. squarrosa* /Retz./ Dumort.) zwischen Gestein am montenegro Platz bei Cattaro (1 h.s.)
29. *E. lappula* Lehm. 9.1893. **S** FN (*L. squarrosa* /Retz./ Dumort.) Brachäcker b. Wr Neustadt (3 h.s.)
30. *Echium italicum* L. 7.6.1875. **S** FD (*E. italicum* L.) wüsten Plätzen um Spalato (2 h.s.)
31. *E. italicum* L. 11.7.1904. **S** FI (*E. italicum* L.) wüste Plätze gegen Isola (1 h.s.)
32. *E. parviflorum* Moench. 4.1875. **S** FD (*E. parviflorum* Moench) bei Toretta und Botticelli nächst Spalato (2 h.s.)
33. *E. plantagineum* L. 22.5.1875. **S** FD (*E. plantagineum* L.) wüsten Plätzen im kloster won Paludi nächst Spalato (2 h.s.)
34. *E. rubrum* Jcq. 22.5.1873. **Fy** U (*E. maculatum* L.) Siebenbürgen. Comitat: Kolas Bergwiesen bei Klausenburg Tert. Tegel 506 m (1 h.s.)
35. *E. vulgare* L. 22.6.1877. **S** FD (*E. vulgare* L.) nächst dem Fort Cerequizze in der Krivošie (1 h.s.)
36. *E. vulgare* L. 25.6.1895. **S** FWN (*E. vulgare* L.) Wälder üm Wöllersdorf (2 h.s.)
37. *E. vulgare* L. 12.6.1902. **S** FT (*E. vulgare* L.) Schutthaufen am Trstenik ober Barcola (1 h.s.)
38. *Eclipta erecta* L. **Frank** U (*E. prostrata* /L./ L.) Ohio (2 h.s.)
39. *Eritrichium nanum* Schrd. 2.8.1870. **St** FO (*E. nanum* /L./ Schrad. ex Gaudin) Auf dem Hochgolling Gl. Schiefer (2 h.s.)
40. *Heliotropium curassavicum* Lin. 6.1874. **T** HT (*H. curassavicum* L.) Sables maritimes Ile S^{t} Lucie (Aude) (2 h.s.)
41. *H. europaeum* L. 8.1875. **S** FD (*H. europaeum* L.) auf Rainen und Aeckern der Halbinsel Lapad (3 h.s.)
42. *H. macrocarpum* Guss. 1.9.1874. **He** HE (*H. dolosum* De. Not) Athen. In campis arenosis halopedi Phaleri (2 h.s.)
43. *Lithospermum apulum* Vahl. 23.5.1875. **Fy** U (*Neatostema apulum* /L./ I.M.Johnst.) In siccis insulae S. Girolamo Istriae australis, solo calc. alt. 5m (3 h.s.)

44. *L. apulum* Vahl. 2.5.1877. **To** S (*N. apulum* /L./ I.M.Johnst.) Habitat in collibus Regionis submontanae (600 m) Lecta prope Madrid (5 h.s.)
45. *L. arvense* L. 5.1880. **S** FD (*Buglossoides arvensis* /L./ I.M.Johnst.) uncult. Orte ober Scagliari bei Cattaro (1 h.s.)
46. *L. arvense* L. 5.1885. **S** FP (*B. arvensis* /L./ I.M.Johnst.) Aecker um Przemysl (4 h.s.)
47. *L. incrassatum* Guss. 5.1875. **S** FD (*B. incrassata* /Guss./ I.M.Johnst.) am Monte Marian; im grösserer Ansehl auf dem Hügel gegenüber der Eremitage (2 h.s.)
48. *L. incrassatum* Guss. 5.1880. **S** FD (*B. incrassata* /Guss./ I.M.Johnst.) am Monte Sella oberhalb Cattaro /:1. monteng. Dorf:/ (4 h.s.)
49. *L. officinale* L. 5.1878. **S** FD (*L. officinale* L.) bei Scagliari nächst Cattaro (1 h.s.)
50. *L. officinale* L. 1893. **S** FWN (*L. officinale* L.) hügel üm Fischaü (2 h.s.)
51. *L. petraeum* DC. 5.1880. **S** FD (*Moltkia petraea* /Tratt./ Griseb.) auf Felsen am Giovanni di Cattaro (10 h.s.)
52. *L. petraeum* A.DC. 6.1880. **S** FD (*M. petraea* /Tratt./ Griseb.) auf Felsen am Vermacz oberhalb Mulla rechts von den serpentinweg zum Fort Vermacz (6 h.s.)
53. *L. purpureo-coeruleum* L. 6.1873. **Ri** FH (*Buglossoides purpurocaerulea* /L./ I.M.Johnst.) Ofen Wälder (2 h.s.)
54. *L. p.-coeruleum* L. 4.1874. **S** FD (*B. purpurocaerulea* /L./ I.M.Johnst.) zwischen Gesträuch bei Salona (2 h.s.)
55. *L. purpureo-coeruleum* L. 5.1894. **S** FN (*B. purpurocaerulea* /L./ I.M.Johnst.) auf der Malleiten bei Fischau (2 h.s.)
56. *Mertensia maritima* Don. 23.6.1875. U (*M. maritima* /L./ Gray) Nova Anglia (1 h.s.)
57. *Myosotis alpestris* Hopp. 23.6.1875. **S** FD (*M. alpestris* F.W.Schmidt) am Bikovo /:höchste Spitze Sveti Juro 5587':/ (2 h.s.)
58. *M. alpestris* Schult. 5.7.1874. **Ay** U (*M. alpestris* F.W.Schmidt) Paturages rocailleux du Jura an Recuilét (Ain) (1 h.s.)
59. *M. balbisiana* Jord. 22.5.1873. **L** P (*M. balbisiana* Jord.) du cour lapidosis montium supra Montbrison alt. 900 m (6 h.s.)
60. *M. caespitosa* Schultz 5.1891. **S** FOl (*M. laxa* Lehm. ssp. *caespitosa* /Schultz/ Hyl. ex Nordh.) sumpfwiesen bei Kloster Hradisch (4 h.s.)
61. *M. hispida* Schlechtd. 5.1894. **S** FN (*M. ramosisisma* Rochel) auf der Malleiten b. Fischau (13 h.s.)
62. *M. intermedia* Link var. *dumetorum* Crepin. 5.1874. **D** SV (*M. arvensis* /L./ Hill) Station: Bois humide Localite: Eegenhoven près de Louvain (Brabant) Altitude: 25 mètres (3 h.s.)
63. *M. intermedia* Lk. 19.5.1880. **S** FD (*M. arvensis* /L./ Hill) steinige Hügel u. Abhänge um Cattaro (2 h.s.)
64. *M. intermedia* Link 17.6.1895. **S** FWN (*M. arvensis* /L./ Hill) im Föhrenwald beim Dillmanhof (2 h.s.)
65. *M. laxiflora* Rchb 6.8.1876. **G** FG (*M. scorpioides* L.) No 1002 Hab. in paludis et fossis as Arnas (2 h.s.)
66. *M. palustris* Withering 6.1882. **S** FB (*M. scorpioides* L.) sumpfige Gräben um die Festung Theresienstadt (2)
67. *M. palustris* Withering 16.5.1885. **S** FP (*M. scorpioides* L.) an Bächen v. r. der strasse nach Buda b. Premysl (2 h.s.)

68. *M. palustris* Roth. α) *grandiflora* Čelak 23.5.1878. **Fy** U (*M. scorpioides* L.) Böhmen. Mensegebirge. Sümpfwiesen (2 h.s.)
69. *M. palustris β laxiflora = M. laxiflora* Reichb 11.6.1885. **S** FP (*M. scorpioides* L.) in Birkenwäldern bei Kruhel (4 h.s.)
70. *M. palustris* Roth. α *strigulosa* 16.6.1897. **S** FWN (*M. strigulosa* Rchb.) an der Fischa bei Wr N. (2 h.s.)
71. *M. strigulosa* Rchb. 5.1878. **K** S (*M. strigulosa* Rchb.) In pratis. Aistersheim. Austria sup. (6 h.s.)
72. *M. pyrenaica* Pourr. 1878. **Bo** S (*M. alpina* Lepeyr.) Pàturages du Pic Blanc, Hautes-Pyrénées. Formation calcaire. Altitude 2500 mètres (13 h.s.)
73. *M. rehsteineri* Wartm. 2.5.1874. **Ay** U (*M. rehsteineri* Wartm.) Graviers¸ humides du lac Léman a Bellerive prés Genéve (7 h.s.)
74. *M. rehsteineri* Wartm. 23.5.1874. **F** SV (*M. rehsteineri* Wartm.) Versoix, Genéve, Suisse. Gräves du Léman 375 m (7 h.s.)
75. *M. sicula* Guss. 20.5.1873. **Rv** U (*M. sicula* Guss.) Ribieres (5 h.s.)
76. *M. silvatica* Hoffm. β *alpestris* 9.5.1878. **S** FD (*M. sylvatica* Hoffm.) auf der neuen Strasse bei Scagliari (3 h.s.)
77. *M. silvatica* Hoffm. var: *grandiflora* 6.1882. **S** FB (*M. sylvatica* Hoffm.) Wälder am Kreutzberg bei Leitmeritz (1 h.s.)
78. *M. sparsiflora* Mikan 5. **H** S (*M. sparsiflora* J.C.Mikan ex Pohl) In nemoribus Danubii (Prater) ad Viennam, Austriae inf. (2 h.s.)
79. *M. sparsiflora* Mikan 31.5.1882. **S** FB (*M. sylvatica* Hoffm.) Wall von Theresienstadt (4 h.s.)
80. *M. stricta* Lk. forma *alpestris* 27.5.1875. **A** U (*M. stricta* Link ex Roem. & Schult.) Simplon (26 h.s.)
81. *M. stricta* Link 29.5.1881. **S** FB (*M. stricta* Link ex Roem. & Schult.) Sandäcker um Travčič bei Leitmeritz (3 h.s.)
82. *M. stricta* Link 11.5.1885. **S** FP (*M. stricta* Link ex Roem. & Schult.) sandige Getreidefelder unterhalb dem Laboratorium n Tatarenhügel b. Przemysl (4 h.s.)
83. *M. verna* Nutt. 10.8.1875. **E** HA (*M. verna* Nutt.) Wald an De Hodiamont Av. (2 h.s.)
84. *M. versicolor* Pers. 29.4.1874. **Ay** U (*M. discolor* Pers.) Champs à terrain liqu à Meyrin prés Geneve (5 h.s.)
85. *Nonnea pulla* DC. 31.5.1881. **S** FB (*Nonea pulla* /L./ DC.) Wall von Theresienstadt (2 h.s.)
86. *N. pulla* DC. 31.5.1892. **S** FWN (*N. pulla* /L./ DC.) Berge um Fischau (3 h.s.)
87. *N. ventricosa* Grieseb. 3.4.1874. **S** FD (*N. echioides* /L./ Roem. & Schult.) steinig Rainen bei Spalato /:gegen Almissa zu und Hügel bei Toretta:/ (3 h.s.)
88. *N. ventricosa* Griesb. 4.1879. **Le** FGr (*N. echioides* /L./ Roem. & Schult.) auf Corfü (4 h.s.)
89. *Omphalodes scorpioides* Lehm. 24.4.1872. **Z** U (*O. scorpioides* /Haenke/ Schrank) Würzberg bei Fernitza Stiria (3 h.s.)
90. *O. scorpioides* Lehm. 14.4.1875. **Ba** U (*O. scorpioides* /Haenke/ Schrank) In silvis prope pag. Blasendorf Transsilvaniae merid. (3 h.s.)
91. *O. verna* Mnch. 4.1876. **R** FC (*O. verna* Moench) Želimlje bei Laibach (6 h.s.)
92. *Onosma arenarium* W.K. 17.7.1873. **Fy** U (*O. arenaria* Waldst. et Kit.) Ungarn. Comitat Pest. Wiesen der Rennbahn bei Pest flugsand 120 m (2 h.s.)

93. *O. echioides* L. 11.6.1895. **S** FWN (*O. echioides* L.) hügel bei Fischau (1 h.s.)
94. *O. stellulata* WK 1874. **S** FD (*O. stellulata* Waldst. et Kit.) auf der südseite des Monte Marian bei Spalato (3 h.s.)
95. *O. stellulata* Wldst & Kit 6.1880. **S** FD (*O. stellulata* Waldst. et Kit.) Monte Vermacz bei Cattaro (1 h.s.)
96. *O. stellulatum* W & Kit. β *angustifolium* 7.6.1900. **S** FTriest (*O. stellulata* Waldst. et Kit.) Barcola & Gretta (3 h.s.)
97. *Pulmonaria angustifolia* L. 9.5.1872. **B** (*P. angustifolia* L.) Koenigsberg: Scherwitt bei Tapiau (4 h.s.)
98. *P. azurea* Bess. 16.5.1882. **S** FB (*P. angustifolia* L.) sumpfige Bäche bei Kamaik nächst Leitmeritz (3 h.s.)
99. *P. mollis* Wolff. 5.1873. **Ho** U (*P. mollis* Wulfen ex Hornem.) Gemein auf Weinbergtriften bei N. Podhrad. Ungarn (2 h.s.)
100. *P. mollis* Wolff. 30.5.1875. **A** U (*P. mollis* Wulfen ex Hornem.) Mayens de Sion, Wallis (2 h.s.)
101. *P. officinalis* Jord. 4.1871. **L** U (*P. officinalis* L.) Gallia. Montbrison (2 h.s.)
102. *P. officinalis* L. 13.4.1874. **Ay** U (*P. officinalis* L.) Dans une haie à Bellerive prés Genéve (2 h.s.)
103. *P. officinalis* L. 5.1875. **H** U (*P. officinalis* L.) in silvaticis ad Hainbach, Austriae inf. (3 h.s.)
104. *P. styriaca* Kern. 4.1870. **Gr** U (*P. stiriaca* A.Kern.) Stiria.Stübing (1 h.s.)
105. *Solenanthus lanatus* DC 3.1879. **G** FA (*Pardoglossum cheirifolium* /L./ E. Barbier & Mathez) No 34 Hab. In ditione urbis Alger, loco dicto Kouba (2 h.s.)
106. *Stenhammaria maritima* Rchnb. 23.7.1875. **Re** U (*Mertensia maritima* /L./ Gray) West Finmarken, Lyngen in litore marino (1 h.s.)
107. *Symphytum bohemicum* Schmidt 15.5.1878. **Fy** U (*S. officinale* L.) 198. Böhmen. Opočno auf Wiesen haüfig (1 h.s.)
108. *S. bohemicum* Schm. 6.1882. **S** FB (*S. officinale* L.) Auen u. Gräben um Theresienstadt (2 h.s.)
109. *S. cordatum* W. et K. 13.4.1873. **Göth** FTr (*S. cordatum* Waldst. et Kit. ex Willd.) In lucis Torda (2 h.s.)
110. *S. floribundum* Schuttleworth. 27.4.1874. **Hu** S (*S.* × *floribundum* Schuttlew. ex Buckn.) Bords des cour à Hyères (Var) dans la propriété de M le Comte de Beauregard (1 h.s.)
111. *S. officinale* L. 5.6.1881. **S** FB (*S. officinale* L.) sumpfwiesen an der Eger bei Theresienstadt (2 h.s.)
112. *S. officinale* L. 11.5.1885. **S** FP (*S. officinale* L.) sumpfge Wiesen um Przemysl /:Tatarenhügel:/ (1 h.s.)
113. *S. tuberosum* L. 15.5.1875. **S** FD (*S. tuberosum* L.) zwischen Gesträuch aüf der Marchesina Gredda bei Clissa (3 h.s.)
114. *S. tuberosum* L. 5.1885. **S** FP (*S. tuberosum* L.) feuchte Wldr, Holzschlge bei Lipowitza n. Przemysl (2 h.s.)
115. *S. tuberosum* L. 9.5.1897. **S** FWN (*S. tuberosum* L.) Wälder aüf der Malleiten bei Fischaü (1 h.s.)
116. *S. tuberosum* L. 15.4.1902. **S** FT (*S. tuberosum* L.) im Wäldchen am Trstenik (2 h.s.)

The total number of the herbarium scheets in analysed family Boraginaceae is 115 (with 338 samples of herbal plants, excluding herbarium scheet *Eclipta erecta* L. with two samples of herbal plants, witch belongs to family Compositae). The majority of the plants was collected in Europe (110 herbarium sheets), 4 sheets originate from North America and one herbarium sheet is from Africa.

Most of the herbarium samples were collected in the area of Austria (20 herbarium sheets) and: Montenegro -15, Czech Republic -15, Croatia -15, France -10, Poland -8, Switzerland -6, Italy -5, USA -4, Romania -3, Hungary -3, Slovenia -2, Greece -2, Norway -2. One herbarium sheet was collected in: Russia, Slovakia, Belgium, Algeria and Spain.

According to the affiliation to a particular herbarium collection in analysed familys or their parts the most represented is Flora Dalmatiens with 28 herbarium sheets. This is followed by other collections, such as: Flora Böhmens -12 (including Flora von Böhmen collection), Flora von Wr. Neustadt -9, Flora von oesterr. Polen -8 (including Flora von Przemysl collection), Flora von Triest -5, Societas Helvetica -5 (including Societas Botanica Helvetica and Société Helvétique collections), Flora von N. Oesterreich -4 (including Flora von Unt. Oesterreich collection), Flora Gallica exsiccata -2, Eggert Herbarium Americanum -2, C. Baenitz Herbarium Europaeum -1, and others. In the part of the herbarium which has already been analysed (compare with: Mitić et al. 2008a, 2008b, 2010-2011, 2011, 2012, 2013, Vladović et al. 2007a, 2007b, 2009b, 2010b, 2013b, 2014bc, Ževrnja et al. 2008b, 2009c, 2010b, 2011, 2013a, 2014ab), one new collection is mentioned and there is: Flora von Griechenland (1 herbarium sheet). There are 24 herbarium sheets that are unmarked and therefore we do not know which herbarium collection they belong to.

Most of the samples of herbarium plants were collected by Studniczka himself (70 herbarium sheets), while others were sent to him by: Freyn -6, Ayasse -4, Gandoger -3, Eggert -2, Halácsy -2, Graf -2, Legrand -2, Alioth -2. Studniczka received one herbarium sheet from 30 collectors or botanists.

The oldest herbarium sheet dates from 1870, where as the newest ones date from 1904. The vast majority of herbarium sheets, 70, was collected during the period from 1871 to 1880. The exact year of collection is missing from 3 herbarium labels.

According to Studniczka, within 116 herbarium sheets (order Boragineen) there are 20 genera with 71 species, and 10 varieties. According to The Plant list and USDA plants Database there are 22 genera with 51 species of plants and 3 subspecies.

References

Mitić, B., Vladović, D., Ževrnja, N. & Anterić, P. (2008a). Analysis of Ord. Berberideen, Nymphaeaceen, Papaveraceen and Fumariaceen from C. Studniczka`s Herbarium. *Hladnikia*, Ljubljana, 22, 61.

Mitić, B., Vladović, D., Ževrnja, N. & Anterić, P. (2008b). *Analysis of Ord. Berberideen, Nymphaeaceen, Papaveraceen & Fumariaceen from C. Studniczka`s Herbarium.* Prirodoslovni muzej i zoološki vrt, Split.

Mitić, B., Vladović, D. & Ževrnja, N. (2010-2011). Crassulaceen, Marsileaceen i Saxifragaceen iz herbara C. Studniczke. The Crassulaceen, Marsileaceen and

Saxifragaceen - Collection of the herbarium C. Studniczke. *Radovi hrvatskoga društva za znanost i umjetnost Sarajevo*, 22-23, 42-48.

Mitić, B., Vladović, D. & Ževrnja, N. (2011). Analysis of Family Rosaceae from Carl Studniczka`s Herbarium. *Natura Montenegrina*, 10(2), 71-85.

Mitić, B., Vladović, D. & Ževrnja, N. (2012). Analysis of some Familys from Carl Studniczka's Herbarium (I). *Natura Montenegrina*, 11(3), 397-404.

Mitić, B., Vladović, D., & Ževrnja, N. 2013. *Ord. Crassulaceen, Marsileaceen i Saxifragaceen iz herbarija C. Studniczke*. Prirodoslovni muzej i zoološki vrt, Split.

The Plant List (2016). *Version 1.1* Retreived September 2016, from: http://www.theplantlist.org/.

USDA Plants Database (2016). Retreived September 2016, from: plants.usda.gov.

Vladović, D., Ževrnja, N., Mitić, B. & Tomasović, D. (2007a). The analysis of the order Ranunculales in the herbarium of C. Studniczka. In Book of Abstracts 20-22. rujna 2007. (pp. 58-59). 2nd Croatian Botanical Congess, Zagreb.

Vladović, D., Ževrnja, N., Mitić, B., Tomasović, D. & Bradarić, D. (2007b). *Die Analyse ord. Ranunculaceen aus dem Herbarium von C. Studniczka*. Prirodoslovni muzej i zoološki vrt, Split.

Vladović, D., Ževrnja, N. & Mitić, B. (2009a). Analysis of Ord. Violarieen, Capparideen, Cistineen and Sileneen from C. Studniczka´s herbarium. In Book of Abstracts, 22-24/25-29 June 2009. (pp. 104). Biodiversity Hotspots in the Mediterranean Area, 45th International Congress of SISV & FIP Cagliari.

Vladović, D., Ževrnja, N. & Mitić, B. (2009b). *L'analisi delle Ord. Violarieen, Capparideen, Cistineen e Sileneen dall' erbario di C. Studnitzka.* Prirodoslovni muzej i zoološki vrt, Split.

Vladović, D., Ževrnja, N. & Mitić, B. (2010a). The Papilionaceen-collection of the Herbarium C. Studniczka. In Book of Abstracts September 6-9, 2010. (pp. 50). 5th Slovenian symposium on plant biology, Ljubljana.

Vladović, D., Ževrnja N. & Mitić, B. (2010b). *The Papilionaceen-collection of the Herbarium C. Studniczka, Natural History Museum Split (Croatia)*. Prirodoslovni muzej i zoološki vrt, Split.

Vladović, D., Ževrnja, N. & Mitić, B. (2011). Analysis of family Umbelliferae form C. Studniczka's herbarium. In Book of programme, abstracts, excursion guide May 24-28, 2011. (pp. 51). 34th International Symposium of the Eastern Alpine and Dinaric Society for Vegetation Ecology, Camerino (Marches, central Italy).

Vladović, D., Ževrnja, N. & Mitić, B. (2012). Analiza redova „Alsineen“, „Xanthoxyleen“ i „Elatineen“ iz herbarija C. Studniczke, In Book of Abstracts 16th.-21th. September 2012. (pp. 37). 11th Croatian Biological Congess, Šibenik.

Vladović, D., Mitić, B. & Ževrnja, N. (2013a). Analysis of some Families from Carl Studniczka's Herbarium (III). In Book of Abstracts 27 – 29 September 2013. (pp. 128-129). 4th Croatian Botanical Symposium Split, Croatia

Vladović, D., Ževrnja, N., & Mitić, B. (2013b). *The Umbelliferae – Collection of the herbarium Carl Studniczka*. Prirodoslovni muzej i zoološki vrt, Split.

Vladović, D., Mitić, B. & Ževrnja, N. (2014a). Analysis of Family Labiaceae from Carl Studniczka's herbarium. Book of Abstracts 16-18th May, 2014 (pp. 59). - International Symposium Floristic Patterns at Different Organisation and Distribution Levels. Babeş-Bolyai University Cluj-Napoca (Romania).

Vladović, D., Mitić, B. & Ževrnja, N. (2014b). Analysis of the Family Labiaceae from Carl Studniczka's herbarium. *Contrib. Bot. Cluj*, /2014/ XLIX, 85-96.

Vladović, D., Mitić, B. & Ževrnja, N. (2014c). Analiza Ord. Acanthaceen, Ericineen, Primulaceen i Vaccinieen iz herbarija C. Studniczke. *Naš krš Sarajevo* XXXIV, 47**,** 200-221.

Vladović, D., Mitić, B. & Ževrnja, N. (2015). Addition to the family Apiaceae from Carl Studniczka's herbarium. Book of Abstracts 14.-18. September 2015. (pp. 121.). 6th Balkan Botanical Congress International Symposium, Rijeka.

Ževrnja, N., Mitić, B., Vladović, D. & Anterić, P. (2008a). Analyse Ord. Cruciferen aus dem C. Studniczka Herbar. *Sauteria*, 16, 415-416.

Ževrnja, N., Mitić, B., Vladović, D. & Anterić, P. (2008b). *Analysis of Ord. Cruciferen from C. Studniczka`s Herbarium*. Prirodoslovni muzej i zoološki vrt, Split.

Ževrnja, N., Mitić, B., Vladović, D. & Anterić, P. (2009a). Die Cruciferen-Sammlung des Herbariums von C. Studniczka im Naturkundemuseum Split (Kroatien). *Sauteria*, 18, 299-307.

Ževrnja, N., Mitić, B. & Vladović, D. (2009b). Analysis of Ord. Geraniaceen, Oxalideen, Rutaceen and Lineen from C. Studniczka`s herbarium, In Book of Abstracts, 17-20 june 2009. (pp. 60). International scientific conference Balkans, Hot Spots of Ancient and Present Genetic Diversity, Sofia, Bulgaria.

Ževrnja, N., Mitić, B. & Vladović, D. (2009c). *Analysis of Ord. Geraniaceen, Oxalideen, Rutaceen and Lineen from C. Studniczka`s herbarium*. Prirodoslovni muzej i zoološki vrt, Split.

Ževrnja, N., Mitić, B. & Vladović, D. (2010a). The new findings from C. Studniczka's herbarium. In Book of Abstracts, 24.-26. rujna, 2010. (pp. 210-211). 3rd Croatian Botanical Congess, otok Murter.

Ževrnja, N., Mitić, B. & Vladović, D. (2010b). *Analysis of Ord. Hippocastanēn, Balsimeinēn, Acerineen, Ampelideen, Malvaceen and Hypericineen from C. Studniczka's herbarium*. Prirodoslovni muzej i zoološki vrt, Split.

Ževrnja, N., Mitić, B. & Vladović, D. (2011). Novosti iz herbarija Carla Studniczke (Sanguisorbeen, Pomaceen, Granateen). *Prirodoslovlje*, 11(1), 19-28.

Ževrnja, N., Mitić, B. & Vladović, D. (2013a). Analysis of some Families From Carl Studniczka's Herbarium (II). *Natura Montenegrina*, 12(1), 241-250.

Ževrnja, N., Mitić, B. & Vladović, D. (2013b). News from C. Studniczka's herbarium (ord. Onagrarieen, Cucurbitaceen and Portulaceen). In Book of Abstracts, 3.–6. 7. 2013. (pp. 46-47). 35th Meeting of Eastern Alpine and Dinaric Society for Vegetation Ecology. Ohrid (Republic of Macedonia).

Ževrnja, N., Mitić, B.,Vladović, D., Cvitanić, R., Mekinić ,S., & Boban, J. (2013c). Analysis of some Familys from Carl Studniczka's Herbarium (IV). In Book of Abstracts, 2-5. 10. 2013. (pp. 88). V International Symposium of Ecologist of the Republic of Montenegro, Tivat.

Ževrnja, N., Mitić, B. & Vladović, D. (2014a). Analysis of some familys from Carl Studniczka's herbarium (V). *GZM (PN), NS* 35, 31-37.

Ževrnja, N., Mitić, B. & Vladović, D. (2014b.) Analiza Ord. Gentianeen, Convolvulaceen, i Ebenaceen iz herbarija C. Studniczke. Book of Abstracts, 16.-19. 10. 2014. (pp. 53). International Scientific Symposium „Man and Karst“, Rama, Šćit, BiH.

Ževrnja, N., Mitić, B. & Vladović, D. (2014b). Analiza Ord. Gentianeen, Convolvulaceen, i Ebenaceen iz herbarija C. Studniczke. *Naš krš Sarajevo*, XXXV, 48, 144-156.

Ževrnja, N., Mitić, B. & Vladović, D. 2015c. The analysis of part of Carl Studniczka's herbarium (ord. Lonicereen, Caprifoliaceen and Stellateen). Book of Abstracts. 18.–23. IX 2015. (pp. 178). 12th Croatian Biological Congress with International Participation Sveti Martin na Muri, 18–23.09.2015.

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 67-78
DOI: 10.1515/ahbb-2016-0006

PLANT SPECIES OF COMMUNITY INTEREST IDENTIFIED IN THE CĂLIMANI-GURGHIU MOUNTAINS (MUREŞ COUNTY, ROMANIA)

SĂMĂRGHIŢAN Mihaela[1], OROIAN Silvia[2*], HIRIŢIU Mariana[3], CALALB Tatiana[4]

Abstract: The aim of this study was to verify in the field the existing information about chorology, to select the category of endangered plants and to highlight the main populations of valuable species. The territory under study is located in the Călimani-Gurghiu Mountains, the area belonging to Mureş County. For each species of community interest data were recorded about the systematics of species, the area, the population, the area of habitat/habitats suitable for species, the conservation status and the general trend of conservation status, abundance. As a result of this field research, nine plant species of community interest were identified (*Angelica palustris, Arnica montana, Campanula serrata, Galanthus nivalis, Iris aphylla, Lycopodium alpinum, L. annotinum, L. clavatum, L. complanatum*). These species belong to phytocenosis framed into 8 types of Natura 2000 habitats of community interest (6520, 6430, 6230*, 7140, 8220, 9410, 9130, 91V0). The general trend of conservation status is unfavourable-inadequate, the populations of the species of community interest are predominantly in a good and very good state of preservation, but in the future the populations are expected to decrease. Besides species of community interest, within the study area 30 plant species important in phyto-geographical terms and rare species present in the national red lists were identified.
Key words: species of community interest, conservation status, population, Călimani-Gurghiu Mountains.

Introduction

The conservation of biodiversity is a well defined, complex process that is underpinned by an international and national legislative framework.

The objectives of this study were to verify in field the existing information about chorology (Oroian 1998, Oroian & Giurgiu 2003, Oprea 2005, Sămărghiţan 2005), to select the category of the endangered plants and to highlight the main populations of valuable species, their spread in the study area and presence in Natura 2000 habitat types.

The territory under study is the mountainous area of Mureş County, Călimani-Gurghiu Mountains, and partially overlaps the two protected areas: The Natural Park

[1] Mureş County Museum, Natural Science Department, 24 Horea Street, RO-540036, Târgu-Mureş, Romania.
[2] University of Medicine and Pharmacy Târgu-Mureş, Facultaty of Pharmacy, 38 Gh. Marinescu street, RO-540139 Târgu-Mureş, Romania.
[3] University of Medicine and Pharmacy, Botanical Garden, 38 Gh. Marinescu Street, RO-540139 Târgu-Mureş, Romania.
[4] *„Nicolae Testimiţeanu"* State University of Medicine and Pharmacy, 165 Ştefan cel Mare şi Sfânt Bd., Chişinău, MD-2004, Republic of Moldavia.
[*] Correspondence: oroian.silvia@umftgm.ro

Mures Gorge between Topliţa and Deda and the Natura 2000 site ROSCI0019 Călimani-Gurghiu.

The study includes research results materialized in the identification and analysis of the conservation status of nine plant species of community interest.

Previous studies in the area mention the presence of these species (Nyárády, 1931, Săvulescu, red.pr. 1952-1976, Oroian 1998, Oroian & Giurgiu 2003, Oprea 2005, Sămărghiţan 2005) in several localities but populational studies on these plants were not published.

Material and methods

The inventory of the flora in the studied area was made using the "itinerant transects" model so the area would be covered throughout (Cristea et al. 2004).

For each species of community interest were recorded data such as: species systematic, area, population, area of habitat/habitats suitable for species, the conservation status and the general trend of conservation status, conservation measures, factors that isolate populations and future prospects, abundance (Mihăilescu et al. 2015).

The evaluation of the conservation status was done by applying the method known as the "semaphore method" (Combroux & Schwoerer 2007). There are no previous studies published regarding the population size. Because of that, the evaluation of the conservation trend namely the decrease of populations was made taking in consideration previous personal field reserch and the authors' experience.

The name of the species was given in accordance to Ciocârlan (2009) and Sârbu et al. (2013).

The habitat types are coded according to the Interpretation Manual of Natura 2000 Romanian Habitats [Gafta & Mountford (eds.) 2008] and according to Habitats in Romania (Doniţă et al. 2005).

In determining endangered, rare or vulnerable species, National Red Lists (Boşcaiu et al. 1994, Dihoru & Dihoru 1994, Oltean et al. 1994, Dihoru & Negrean 2009) were consulted, as well as the latest version of sozological categories published in the *European red list of vascular plants* (Bilz et al. 2011).

Results and discussion

Of the investigated area, nine species of community interest were identified: *Angelica palustris* (Besser) Hoffm., *Arnica montana* L., *Campanula serrata* (Kit. ex Schult.) Hendrych, *Galanthus nivalis* L., *Iris aphylla* L., *Lycopodium alpinum* L., *Lycopodium annotinum* L., *Lycopodium clavatum* L., *Lycopodium complanatum* L..

Besides the nine species of community interest, within the study area, a number of 30 plant species important in phyto-geographical terms and rare, endangered species listed in national red lists were identified.

***Angelica palustris* (Besser) Hoffm.** (Ann. IIb, IVb, Habitat Directive) (Fig. 1). This plant is a biennial or perennial species that prefers moist soils rich in nitrogen. It is a relict species in eutrophic wetlands (Dihoru & Negrean 2009).

Angelica palustris can be found in meadows and wet meadows, wetlands, thickets, eutrophic bogs and at the edges of humid forests. The species is rare in the hornbeam and spruce belts (boreal) (Săvulescu, red.pr. 1956, Sârbu et al. 2013).

The species is mentioned in bibliography on the studied territory at Ciobotani (Oroian, 1998), Topliţa (Nyárády 1931, Săvulescu, red.pr. 1956), Mureş Gorge (Oroian et al. 2005). We have no knowledge, so far, of population-based studies, but the presence of the species has been reported on several occasions in the study area. In the studied area there were identified, in some observation points, both vegetative individuals (about 3 individuals / m^2) and individuals in the first year of vegetation. Populations were relatively small, up to 10 vegetative individuals (in the inventory points).

In the studied area the species was identified in 10 locations: in Ghurghiului river basin near Lăpuşna (Pârâul Negru valley) and Ibăneşti (Fâncel), also in Mureş Gorge near Răstoliţa (Costeasa Valley, Vişa Valley) Stânceni and Ciobotani.

The specimens were identified in three types of Natura 2000 habitats:

- 6430 Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels. On the observation points, in the species composition of this habitat we noticed following species: *Filipendula ulmaria, Mentha longifolia, Cirsium oleraceum, Lythrum salicaria, Lycopus europaeus, Calamagrostis pseudophragmites, Urtica dioica*;
- 7140 Transition mires and quaking bogs with accompanying species: *Carex rostrata, Calla palustris, Galium uliginosum, Lysimachia vulgaris, Myosotis scorpioides, Lycopus europaeus, Valeriana officinalis, Deschampsia caespitosa, Lythrum salicaria, Salix cinerea*;
- On the edge of habitat 6520 Mountain hay meadows in the observation point besides *Angelica palustris* species such as *Trisetum flavescens, Poa pratensis, Lythrum salicaria, Salix cinerea* were identified.

Potential threats estimated in the field are related to water supply and soil moisture levels. These threats could be very important as *Angelica palustris* is a species that requires high level of groundwater, even puddles. If these conditions are influenced by various abiotic human activity, decreased soil moisture levels lead to changes in the floristic structure of phytocenoses of the habitat where the individuals of species vegetate and staff population decline.

The main threats, but also anthropogenic pressure observed on populations of *Angelica palustris* are the expansion of agriculture and changing land into arable land.

Field observations on populations and the human factors (pressure / threats) shows that, six populations are in a good state of conservation, but is expected to decrease the populations in the future.

The last years drought and low rainfall, in the Călimani-Gurghiu Mountains (described by locals as one not seen in last 60 years), affected drastically the populations of *Angelica palustris*, by diminishing the moisture level in the soil and therefore, we expect future decreasing of the six populations in a good state of conservation.

Even though four populations show signs of decreasing they are in a very good state of preservation.

***Arnica montana* L.** (Ann. IVb, Habitat Directive) prefers moderately acidic soils poor in nutrients (oligotrophic). Typical habitats, where this species is found, are mountain meadows generally used in a mixed system (mowing and grazing). It can be found in the squat bushes at the edge of peatlands. The vertical distribution of the

species stretches from the montane level to the subalpine level and rarely in alpine level (Săvulescu, red.pr. 1964, Sârbu et al. 2013).

In the studied area, the species was identified in one point, several sporadic individuals. Although in the observation point no orchids species were found we framed the phytocoenosis in the habitat 6230*, according to Gafta & Mountford (2008). Besides the target species in this observation point can note: *Nardus stricta, Festuca rubra, Agrostis capillaris, Viola canina, Hieracium pilosella*.

The main pressures and threats targeting both the species' habitat and the dynamics of effective population size are related to land use by abandoning land (in particular the lack of mowing) and intensive sheep grazing.

The conservation status of the identified population is unfavourable-bad.

***Campanula serrata* (Kit.) Hendrych** (Ann. IIb, IVb, Habitat Directive) (Fig. 2) is a mesophilic, oligotrophic-mesotrophic moderate acidophile species (Sârbu et al. 2013). It is a Carpathian floristic element (endemic) and considered a common species that occurs in grasslands, bushes, debris from beech level to alpine one, in *Potentillo ternatae-Nardion* alliance respectively *Molinio-Arrhenatheretea* class (Sârbu et al. 2013).

The bibliographic data mention the presence of this species in Gurghiu Mountains (Fl.R.P.R. IX, 1964), Piatra Orşova (Sămărghiţan 2005), Scaunul Domnului-spruce forest fringe (Höhn 1998). Also, in his PhD thesis, Giurgiu (1981) noted this species in Becheci, on Nirajul Mare and Nirajul Mic Valleys (Brădăţel, Poiana Ţigle, Poiana Coţa Mică).

In the study area, the species was found in 14 locations: Scaunul Domnului, Meştera-Stânceni (Borta), Gudea Mare, near Lăpuşna and in hydrographic basin of Sovata River (Platoul Repaş, Poiana Rusu, Poiana Cerepeş).

The species is rare in the territory under study and in the 14 observation points mainly vegetative individuals have been identified.

In some observation points, the identified populations were well developed, including vegetative individuals (5-10 / $1m^2$ to 50-150 / $1m^2$). In other observation points the abundance of individuals was much lower (5 individuals /1 m^2). In the points with compact populations also generative individuals have been identified (15/5 m^2). We mention that no data estimating the population size of this species in the studied area were published.

In the study area the species occur most frequently in 6520 Mountain hay meadows habitat, but we also found it in 6230* Species-rich *Nardus* grasslands, on silicious substrates in mountain areas habitat (3 observation points).

For the populations that grow in 6520 habitat (11 populations) the most frequent species in the inventory points are: *Agrostis capillaris, Festuca rubra, Nardus stricta, Lotus corniculatus, Potentilla erecta, Viola canina, Alchemilla xanthochlora.*

In the composition of phytocoenosis belonging to 6230* habitat, besides *Campanula serrata,* the following species were noted: *Nardus stricta, Festuca rubra, Agrostis capillaris, Viola canina, Hieracium pilosella, Hypericum maculatum* in the observation points.

The main pressures/threats that affect the habitat of this species and also the effective population size are connected to the land use. Both abandoning land (especially the lack of mowing) and the intensive grazing of sheep have a negative

effect on the conservation status of the species. These threats could lead to decay and replacement of habitats and affecting long-term perpetuation of the species individuals.

Based on the field observation of pressures, threats and the dynamics of effective population size we conclude that six populations are in a very good state of conservation with a stable future trend of population size, seven populations are in a good state of conservation but is expected to decrease the size population in the future and one population (the one identified on Scaunul Domnului) is in a unfavourable-bad state of conservation and the size of population is declining. For this population a negative impact has the tourism which affected also the habitat of species.

***Iris aphylla* L.** (Ann. IIb, IVb, Habitat Directive) (Figs. 3,4) is a xerophilous, eurithermic species that can be found in dry grasslands, thermophilic forest glades, bushes, sandy-rocky places, sunny limestone cliffs, grassy slopes of the hills to the subalpine belt (Săvulescu, red.pr. 1966, Sârbu et al. 2013).

This species is mentioned in previous studies made in the area (Haltrich 1982, Oroian 1995). The species was identified in three inventory points at Stânceni (Leu Mountain). In the three viewpoints the species is abundant. *Iris* populations are stable, including a large number of individuals and they are in a very good state of preservation. As the rocky slopes that were identified *Iris* populations were fenced with wire mesh designed to protect the E578 road against falling rocks, estimation of population size was carried out taking into account the experience of the authors.

The species individuals were identified in phytocoenosis belonging to 8220 Siliceous rocky slopes with chasmophytic vegetation habitat. Beside the target species in the inventory points following species were recorded: *Asplenium trichomanes, Asplenium viride, Cystopteris fragilis, Thymus dacicus, Libanotis montana, Sedum maximum, Sempervivum marmoreum, Poa nemoralis, Spiraea chamaedryfolia.*

Because the populations are located on the side of road E578 they are relatively accessible. The main anthropic factor affecting the species is tourists or locals collecting the flowers during anthesis or even whole plants with rhizomes, for ornamental purposes.

Field observations on population size and on the human factors (pressures / threats) shows that the population is stable and in a very good state of preservation.

***Galanthus nivalis* L.** (Ann. IVb, Habitat Directive) (Figs. 5, 6). Snowdrop is a mesophilic species that prefers moist, sandy, loamy, moderately acidic soils. It can be found through forests, glades, thickets and meadows, from lowlands to alpine areas. It occurs frequently in deciduous forests or occasionally in coniferous forests, meadows, pastures, thickets, or near water courses. In Romania, except Dobrogea region, this plant is widespread (Săvulescu, red.pr. 1966, Sârbu et al. 2013, Mihăilescu et al. 2015). In the studied area the species was identified at Sălard (two points), Gălăoaia, Andreneasa, Orşova Pădure, Ibăneşti, Gura Fâncel, Brădeţel.

The *Galanthus nivalis* populations were recorded in three types of Natura 2000 habitats: 9410 Acidophilous *Picea* forests of the montane to alpine levels (*Vaccinio-Piceetea*), 9130 *Asperulo-Fagetum* beech forests, 91V0 Dacian Beech forests (*Symphyto-Fagion*). Among the species recorded alongside *Galanthus nivalis* we mention: *Picea abies, Fagus sylvatica, Hieracium transsylvanicum, Corylus avellana, Daphne mezereum, Luzula sylvatica, Anemone nemorosa, Erythronium dens-canis, Pulmonaria rubra* etc.

The main threat factor is the anthropic factor namely gathering for ornamental purpose or trading.

Concerning the conservation status, the field observations show that the populations are decreasing but they are in an average or good state of conservation.

***Lycopodium alpinum* L.** (Ann. IVb, Habitat Directive) (Fig. 7) is an oligotrophic, mesophilic species encountered sporadically in spruce level – juniper level, in meadows and thickets in communities of alliance *Potentillo ternatae-Nardion* and order *Piceetalia excelsae* (Sârbu et al. 2013).

The species was identified at Răstoliţa on Iod Valley at altitudes between 1154-1220 m. Six observation points were set. These specimens were identified in the phytocoenosis belonging to 6230* Species-rich *Nardus* grasslands, on silicious substrates in mountain areas habitat, the association *Violo declinatae-Nardetum* Simon 1966.

The species identified on the observation points are the ones characteristic to this habitat type: *Nardus stricta, Festuca rubra, Agrostis capillaris, Viola canina, Hieracium pilosella, Hypericum maculatum, Carex pallescens, Cruciata glabra, Hieracium aurantiacum, Polygala vulgaris, Potentilla ternata*.

The main pressures and threats while targeting both species' habitat and population dynamics are related to intensive grazing of sheep and ruderalisation. It has found the presence of the species *Veratrum album* in this habitat.

Considering these treats and previous observations in the field we can conclude that the studied populations are in a very good state of preservation but decreasing.

***Lycopodium annotinum* L.** (Ann. IVb, Habitat Directive) (Fig. 8) is an acidophile, oligotrophic, mesophilic to mezo-higrophilic, heliosciaphilic-sciaphilic and calcifugous species.

It is a sporadic species that occurs in beech level - spruce level (up to 1800 m altitude), in bogs, thickets, wet forests, plant communities in Piceetalia excelsae order respectively Vaccinio-Piceetea class (Sârbu et al. 2013).

Previous publications mention the presence of this species in Călimani and Gurghiu Mountains (Săvulescu, red.pr. 1952), Sălardului Valley (Haltrich 1982), Lunca Bradului-Neagra, Sălard, Răstoliţa (Oroian 1995), Mureş Gorge (Oroian et al. 2005), Gurghiului Mountains, Lăpuşna, Bătrâna Valley, Secuieu Valley, Creanga Albă Valley (Sămărghiţan 2005), Gurghiului Mountains-Saca (Coldea & Wagner 1997), Mureş Valley sector II (Drăgulescu 1995).

In the study area the species was recorded in 91 observation points located on the valleys of Mureş and Gurghiu rivers tributaries and also on Nirajul Mic and Nirajul Mare rivers. The populations are representatives and occupy large areas especially on tributaries of Gurghiu River, namely Secuieu and Sirod.

The *Lycopodium annotinum* populations lie on the edge of spruce forests belonging to 9410 Acidophilous *Picea* forests of the montane to alpine levels (*Vaccinio-Piceetea*) habitat. The plant species recorded in the inventory points are: *Picea abies, Hieracium transsylvanicum, Vaccinium myrtillus, Huperzia selago, Senecio nemorensis, Mycelis muralis, Leucanthemum waldsteinii.*

The plant associations to which *Lycopodium annotinum* belong in the study area are: *Hieracio rotundati-Piceetum* Pawł. *et* Br.-Bl. 1939 and *Leucanthemo waldsteinii-Piceetum* Krajina 1933.

Also the species was identified in the 91V0 Dacian Beech forests (*Symphyto-Fagion*) habitat. Besides *L. annotinum* in the inventory point we noticed characteristic species for this habitat such as: *Symphytum cordatum, Cardamine glanduligera, Pulmonaria rubra, Leucanthemum waldsteinii, Ranunculus carpaticus, Euphorbia carniolica, Aconitum moldavicum.*

Lycopodium annotinum is the most prevalent of the identified species of community interest in the study area.

The main anthropogenic factor affecting the species is plant collection for medicinal purposes. Also, the work for improving the forest roads in the area has a negative impact on these populations.

Field observations on effective population size shows that populations are predominantly in a very good (73) and good (17) state of conservation, only one of the studied populations are severly dropping and in an unfavourable-bad conservation status (at Răstoliţa).

***Lycopodium clavatum* L.** (Ann. IVb, Habitat Directive) (Fig. 9) is an acidophile, oligotrophic, euriphilous, helio-sciaphilic and calcifugous species. It is a sporadic species that occurs from montane level to subalpine belt in thickets, forests edges, spruce fire forests, meadows. (Sârbu et al. 2013)

In the bibliography this species was mentioned in: Călimani Mt., Gurghiu Mt. (Săvulescu, red.pr. 1952), Răstoliţa-Podirei (Oroian 1995), Gurghiu Valley-Măgura; Gurghiului Mt. (Măgura Mare Peak); Lăpuşna, Bătrâna Valley (Sămărghiţan 2005), Mureş Gorge (Oroian et al. 2005), spruce forest on the upper course of Niraj River (Giurgiu 1981).

The species was recorded in 43 observation points at Răstoliţa (Listeş), Valea Iodului, Stânceni-Meştera, Valea Bistrei, Ilişoara Mică, Ilişoara Mare, Gudea Mare, Gropuşoara, Hidegag, Şolea, Ţiba Mare, Ţiba Mică, Jârca, Scaunul Domnului, Nirajul Mare, Poiana Deniş, Poiana Brădeţel, Nirajul Mic, Valea Sebeş, Valea Sovata, Pârâul Mocirlosu, Lăpuşna, Fâncel, Bătrâna, Şirodul Mic, Poiana Meştera (Gurghiu), Vârful Zambrinii (Gurghiu).

Lycopodium clavatum populations often occur in the studied area together with *L. annotinum*, in some cases the two species have been recorded in the same observation points. Identified populations are represented by vigorous, well-developed individuals but on relatively small areas, ranging from a few square meters to tens of square meters.

The species was identified in meadows on the edge of spruce forests, mountainous plateaus, in micro-depressions with higher humidity where specific conditions for development of the species are met. The species is present in the studied area in the following habitat types:

- 6520 Mountain hay meadows, the species recorded in observation points alongside *Lycopodium clavatum* are: *Festuca rubra, Agrostis capillaris, Anthoxanthum odoaratum, Briza media, Viola canina, Potentilla erecta, Alchemilla xanthochlora, Veronica officinalis, Tragopogon pratensis* subsp. *orientalis, Trifolium repens, T. pannonicum, Campanula patula, Achillea millefolium, Thymus pulegioides, Stellaria graminea, Carlina acaulis*;
- 6230* Species-rich *Nardus* grasslands, on silicious substrates in mountain areas habitat, with the plant associations *Violo declinatae-Nardetum* Simon 1966 and *Hieracio pilosellae-Nardetum strictae* Pop et al. 1988. In their composition, beside de

characteristic species *Nardus stricta, Hieracium pilosella, Viola canina,* we noticed species characteristic to superior taxa such as: *Alchemilla xanthochlora, Carex pallescens, Festuca rubra, Hypericum maculatum, Polygala vulgaris, Carex leporina, Genista tinctoria, Hieracium umbellatum, Luzula campestris, Potentilla erecta, Veronica officinalis, Viola canina*;

- 9410 Acidophilous *Picea* forests of the montane to alpine levels (*Vaccinio-Piceetea*) accompanying species in the observation points being: *Picea abies, Fagus sylvatica, Hieracium transsylvanicum, Corylus avellana, Daphne mezereum, Luzula sylvatica, Anemone nemorosa, Erythronium dens-canis, Pulmonaria rubra*.

The main anthropogenic factor facing the populations is collecting plants for medicinal purposes. Also an important threat / pressure is grazing, especially intensive grazing which leads to depletion of species of phytocoenoses.

Field observations on the effective population size show that the populations are declining but in an average (seven) or good (10) state of preservation. 26 populations are stable and in very good state of preservation.

***Lycopodium complanatum* L.** (Ann. IVb, Habitat Directive) (Fig. 10) is an oligotrophic species, calcifugous, glacial relict in flora of Romania (Sârbu et al. 2013). It is a rare, species that occurs on beech-spruce level in forests and thickets in the following plant communities: *Dicrano-Pinion* and in *Piceion excelsae* (Sanda et al. 2008, Sârbu et al. 2013).

The species was identified in 9 locations at Răstoliţa (Peşcoasa Mare) Stânceni on Gudea Mare, Nirajul Mic (three points), Poiana Brădeţel (two points), Gurghiu-Poiana Căpităneasa, Secuieu.

The species is rare in the studied area. The identified populations are characterised by vigorous and well developed individuals but they lay on relatively small area.

L. complanatum populations were observed on the edge of spruce forest belonging to 9410 - Acidophilous *Picea* forests of the montane to alpine levels (*Vaccinio-Piceetea*) habitat. The species recorded in the observation points are: *Picea abies, Hieracium transsylvanicum, Vaccinium myrtillus, Huperzia selago, Senecio nemorensis, Mycelis muralis.*

In the study area the species was also found in phytocoenosis framed in 6520 Mountain hay meadows habitat. In the floristic structure of this habitat can meet on the observation points, species such as: *Festuca rubra, Agrostis capillaris, Cynosurus cristatus, Lotus corniculatus, Dactylis glomerata, Pimpinella saxifraga, Anthoxanthum odoratum, Cerastium holosteoides, Holcus lanatus, Trifolium pratense, Briza media, Carex pallescens.*

The main anthropic factor that affects the population is collecting for medicinal purpose. Also the main pressure/threat is grazing mainly over-grazing. This can lead to an impoverishment of species number in 6520 habitat and to the development of *Nardus stricta* causing a succession to 6230* habitat. These threats lead to a decrease of populations.

In term of conservation status the field observations show that the populations are decreasing and they are in an unfavourable-bad conservation status in two observation points and in seven points the populations are in a good stage of conservation but they are continuously decreasing.

We mention that the population cited from Răstoliţa-Podirei (Oroian 1995) vanished, probably as a result of collecting for medicinal purpose.

Conclusion

Of the investigated area, nine species of community interest were identified: *Angelica palustris* (Besser) Hoffm., *Arnica montana* L., *Campanula serrata* (Kit. ex Schult.) Hendrych, *Galanthus nivalis* L., *Iris aphylla* L., *Lycopodium alpinum* L., *L. annotinum* L., *L. clavatum* L., *L. complanatum* L..

Among the identified species, the most widespread are *Lycopodium* spp., which occur most frequently on the edge of spruce forests.

These nine species of community interest belong to phytocoenosis framed into eight types of Natura 2000 habitats of community interest (6520 Mountain hay meadows, 6430 Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels, 6230* Species-rich *Nardus* grasslands, on silicious substrates in mountain areas, 7140 Transition mires and quaking bogs, 8220 Siliceous rocky slopes with chasmophytic vegetation, 9410 Acidophilous *Picea* forests of the montane to alpine levels (*Vaccinio-Piceetea*), 9130 *Asperulo-Fagetum* beech forests, 91V0 Dacian Beech forests (*Symphyto-Fagion*).

The general trend of conservation status is unfavourable-inadequate, the populations of species of community interest are predominantly in a good and very good state of preservation, but in the future the populations are expected to decrease.

Besides species of community interest, within the study area 30 plant species important in phyto-geographical terms and rare species present in the national red lists were identified.

In the observation points 2 alien species were identified (*Erigeron annuus* and *Solidago canadensis*), without a significant impact on the studied species, thus we conclude that the phenomenon of presence and spread of invasive neophytes is restricted.

References

Bilz, M., Kell, S.P., Maxted, N. & Lansdown, R.V. (2011). *European Red List of Vascular Plants*. Luxembourg: Publications Office of the European Union.

Boşcaiu, N., Coldea, G. & Horeanu, C. (1994). Lista roşie a plantelor vasculare dispărute, periclitate, vulnerabile şi rare din flora României, *Ocrotirea Naturii şi a Mediului înconjurător*, Bucureşti, 38 (1), 45-56.

Ciocârlan, V. (2009). *Flora ilustrată a României. Pteridophyta et Cormophyta*. Bucureşti: Edit. Ceres.

Coldea, G. & Wagner, I. (1997). Pflanzensoziologische Untersuchungen im Gurghiu – Gebirge (Ostkarpaten). *Verh.Zool.-Bot.Ges.Österreich*, 134, 357-371.

Combroux, I. & Schwoerer, C. (2007). *Evaluarea statutului de conservare a habitatelor şi speciilor de interes comunitar din România-Ghid metodologic*. Timişoara: Edit. Balcanic.

Cristea, V., Gafta, D. & Pedrotti, F. (2004). *Fitosociologie*. Cluj-Napoca: Edit. Presa Universitară Clujeană.

Dihoru, G. & Dihoru, A. (1993 – 1994). Plante rare, periclitate şi endemice în flora României – Lista Roşie. *Acta Botanica Horti Bucurestiensis*, /1994/, 173-179.

Dihoru, G. & Negrean, G. (2009). *Cartea roşie a plantelor vasculare din România*. Bucureşti: Edit. Academiei Române.

Doniţă, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S. & Biriş Iovu, A. (2005). *Habitatele din România.* Bucureşti: Edit. Tehnică Silvică.

Drăgulescu, C. (1995). The flora and vegetation of Mureş (Maros) Valley, in The Maros/Mureş River Valley, A study of the geography, hydrobiology and ecology of the river and its environment, *TISCIA monograph series*, Szolnok-Szeged-Târgu-Mureş.

Gafta, D. & Mountford, O. (eds.). (2008). *Manual de interpretare a habitatelor Natura 2000 din România*. Cluj-Napoca: Edit. Risoprint.

Giurgiu, M. (1981). *Plante medicinale din Valea Nirajului*. Unpublished doctoral dissertation. Institute of Medicine and Pharmacy Târgu-Mureş, Faculty of Pharmacy (Coordinator Prof. dr. G. Rácz).

Haltrich, I. (1982). Contribuţii la cunoaşterea florei din Valea Sălardului (Munţii Gurghiului), *Studii şi comunicări*, Reghin, 2, 205-218.

Höhn, M. (1998). *A Kelemen-havasok növényzetéről*. Mentor Kiadó, Marosvásárhely.

Mihăilescu, S., Anastasiu, P., Popescu, A. et al. (2015). *Ghidul de monitorizare a speciilor de plante de interes comunitar din România*. Constanţa: Edit. Dobrogea.

Nyárády, E.I. (1931) Die Vegetation des andesitischen Muresdurchbruchthales zwischen Toplita und Deda, In: Borza, A., *Guide de la sixieme Excursion Phytogeografic International Roumanie*, Cluj: 185-195.

Oltean, M. & al. (1994). Lista roşie a plantelor superioare din România, *Studii, sinteze, documentaţii de ecologie*, Acad. Română, Instit. de Biologie, Bucureşti, 1, 1-52.

Oprea, A. (2005). *Lista critică a plantelor vasculare din România*. Iaşi: Edit. Univ. „Al. I. Cuza".

Oroian, S. (1998). *Flora şi Vegetaţia Defileului Mureşului între Topliţa şi Deda*. Târgu-Mureș: Casa de Editură Mureș.

Oroian, S. & Giurgiu, M. (2003). Plante medicinale spontane din Valea Nirajului. *Rev. Medico-Chirurgicală a societăţii de Medici Naturalişti din Iaşi*, 107(2), Supliment 1: 124-130.

Oroian, S., Ştefănescu D. & Sămărghiţan, M. (2005). *Ariile protejate din judeţul Mureş*. Târgu-Mureș: Edit. University Press.

Sămărghiţan, M. (2005). *Flora şi vegetaţia Văii Gurghiului*. Târgu-Mureș: Edit. University Press.

Săvulescu, T. (red. pr.), (1952-1976). *Flora R.P.Romania-R.S.Romania*, vol. I-XIII. Bucureşti: Edit. Academiei Române.

Sârbu, I., Ştefan, N. & Oprea, A. (2013). *Plante vasculare din România, determinator ilustrat de teren*. Bucureşti: Edit. VictorBVictor.

***Habitats Directive 92/43/EEC – Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (OJ L 206, 22.7.92).

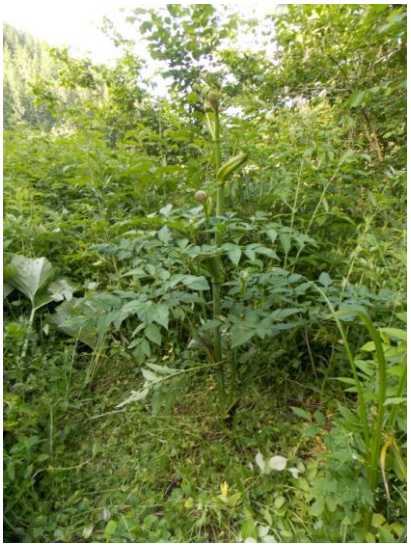

Fig. 1. Angelica palustris habitus and characteristic habitat in Gurghiu Valley (photo: M. Sămărghiţan)

Fig. 2. Campanula serrata in 6520 habitat (photo: M. Sămărghiţan)

Figs. 3, 4. Galanthus nivalis L. (photo: M. Sămărghiţan)

Figs. 5, 6. Iris aphylla L. in 8220 habitat (photo: S. Oroian)

Fig. 7. Lycopodium alpinum in 6520 habitat (photo: M. Sămărghiţan)

Fig. 8. Lycopodium annotinum (photo: M. Sămărghiţan)

Fig. 9. Lycopodium complanatum (photo: M. Sămărghiţan)

Fig. 10. Lycopodium clavatum (photo: M. Sămărghiţan)

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 79-84
DOI: 10.1515/ahbb-2016-0007

SYMPHYOTRICHUM SQUAMATUM – A NEW ALIEN PLANT IN ROMANIA

CAMEN-COMĂNESCU Petronela[3*], NEGREAN Gavril[1], NAGODĂ Eugenia[1], ANASTASIU Paulina[2]

Abstract: *Symphyotrichum squamatum* (Compositae) is reported as a new alien species to Romania. The species is native to South America and the way of introduction in Romania remains unknown. Data about habitat and population of the taxon are presented. The specimens of this plant were collected in Bucharest (Romania) and deposited in the Herbarium of the University of Bucharest (BUC).
Key words: alien species, invasive plants, Romanian flora, *Symphyotrichum squamatum.*

Received 15 November 2016 *Accepted 22 November 2016*

Introduction

Symphyotrichum is a genus with about 90 species native to the Americas and one species (*S. ciliatum*) native to Eurasia (Verloove 2016). Many of them are widely cultivated as ornamental plants in Europe and have become naturalised in many countries (Verloove 2016). In Romania, nine non-native species of the genus have so far been reported, many of them cultivated and naturalised in many regions of the country (Sîrbu & Oprea 2011).

In the present paper we report a new alien species for the flora of Romania, namely *Symphyotrichum squamatum* (Compositae), which is considered invasive in large parts of the Mediterranean area (Celesti-Grapow et al. 2009).

Material and methods

The species was recorded during our field works on alien plants, in Bucharest (Romania). The geographic coordinates were recorded using a handheld Garmin GPS model eTrex Legend C, using WGS84 system. Voucher specimens were deposited in the Herbarium of the Botanic Garden "D. Brandza", University of Bucharest (BUC). The morphological characters of the species and ecological features were compared with data from the literature (Yeo 1976, Nesom 2005, Brouillet et al. 2006). The taxonomy and nomenclature of species follow The Euro+Med Plantbase (Greuter 2006+).

[3] University of Bucharest, Botanic Garden „D. Brandza", 32, Şos. Cotroceni, Sector 6, 060101 – Bucureşti, Romania.
[2] University of Bucharest, Faculty of Biology, 1-3, Intr. Portocalelor, Sector 6, 060101 – Bucureşti, Romania.
[*] Correspondence: vpetronela@yahoo.com

Results and discussion

Symphyotrichum squamatum (Spreng.) G.L. Nesom belongs to the Asteraceae (Compositae) family, tribe Astereae, subtribe Symphyotrichinae and is native to South America (Nesom 1994, Greuter 2003, Nesom 2005, Hind 2011).

Nomenclature:

Symphyotrichum squamatum (Spreng.) G.L. Nesom, Phytologia 77(3): 292, (1994) 1995.

= *Conyza squamata* Spreng., Syst. Veg., ed. 16, 3: 515, 1826.

= *Aster squamatus* (Spreng.) Hieron., Bot. Jahrb. Syst. 29(1): 19, 1900.

= *Aster barcinonensis* Sennen, Bull. Acad. Int. Géogr. Bot. 24: 242. 1914

= *Conyzanthus squamatus* (Spreng.) Tamamsch., Fl. U.R.S.S. 25: 186, 1959.

= *Symphyotrichum subulatum* var. *squamatum* (Spreng.) S. D. Sundb., Sida 21(2): 908, 2004

Type: URUGUAY. MONTEVIDEO.

Voucher specimen: Romania, Bucharest: Cotroceni (44°26'12.27"N, 26°4'0.82"E), 72 m alt., 28.10.2016, leg. E. Nagodă, G. Negrean, P. Camen-Comănescu; det. P. Camen-Comănescu [BUC 405988].

Description (Fig. 1): Annual or biennial plant, usually 30-100 cm height (Yeo 1976). The recorded specimens in Bucharest have 50-70 cm, except one which is 170 cm tall, very branched in the upper part. Erect and glabrous stems, often reddish, with lateral ascending branches. Leaves sessile, alternate, dark green, entire or distantly toothed. The lower leaves spatulate, 1.5 to 15 cm long, most of them dried during the flowering time. The leaves on the floral branches sessile, linear-lanceolate and scale-decreasing.

Inflorescences are elongated, with corymbiform to thyrsiform arrangement of heads and compound branching. Tiny heads, up to 500 to an individual of about 170 cm height. Involucres 3-4 x 5–7(–8) mm, with 3 to 5 rows of unequal bracts. Phyllaries 18–24(–30), subulate to lanceolate, hyaline margins and ± mucronulate, distinctly demarcated, with apical green zone and dark apex. Ray florets numerous (20-30), white or lavender. Disk florets (7-14) hermaphrodite, yellow. Achenes pubescent, 3-5-nerved, 1.5 to 3 mm long. Pappus 1-seriate, accrescent, hairs about 4-5 mm long, longer than ray corollas.

Symphyotrichum squamatum is tetraploid, 2n=20 (Yeo 1976, Nesom 2005).

Distribution: Yeo (1976) indicated *Symphyotrichum squamatum* as widely naturalized in SW Europe and also in the C & E Mediterranean region (Azores, Baleares, Corse, Kriti with Karpathos, France, Greece, Spain, Italy, Former Jugoslavia, Portugal, Sardagena). According to Nesom (2005), the species is naturalized in Australia, Japan, Iraq, Africa (Algeria, Egypt), France and probably other regions of the world. The species is listed as alien in Europe (DAISIE 2009) and widely distributed in many countries: Italy, France, Malta, Cyprus, Slovenia, Greece (Šajna 2014).

Nowadays, according to Greuter (2006+), in Europe it is spread in the west and south of the continent. Among neighbouring countries of Romania, until now, it has been reported only from Bulgaria (Dimitrov & Assyov 2003). Even in the Flora Europaea, *Symphyotrichum squamatum* is mentioned from Former Yugoslavia, there are data only for Slovenia (Greuter 2006+, Šajna 2014) and Croatia (Greuter 2006+), but not for Serbia, neighbouring country of Romania.

In 2015, we recorded four individuals of *Symphyotrichum squamatum* in Romania, Bucharest, on the Şoseaua Cotroceni Street. In 2016, over 100 individuals were recorded on the right side of the Dâmbovița River, between Cotroceni Bridge and Unirii Square, as well as alongside of Şoseaua Cotroceni Street.

Biology, ecology: The species flowers from June to October, fruiting until November. It produces up to 70.000 seeds/plant (Šajna 2014), efficiently dispersed by wind and with high germination rates.

The typical habitat is represented by salt marshes, brackish marshes (Brouillet et al. 2006, Šajna 2014) and prefers soft climates along the sea surface or over lowlands (Invasive Plants in Portugal 2016).

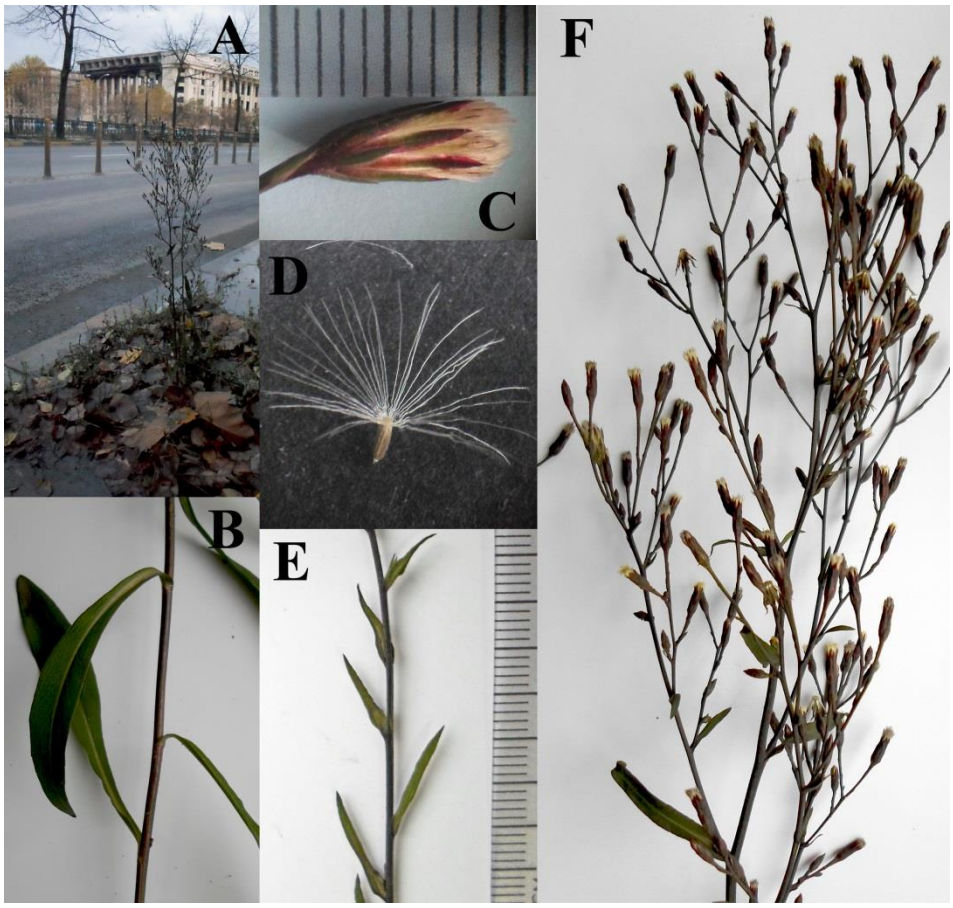

Fig. 1. Symphyotrichum squamatum. A - habitat; B - basal leaves; C - floral head; D - achene; E - upper leaves; F – inflorescences (photos: Camen-Comănescu Petronela).

Symphyotrichum squamatum is an urbanophile species and can be found in wet crops, waste ground, roadsides, abandoned gardens, ruins, harbours (Nesom 2005). It

presents great ecological resilience, adapting to different conditions as long as soil humidity is assured. Therefore it is presented as a successful weed in the disturbed saline mashes (Zelnik 2012, Schembri & Lanfranco 1996), but also in anthropogenic habitats (Šajna 2014).

In Bucharest, we found *Symphyotrichum squamatum* on the roadsides, near the fences, between concrete and fences, around the trees cultivated alongside of the streets.

Some sources indicate its **introduction** in European countries as accidental: in agriculture for Portugal (Silva et al. 2008), sometimes brought by ships for Slovenia, Ireland and England (Green 2007, Šajna 2014). Schembri & Lanfranco (1996) indicate the Argotii Botanic Garden as the way of introduction for *Symphyotrichum squamatum* in the Maltese Islands.

For Romania, the way of introduction is unknown. *Symphyotrichum squamatum* is spread around the Botanic Garden "D. Brandza", but the species is neither in the collections of this garden, nor as subspontaneous here. Moreover, checking the seed exchanges for the last five years, we found out that *Symphyotrichum squamatum* was not subject for exchange.

Symphyotrichum squamatum is reported as invasive in different countries of Europe: Portugal (Bernez et al. 2006), Italy (Pace & Tammaro 2001), Slovenia (Zelnik 2012), Montenegro (Stešević & Caković 2013), Spain, Malta and Greece (EPPO 2016).

The high salt tolerance, the huge number of achenes dispersed by wind and adaptability to anthropogenic habitats, are reasons that this species to be able to expand in a short period of time.

Our field observations defend the idea expressed by Invasive Plants in Portugal (2016) that the plant has a „strong invasive behaviour, able to expand and colonise new habitats in a short period of time".

Conclusions

A new alien plant species is reported from Romania. At the moment, *Symphyotrichum squamatum*, could be considered naturalised, but able to become invasive. Given its high dispersion capacity and the success even in natural habitats such as saline mashes, the early detection and control of *Symphyotrichum squamatum* is very important.

References

Bernez, I., Aguiar, F., Violle, C. & Ferreira, T. (2006). Invasive river plants from Portuguese floodplains: What can species attributes tell us?, *Hydrobiologia*, 570(1), 3-9.

Brouillet, L., Semple, J.C., Allen, G.A., Chambers, K.L. & Sundberg, S.D. (2006) *Symphyotrichum*. In Flora of North America Editorial Committee (eds.). *Flora of North America*. Vol. 20. (pp. 465-539). New York-Oxford: Oxford University Press.

Celesti-Grapow, L., Alessandrini, A., Arrigoni, P.V., Banfi, E., Bernardo, L., Bovio, M., Brundu, G., Cagiotti, M.R., Camarda, I., Carli, E., Conti, F., Fascetti, S., Galasso, G., Gubellini, L., La Valva, V., Lucchese, F., Marchiori, S., Mazzola, P., Peccenini, S., Poldini, L., Pretto, F., Prosser, F., Siniscalco, C., Villani, M.C.,

Viegi, L., Wilhalm, T. & Blasi, C. (2009). Inventory of the non-native flora of Italy. *Plant Biosystems*, 143 (2), 386-430.

DAISIE (2009). Handbook of alien species in Europe. Dordrecht: Springer Verlag.

Dimitrov, D. & Assyov, B. 2003. New taxa for the Bulgarian Flora. *God. Sofiisk. Univ. "Kliment Okhridski" Biol. Fak.*, 95(4), 269-272.

EPPO (2016). EPPO Global Database. Retrieved November 11, 2016 from: https://gd.eppo.int.

Green, P.R. (2007). *Aster squamatus* could be on its way to you. A new alien for Ireland and England. *BSBI News*, 105, 31-32.

Greuter, W. (2003). The Euro+Med treatment of Astereae (Compositae) – generic concepts and required new names. *Willdenowia*, 33, 45-47.

Greuter, W. (2006+): Compositae (pro parte majore). In Greuter, W. & Raab-Straube, E. von (ed.): Compositae. Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. Retrieved November 20, 2016, from: http://ww2.bgbm.org/EuroPlusMed/.

Hind, D.J.N. (2011). *An annotated preliminary checklist of the Compositae of Bolivia. Vers. 2.* Retrieved November 11, 2016, from: http://www.kew.org/science/tropamerica/boliviacompositae/checklist.pdf.

Invasive Plants in Portugal (2013). *Aster squamatus* (annual saltmarsh aster). Retrieved November 11, 2016, from: http://invasoras.pt/wp-content/uploads/2012/10/Aster-squamatus_en.pdf.

Nesom, G. L. 1994. Review of the taxonomy of *Aster* sensu lato (Asteraceae; Astereae), emphasizing the New World species. *Phytologia*, 77, 141–297.

Nesom, G.L. 2005. Taxonomy of the *Symphyotrichum* (*Aster*) *subulatum* group and *Symphyotrichum* (*Aster*) *tenuifolium* (Asteraceae: Astereae). *Sida*, 21, 2125–2140.

Pace, L. & Tammaro, F. (2001) The Main Invasive Alien Plants in the Protected Areas in Central Italy (Abruzzo). In Visconti G. et al., Global change and protected areas. Advances in Global Change Research, 9: 495-504. Kluwer academic publ.

Šajna, N., Kaligarič, M. & Ivajnšič, D. (2014). Reproduction biology of an alien invasive plant: a case of drought-tolerant *Aster squamatus* on the Northern Adriatic seacoast, Slovenia. In Rannow, S., Neubert, M., (eds.). *Managing protected areas in Central and Eastern Europe under climate change* (Advances in global change research 58). Dordrecht: Springer.

Schembri, P.J. & Lanfranco, E., 1996. Introduced species in the Maltese Islands. p. 29-54. In: Introduction of alien species of flora & fauna. Proceedings of seminar held at Qawra, Malta, A.E. Baldacchino & A. Pizzuto (Eds), Floriana, Malta, Environment Protection Department.

Silva, L., Ojeda, L.E., Rodríguez, L.J.L. (eds.) (2008). *Flora e Fauna Terrestre Invasora na Macaronésia*. TOP 100 nos Açores, Madeira e Canárias. ARENA, Ponta Delgada.

Sîrbu, C. & Oprea, A. (2011). *Plante adventive în flora României*. Iași: Edit. Ion Ionescu de la Brad.

Stešević, D. & Caković, D. (2013). Contribution to the alien flora of Montenegro and Supplementum to the Preliminary list of plant invaders. *Biologica Nyssana*. 4(1-2), 1-7.

Verloove, F. (*Symphyotrichum* Nees). On: Manual of the Alien Plants of Belgium. Botanic Garden of Meise, Belgium. Retrieved November 11, 2016, from: alienplantsbelgium.be.

Yeo P.F. (1976). *Aster squamatus* (Sprengel) Hieron.. In T.G. Tutin, D.H. Heywood, N.A. Burges, D M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb (eds.), assit. by A. O. Chater, R.A. DeFilipps & I.B.K. Richardson. *Flora Europaea*. Vol. 4. (p. 115). Cambridge: Cambridge University Press.

Zelnik, I. (2012). The presence of invasive alien plant species in different habitats: case study from Slovenia. *Acta Biol. Slov*., 55, 25–38.

DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2016, **43**: 85-96
DOI: 10.1515/ahbb-2016-0008

VERBESINA ALTERNIFOLIA – A NEW ALIEN PLANT IN ROMANIA'S FLORA

OPREA Adrian [1*], SÎRBU Culiţă [2]

Abstract: In the recent decades, there was reported, with an increasing frequency, the presence of more and more alien plant species in Romania. These newly arrived species are added to the inventory of wild, spontaneous/subspontaneous species of Romania. In this regard, the authors signaled out the presence of a new alien plant species of genus *Verbesina* in Romania, namely *Verbesina alternifolia*, identified during the year of 2016, along the Mureş river floodplain, in Simeria town (Hunedoara County).
Key words: new alien species, *Verbesina alternifolia*, Simeria, Romania, identification key.

Received 24 November 2016 *Accepted 28 November 2016*

Introduction

The genus name of *Verbesina* L. is considered to be derived from the genus name *Verbena* (*Verbenaceae* family) and the ending Latin *-ina*, meaning resemblance, with reference to the similarity of the leaves of some species of *Verbesina* genus, with those of *Verbena* genus (Robinson & Greenman 1899).

The genus *Verbesina* is now on the whole accepted to be exclusively an American one, naturally distributed from southern parts of Canada in North to Argentina in South (Coleman 1977), except so far as those plant species which have been introduced or/and naturalized in some parts of the Old World (Robinson & Greenman 1899). For instance, the current distribution of *V. encelioides* (Cav.) A. Gray includes North America, Central America, South America, Africa, Asia, Oceania, and Europe, including Romania, though the origin of this species is North America (Hansen 1976, Anastasiu et al. 2009).

Over the time, to this genus have been assigned more and more species, as they are: 109 species (Robinson & Greenman 1899); ca 150 species (Corell & Johnston 1970); 250 specii (Coleman 1977); over 300 species (Flann 2009+).

Among the species of *Verbesina*, over 70 are strictly located but over 90% of them are distributed in several regions of the America's (Robinson & Greenman 1899), as follows: S. E. of United States of America (S Carolina to Florida, and Alabama) = 3 species; Region of Lower Rio Grande Sonora = 4 species; Region Sonoran (W Texas to S California and N. W. Mexico) = 16 species; Central and S Mexico = 44 species; - Central America = 9 species; N part of S America (Colombia to Guyana) = 4 species;

[1] Botanic Garden ″Anastasie Fătu″, University ″Alexandru Ioan Cuza″ Iaşi, Romania.
[2] University of Agricultural Sciences and Veterinary Medicine ″Ion Ionescu de la Brad″ Iaşi, Romania.
[*] Correspondence: a_aoprea@yahoo.co.uk

Brasil, Uruguay, Paraguay, and Argentine = 11 species; Region of Andes (Ecuador to Chili) = 8 species; W Indies = 2 species.

However, it is thought that the highest specific diversity of this genus is to be met in uplands of Central and Southern Mexico (Coleman 1977), where ca 40% of its species are endemics (Robinson & Greenman 1899).

One of the species of this genus, is *V. alternifolia*, whose name has been given by the Swedish naturalist Carl Linnaeus, as *Coreopsis alternifolia* (Linnaeus 1753), this name being the basionym (the specific epithet *alternifolia* is referring to the alternate leaf pattern of this species, other species within the genus having opposite leaves). In the 1st half of the 19th century, to this name have been awarded others, such as: *Actinomeris squarrosa* (heterotypic synonym), by the English botanist Thomas Nuttal (Nuttal 1818); *Pterophyton alternifolium* (heterotypic synonym) by the French botanist Alexandre Henri Gabriel de Cassini (in Cuvier 1826), but this one is an unresolved name (http://www.theplantlist.org); *Actinomeris alternifolia* (homotypic synonym), by the Swiss naturalist Augustin Pyramus de Candolle (de Candolle 1836); *Ridan alternifolius* (heterotypic synonym), by the American botanist and taxonomist Nathaniel Lord Britton (in Britton & Brown 1913).

Botanical description of this species was made in a book of the American botanist John Torrey (Torrey 1843), basen on a previous description made by Th. Nuttal (Nuttal 1818), preserving also the name as *Actinomeris squarrosa* of Nuttal. Later on, who gave the actual name of the species by transferring it from genus *Actinomeris* (Nuttal 1818) to *Verbesina* L. (Linnaeus 1753) is the American botanist Thomas Henry Kearney (Kearney 1893).

Summarizing, the names of this species are:

Verbesina alternifolia (L.) Kearney 1893. *Bull. Torrey Bot. Club*, **20**(12): 485.

– basionym: *Coreopsis alternifolia* L. (1753, Sp. Pl. **2**: 909)

– homotypic synonym: *Actinomeris alternifolia* (L.) DC. (1836, Prodr. **5**: 575)

– heterotypic synonyms: *A. squarrosa* Nutt. (1818, Gen. **2**: 181); *Pterophyton alternifolium* Cass. (Dict. Sci. Nat., ed. 2. [F. Cuvier], **44**: 49. 1826); *Ridan alternifolia* (L.) Britton (1913, Fl. N. U. S. ed. 2, **3**: 487, fig. 4487) /*R. alternifolius* in orig./

Other authors transferred the name *V. alternifolia* (L.) Kearney to *Actinomeris squarrosa* (L.) DC., keeping this last name and authority as valids (Robinson & Greenman 1899).

Material and methods

A field work in Simeria Arboretum (Hunedoara county, Romania) led us to identify a newcomer, unknown plant species for Romania's flora. The time of that trip was in the 2nd half of August, 2016. At the time of our arrival, this newly alien species was in full blossom, with many flower heads but no any mature fruit (achenae). Later on, in November, mature achenae were collected in the field. Both, the herbarium sheets and achenae were examined using a stereomicroscope (BEL, model STMPRO-T) and were photographed, using a camera Nikon Coolpix P330. In order to identify this plant, the collected specimens were examined using several local North American florae books, as: *An illustrated flora of the Northern United States, Canada and British possesions* (Britton & Brown 1913), *Manual of the vascular plants of Texas* (Corell &

Johnston 1970), *Flora Europaea* (Hansen 1976), *Seed Identification Manual* (Martin & Barkley 1961/1973/), *Synopsis of the genus Verbesina* (Robinson & Greenmanm 1899), *A flora of the State of New York* (Torrey 1843). To document our paper, there were took several digital pictures of living plants, inserted in here. The collected voucher specimens were deposited in the herbaria of the Botanic Garden ″Anastasie Fătu″, University ″Alexandru Ioan Cuza″ in Iaşi (IAGB) (abbreviation according to Thiers 2009), sheets no 47695 and 47696, and University of Agricultural Sciences and Veterinary Medicine ″Ion Ionescu de la Brad″ (I), sheets no 17978 and 17979, also in Iaşi, Romania. We also asked the forestry engineer, Corina Coandă, for a possible introduction on demand of this species in Simeria Arboretum plant collections. There were also examined all seed indexes, edited ceaselessly by the same institution, between 1955 and 2015 (*Index Seminum*, Simeria Arboretum 1955-2015).

The current taxonomy and nomenclature of *V. alternifolia* follow *Flora Europaea*, a database continuously updated, edited at Botanischer Garten und Botanisches Museum in Berlin (Greuter 2006+).

Results and discussion

Verbesina alternifolia (L.) Kearney (commonly called wingstem or yellow ironweed) is included currently in tribe *Heliantheae*, subtribe *Ecliptinae* (Lessing 1831); other treatments included *Verbesina* genus under tribe *Verbesininae* (Karis & Ryding 1994).

Short description of the species, according to some of the America's Florae (Britton & Brown 1913, Corell & Johnston 1970): perennial, weedy, (30-) 100-200+ cm in height, ± erect or horizontal rhizomes, perennating bases; stems ± erect, stiff (Fig. 1), glabrous at least proximal but soft haired in upper part, slightly branched (except in the upper 10-40 cm); internodes narrow winged (Fig. 2); leaves all or mostly alternate (those proximal are sometimes opposite), with lance-elliptic or lanceolate to lance-linear blades, of 10-25+ × 2-8+ cm, bases narrowly cuneate and decurrent along the stem (hence the common name as the wingstem), margins coarsely toothed to subentire, apices attenuate, faces scabrellous; flower heads (3-) 8-25 (-50+) in corymbiform to paniculiform arrays (Fig. 3); receptacle ± saucerlike at beginning, becoming ± conical or round shape later, 10-12+ mm in diameter; phyllaries 8-12+ in 1 (-2) series, ± spreading to reflexed, squarrous, spatulate or lance-linear to linear, 3-8+ mm long; ray florets (2-) 6-8+, bright yellow, laminae 15-25+ mm long, neutral, reflexed, shallowly 3-toothed distal; disc flowers 40-60+, with slightly darker yellow corollas (Fig. 4); achenes dark brown to blackish, oblanceolate to ± orbiculate, 4.5-5.5 mm, squarrous at maturity, sparsely hirtellous to glabrate faces, with prominent wings around, laterally compressed; pappus 1.5-2 mm long, with 2 divergent, stiff, persistent awns (Fig. 5).
Obs.: the flower heads sometimes appear rayless, with only disk flowers, hence the common name as the yellow ironweed (http://www.missouribotanicalgarden.org).

Origin: it is native in eastern and central North America: Texas (presumably adventitious there !), Missouri, Louisiana, Florida, Iowa, Kansas, New Jersey, in North to Ontario (Corell & Johnston 1970).

Chromosome number: 2*n* = 34 (Heiser & Smith 1954, Coleman 1977) or 2*n* = 68 (Strother 2016); it seems to be a tetraploid species (Coleman 1977).

Hybridization between species of *Verbesina* naturally occurs in USA, as *V. alternifolia* × *V. helianthoides*, and *V. alternifolia* × *V. walteri* (Coleman 1977), and

according to the cited author, *V. alternifolia* could resulted from hybridization between *V. walteri* and *V. helianthoides* or between *V. walteri* and *V. occidentalis*.

Flowering time: from August to October.

Seed ripening: October to November.

Ecology: alluvial flats, in woods, forest edges, on meadows, or in thickets, along streams, sloughs and ditches, throughout most of the range (Steyermark 1963), between 10 and 600 m a.s.l. in USA (Strother 2016), on rich, alluvial wet soils.

Occurence in Europe:

France: 1969 – Sessenheim toward Schirrhoffen (Charpin 1971, 1972, 1973); after 1969: ″…«pont de l'Arve à Bonneville», Haute-Savoie, mais cette localité s'est éteinte aprés 1970″ (Charpin 1972, 1973); an important colony between Kauffenheim and Forstfeld (Schneider 1973); Soufflenheim, ″…jusqu'au petit pont sur le Landgraben…″ (Geissert 1988); Kauffenheim (Geissert 1988); river Sambre in Marpent (Dép. Nord) (Geissert 1988).

The occurence of the species in France is also documented in some review papers, as an introduced one: naturalized, or introduced: adventitious (casual) plant (Kerguélen 1993, Anonymous 2007-2014).

Germany: 1972 - Wiesbaden (Weimer 1972).

Belgium: It is a very rare, locally, introduced and more or less naturalized species, escaped from cultivation (Verloove 2002); 1984 - Oud-Heverlee alongside river Dijle (Verloove 2002, Blink & Harle 2012); 1986 - a forest at Paal near Beringen (Verloove 2002).

Czech Republic: commonly cultivated (Slavík et al. 2004).

Romania: Simeria town, Hunedoara county, in Arboretum (parcels no 27, 37, and 41), where it seems to grow ″in abundance″, especially in parcel no 41 (Corina Coandă, pers. comm.)

This new alien species was identified in those parcels, where the vegetation is a natural one, consisting of riverside coppices, with white (*Populus alba* L.) and black (*Populus nigra* L.) poplars, willows (*Salix alba* L.), but also there are some fragments of the former forest glades of elms (*Ulmus glabra* Huds.) and oaks (*Quercus robur* L.), along the Mureş river banks, installed on recent alluvial deposits and/or alluvial soils (Coandă 2015).

In other European countries (listed above), *Verbesina alternifolia* is sometimes cultivated as a honey producer plant, and at least the populations in north-eastern France have been introduced on this purpose, thanks to the late flowering period. They said there are a harvest of one tone honey/ha/normal climatic year (Geissert 1988). Also, due to the fact the seeds are attractive to birds, a possible way to transport seeds/fruits is this (http://www.missouribotanicalgarden.org).

The ways of introduction of *Verbesina alternifolia* in Romania are still unclear. We suppose this species could be grown as an ornamental one upstream on the Mureş river, from where, the seeds were carried downstream by waters, setting up on Mureş river banks, within the Simeria Arboretum. It has natural tendencies of invasion across the natural vegetation (Coandă pers. comm.)

An other species in *Verbesina* genus, present as an already alien naturalised in Romania, is *Verbesina encelioides* (Cav.) Benth. & Hook. fil. ex A. Gray, identified quite recently in central parts of Dobrogea (Anastasiu et al. 2009).

Below, it is presented a key identification of those genera within the tribe *Heliantheae* Cass., reported so far in Romania's flora (compilation according to Barkley et al. 1993+, Corell & Johnston 1970, Moore et al. 2010, Nyárády 1964), including those two species of *Verbesina* (i.e. *V. alternifolia* and *V. encelioides*):

1a All florets unisexual; ligules of ray florets absent 2
1a At least the disk florets bisexual, ray florets female, fertile or infertile; ligules of ray florets present 4
2a Capitula campanulate, bisexual (female florets marginal, the male ones central); the involucral bracts ± smooth, hairy ..... *Iva*
2b Capitula unisexual; the female florets tightly closed by the concrescent involucral bracts 3
3a Leaves alternante; the female capitula with 2 florets; involucral bracts in fruit with numerous uncinate, stiff thorns ..... *Xanthium*
3b Leaves opposite; the female capitula with 1 floret; involucral bracts in fruit ± spiny-toothed, without uncinate, stiff thorns ..... *Ambrosia*
4a Outher involucral bracts linear to linear-spatulate, patent, much longer than the capitulum, with dense stipitate glands ..... *Sigesbeckia*
4b Involucral bracts with other features, never with stipitate glands 5
5a Disk florets bisexual but infertile, functionally staminate (only ray florets produce achenes); leaves strongly connate (plants only in culture) ..... *Silphium*
5b Disc florets fertile; leaves not connate 6
6a Ligulae of ray florets sessile, persistent on the achenes, becoming papery 7
6b Ligulae of ray florets seldom sessile, never persistent on the achenes, never becoming papery 9
7a Achenes not flattened; leaf margins toothed (plants only in culture) ..... *Heliopsis*
7b Achenes flattened or 3-4 angled; leaf margins entire or nearly so 8
8a Receptacle conic (plants in culture or casual alien) ..... *Zinnia*
8b Receptacle nearly flat (plants only in culture) ..... *Sanvitalia*
9a Disk pappus of 15-20 narrow minutely fimbriate persistent scales ..... *Galinsoga*
9b Disk pappus of few scales, setae, teeth, or a small corona, or absent 10
10a All achenes flatened dorsiventrally 11
10b At least the disk achenes flattened laterally 14
11a Pappus of 2 barbless awns or teeth, a mere crown or absent 12
11b Pappus of retrorsely or antrorsely barbed or hispid awns or teeth 13
12a Plants with underground tubers. Ligulae of ray florets unicolor, red to pink or purple, or white; achenes ± fusiform; pappus absent (plants only in culture) ..... *Dahlia*
12b Plants without underground tubers. Ligulae of ray florets usually yellow, sometimes red-brown to purple proximally, or wholly purple or pink to white; achenes orbiculate to oblong; pappus present or absent (plants in culture or casual alien) ..... *Coreopsis*
13a Achenes beaked, ± 4-angled, not winged (plants in culture or casual/naturalised alien) ..... *Cosmos*
13b Achenes not beaked, dorsiventrally flatened or unequally 3-4-angled, sometimes winged ..... *Bidens*

14a Chaff of the receptacle linear-filiform. Corollas white or whitish ....…… *Eclipta*
14b Chaff of the receptacle of concavo-convex or folded pales. Corollas of other colours 15
15a Receptacle strongly conic or columnar 16
15b Receptacle flat or slightly convex 17
16a Receptacle conic; receptacular pales with stout subuliferous apexes; leaves alternate (plants only in culture) ..... *Echinacea*
16b Receptacle conic or columnar; receptacular pales acute; leaves alternate or the extreme basal ones opposite (plants in culture or naturalised-invasive alien) ..... *Rudbeckia*
17a Disk achenes somewhat flattened but not winged (plants in culture or naturalised-invasive alien) ..... *Helianthus*
17b Disk achenes obvious winged ..... *Verbesina* (18)
18a Annual; internodes not winged; leaves mostly alternate (proximalmost usually opposite); stems and leaves (especially on the underside) greyish-green; ray florets 3-toothed or 3-lobed distal, with teeth/lobes of 1.5-3 (-4) mm long ..... *Verbesina encelioides*
18b Perennial; internodes winged; all leaves alternate (rarely opposite); stem and leaves green; ray florets shallowlly 3-toothed distal ..... *Verbesina alternifolia*

It is mentioned that *Chrysopsis graminifolia*, *Solidago erecta*, and *Verbesina alternifolia* were identified as rubber-producing species with 0.4-0.7% hydrocarbon (Carr & Bagby 1987).

The other species of *Verbesina* genus identified in Romania (*V. encelioides*), was previously investigated from anatomically and histologically points of view, concluding that the only structural features of vegetative organs cannot explain the invasive potential of this last species (Smarandache & Mihai 2011).

Conclusions

There is reported the presence of a newcomer, an alien plant species in Romania, namely *Verbesina alternifolia*, a member of the Asteraceae family.

It is originated in eastern and central North America, being naturally spreaded in Texas (presumably adventitious over there), Missouri, Louisiana, Florida, Iowa, Kansas, New Jersey, toward North on to Ontario (Canada).

Verbesina alternifolia was identified in Romania, in Simeria town (Hunedoara county), along the Mureş river floodplain, in August, 2016. It seems to be abundant (in terms of the number of individuals) on the field, at least in some of the parcels of the Simeria Arboretum. It has natural tendencies of invasion across the natural vegetation along the Mureş river, in Simeria Arboretum.

***Aknowledgements*.** We are fully indebted to Forestry Eng. Mrs. Corina Coandă for her valuable help in fruit harvesting, as well as in documentation on the seed indexes and desideratae of the Simeria Arboretum.

References

Anastasiu, P., Sîrbu, I. & Negrean, G. (2009). *Verbesina encelioides* - a new alien plant in Romania. *Analele Univ. Craiova*, *Ser. Agricult*., *Montan*., *Cadastru*, 39/B, 15-19.

Barkley, T. M., Brouillet, L. & Strother, J.L. (1993+). Fam. *Asteraceae* Martinov. In *Flora of North America* (FNA). Editorial Committee (eds.), Vol. 19-21. Retrieved November 22, 2016, from: /http://www.efloras.org/.

Blink, E. & Harle, N. (2012). *Verbesina alternifolia*: een potentiële blijver. *Natuurh. Maandb.* 101(7), 131-133.

Britton, N. L. & Brown, A**.** (1913). *An illustrated flora of the Northern United State*s, *Canada and British possesions from Newfoundland to the parallel of the southern boundary of Virginia, and from the Atlantic Ocean westward to the 102Cmeridian. 2nd edition revised and enlarged.* Vol. **III**. *Gentianaceae* to *Compositae*/Gentian to thistle/2nd edition, revised and enlarged, p. 487. New York: Charles Scribner's sons.

Candolle, A.P. de. (1836). *Prodromus Systematis Naturalis Regni Vegetabilis, sive enumeratio contracta Ordinum, Generum, Specierumque Plantarum huc usque cognitarum, juxta methodi naturalis normas digesta*. Pars quinta: *Systens Calycereas et Compositarum tribus priores*. Parisiis, Sumptibus Sociorum Treuttel et Würtz, p. 575.

Carr, M.E. & Bagby, M.O. (1987). Tennessee plant species screened for renewable energy sources. *Economic Botany*, 41(1), 78-85.

Charpin, A. (1971). Observations sur la flore de la Haute-Savoie: *Saussurea*. *Travaux de la Société Botanique de Genève*, 2, 19-24.

Charpin, A. (l972). Observations sur la flore de la Haute Savoie (2). *Saussurea*, 2, l8-24.

Charpin, A. (l973). A propos de *Verbesina alternifolia* (L) Britton. *Monde des Plantes*, 379, 3.

Coandă, C. (2015). *Index seminum*. National Institute for Research and Development in Forestry ″Marin Drăcea″, Timişoara Forest Research Station - Simeria Division, 9 pp.

Coleman, J.R. (1977). A summary of experimental hybridization in *Verbesina* (Compositae). *Rhodora*, 79(817), 17-31.

Corell, D.S. & Johnston, M.C. (1970). *Manual of the vascular plants of Texas*. Renner, Texas: Texas Research Foundation, pp. 1056-1057.

Cuvier, G.-F. 1826. *Dictionnaire des Sciences Naturelles, dans lequel on traite méthodiquement des différens êtres de la nature, considérés soit en eux-mêmes, d'aprés l'état actuel de nos connoissances, soit relativement à l'utilité quén peuvent retirer la médecine, l'agriculture, le commerce et les arts*. Strasbourg. ed. 2, vol. 44, p. 49.

Flann, C. (ed.), (2009+). *Global Compositae Checklist* (permanent update). Retrieved November 22, 2016, from: /http://www.compositae.org/checklist.

Geissert, F. (1988). A propos de trois plantes adventices en Alsace. *Bull. Assoc. Amis Jard. Bot. Col de Saverne*, 9-14.

Greuter, W. (2006+). *Compositae* (pro parte majore). In W. Greuter & E. von Raab-Straube (eds.), *Compositae*. Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. Retrieved November 22, 2016, from: /http://ww2.bgbm.org/europlusmed/.

Hansen, A. (1976). *Verbesina* L.. In T.G. Tutin, D.H. Heywood, N.A. Burges, D M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb (eds.), assit. by A. O. Chater, R.A. DeFilipps & I.B.K. Richardson. *Flora Europaea*, Vol. 4, (p. 142), 1st paperback printing. Cambridge: Cambridge University Press.

Heiser, CH.B. & Smith, D.M. (1954). New chromosome numbers in *Helianthus* and related genera (Compositae). *Proc. Indiana Acad, Sci., Taxonomy*, 64, 250-253.

Karis, P.O. & Ryding, O. (1994). Tribe *Heliantheae*. In Bremer K. (ed.). *Asteraceae. Cladistics* & *classification* (p. 521-558). Portland, OR: Timber Press.

Kearney, T.H. Jr. (1893). Notes on the Flora of Southeastern Kentucky, with a List of Plants collected in Harlan and Bell counties in 1893. *Bull. Torrey Bot. Club*, 20(12), 474-485.

Kerguélen, M. (1993). *Index synonymique de la flore de France.* Paris: Muséum National d'Histoire Naturelle, Patrimoines naturels, 8, 228 pp.

Lessing, Chr.Fr. (1831). *Synantherae* Rich. *Linnaea*. *Ein Journal für die Botanik in ihrem ganzen Umfange*, 6, 153.

Linnaeus, C. (1753). *Species Plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas*. Holmiae, Impensis Laurentii Salvii. [L. Salvius, Stockholm], 2, 909.

Martin, A.C. & Barkley, W.D. (1961) /Second edition 1973/. *Seed Identification Manual*. Berkeley and Los Angeles: University of California Press.

Moore, D.M., Tutin, T.G. & Walters S.M. 2010. Fam. *Compositae*. In T.G. Tutin, D.H. Heywood, N.A. Burges, D M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb (eds.), assit. by A. O. Chater, R.A. DeFilipps & I.B.K. Richardson. *Flora Europaea*, Vol. **4** (p. 103-410), 1st paperback printing, Cambridge: Cambridge Univ. Press.

Nuttal, T. (1818). *The Genera of North American Plants and a Catalogue of the Species, to the Year 1817*. Philadelphia, 2, 181.

Nyárády, E.I. (1964). Fam. *Compositae*. In T. Săvulescu & E.I. Nyárády (eds.). *Flora R. P. Române*, Vol. **9** (p. 154-977). Bucureşti: Edit. Acad. R.P. Române.

Robinson, B.L. & Greenman, J.M. (1899) (continuous updated). Synopsis of the genus *Verbesina*, with an analytical key to the species. *Contributions from the Gray Herbarium of Harvard University*, 16, 534-566, published by: Harvard University Herbaria. Retrieved November 22, 2016, from: /http://www.jstor.org/stable/41763782/.

Schneider, A. (1973). Découverte d'une deuxième localité de *Verbesina alternifolia* (L.) Britton, composée radiée. *Monde des Plantes*, 377, 5.

Slavík, B., Štěpánková, J. & Štěpánek, J. (eds.) (2004). *Květena České republiky*, Vol. **7**: 152. *Asteraceae*, 153. *Ambrosiaceae*, 154. *Cichoriaceae* (excl. *Taraxacum*). Praha: Academia.

Smarandache D. & Mihai D.C. (2011). Contribution to the knowledge of the vegetative organs structure of *Verbesina encelioides*. *Acta Horti Bot. Bucurest*., 38, 5-15.

Steyermark, J.A. (1963). *Flora of Missouri*. Ames, Iowa: The Iowa State University Press.

Strother, J.L. (2016). *Flora of North America*. 21, 65, 66, 67, 106. Retrieved November 22, 2016, from: www.eFloras.org.

Thiers, B. (2009) [continuously updated]. *Index Herbariorum*: *A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Retrieved November 22, 2016, from: http://sweetgum.nybg.org/ih/.

Torrey, J. (1843). *A flora of the State of New York, comprising a full description of all the indigenous and naturalized plants hitherto discovered in the State, with remarks on their economical and medicinal properties.* Albany: Carol and Cook, printers to the Assembly, Vol. I (p. 384).

Verloove, F. (2002). Ingeburgerde plantensoorten in Vlaanderen. *Mededeling van het Instituut voor Natuurbehoud*, 20, 227 pp.

Weimer, K. (1972). *Verbesina alternifolia* (L.) Britton – ein Neufund auf grenznahem, elsässischem Gebiet. *Hess. Flor. Briefe*, 3, 44-46.

***1955-2015. *Catalog de seminţe, puieţi şi butaşi oferiţi la schimb* (1955-1968, 1973-1986), *Catalog de seminţe şi puieţi* (1969, 1971, 1987-2000), *Catalog de plante lemnoase pentru spaţii verzi* (1970), *Index Seminum* (2001-2015). Inst. Naţ. Cerc.-Dezv. Silvicult. ″Marin Drăcea″, Staţ. Cerc. Silvice Timişoara – Secţ. Simeria.

***Anonymous [Association Tela Botanica]. (2000+/2007-2014/). Tela Botanica. Flore électronique. Flores en ligne, France métropolitaine. Retrieved November 22, 2016, from: http://www.tela-botanica.org/page:eflore 2000+ (continuously updated).

***http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a753 [accessed 22 Nov 2016]

***http://www.theplantlist.org/tpl1.1/record/gcc-78837 [accessed 22 Nov 2016].

Fig. 1. Verbesina alternifolia in Simeria Arboretum: general habitus (orig.).
Scale bar: 25 cm

Fig. 2. Verbesina alternifolia in Simeria Arboretum: stem details (orig.).
Scale bar: 1 cm

Fig. 3. Verbesina alternifolia in Simeria Arboretum: heads in corymbiform to paniculiform arrays (orig.). Scale bar: 1 cm

Fig. 4. Verbesina alternifolia in Simeria Arboretum: heads (orig.). Scale bar: 0.5 cm

Fig. 5. Verbesina alternifolia in Simeria Arboretum: achenes (orig.). Scale bar: 0.5 cm

INSTRUCTIONS TO THE AUTHORS

Acta Horti Botanici Bucurestiensis publishes original research papers and critical reviews on plant anatomy and morphology, systematic botany, plants, algae and fungi biodiversity, plant physiology and biochemistry, plant genetic and bio technology, plant pathology, horticulture.

The manuscript (in English) should be submitted electronically as MS-Word file to the editor, Paulina Anastasiu, e-mail: anastasiup@yahoo.com

The paper should be of maximum 10 pages edited according to the journal's requirements. **Authors are expected to cover the cost of supplementary pages as well as the colour reproductions**.

The title of the paper should be informative and as short as possible. Write the title centred, with bold capitals, size font 10, Times New Roman. Use *italics* only for the plant names and do not add the authority to species names in the title.

Below the title, aligning right, list the **authors' names** with font size 10, bold capitals. For each author include a superscription number to indicate, as footnote, the affiliation, complete address and e-mail of the corresponding author.

The abstract should be written as a single paragraph and should not exceed 200 words, font size 9, Times New Roman, justify. Do not include authority in the names of taxa.

The keywords should not be more than 8 words or phrases identifying the subject matter of the paper. Use Times New Roman, font size 9, justify.

The text of paper must be typed using 10 Times New Roman, one spaced, justify, on A4 format with the next margins: top 57 mm, bottom 50 mm, left 42.5 mm, right 42.5 mm. First line of each paragraph should be at 10 mm. It is recommended to divide the text into: **Introduction**, **Material and methods**, **Results and discussion**, **Conclusions**, **Acknowledgements** and **References**. The critical reviews are excepted from this rule. Write all main headings in bold.

Scientific plant names should be given in italics. The author's name should be written in normal print at least once, when mentioned for the first time in the text or in a table, and should be omitted subsequently. They should be abbreviated according to *Authors of Plant Names*, Royal Botanic Gardens, Kew (Brummitt & Powell 1992). After the first mention, the generic name should be abbreviated to its initial, except where its use causes confusion.

References in the text should be cited in the following form: (Petrescu 1997) or Petrescu (1997) for one author, (Metcalfe & Chalk 1950) or Metcalfe & Chalk (1950) for two authors, (Popescu et al. 1999) or Popescu et al. (1999) for more than two authors (Dumitrescu 2000a, b) or Dumitrescu (2000a, b) for several references by the same author(s) published in the same year. References in the text should be cited chronologically, not alphabetically: (Metcalfe & Chalk 1950, Popescu et al. 1999, Dumitrescu 2000). All references quoted in the text, and only those quoted, must be listed at the end of the manuscript, under the heading **References**, in a format strictly analogous to the examples below. The material in *preparation* or *unpublished* should be referred in the text using the author(s) name(s) followed by "unpubl." or "pers. comm." and cannot be included in the reference list.

Tables should be numbered with Arabic numerals in the order in which they are cited in the text (e.g. Table 3). They must have brief, concise titles and legends that will make the general meaning of the table comprehensible. The titles should be placed at the top of the tables. Explanatory footnotes may be placed below the table written with lowercase letters. All abbreviations must be explained in the legends. The size of table should be proportional to the journal's page (125 × 190 mm).

The illustrations could be represented by photographs, graphs, diagrams, maps, schemes and must be sharp and of high quality. They should be referred as figures (abbreviations: Fig., Figs) and numbered with Arabic numerals (e.g. Fig. 1). All illustrations must be submitted electronically as distinctive files. Their titles and /or legends should be written consequently on a separate sheet. If the photographs are arranged in plates, these should be designated by Roman numerals, while the individual photographs are designated by Arabic numerals (e.g. Plate II, Fig. 2). The bar scale is required for the figures. Any signs and letters in the illustrations must be enough large to be read without problem. Hand-written signs and letters are not accepted. The final size of illustrations should be proportional to the journal's page (125 × 190 mm).

Printed copies of all figures, tables and plates must also be submitted with the manuscript, indicating their place in the text.

References at the end of the paper must be in the **APA Reference Style**:

- **For periodic journals**

1. Bechet, M. & Coman, N. (1964). Contribuţii la cunoaşterea micromicetelor parazite pe plante rare din flora R.P.R. (Contribution á la connaissance des micromycétes parasites sur plantes rares de la flore de Romanie). *Studiu Univ. Babeş-Bolyai, Ser. Biol.*, /1964/(1), 49-57.
2. Borza, A. (1966). Cercetări asupra florei şi vegetaţiei din Câmpia Română (i). *Contrib. Bot. Cluj*, /1966/(2), 141-162.
3. Buttler, K.P. (1969). Chromosomanzahlen und Taxonomische bemerkungen zu einigen Rumanischen Angiospermen. *Rev. Roumaine Biol., Bot.*, 14(5), 275-282.

- **For books**

1. Brandza, D. (1879 – 1883). *Prodromul Florei Române sau enumeraţiunea plantelor până astă-di cunoscute în Moldova şi Valachia*. Bucuresci: Tipogr. Academiei Române.
2. Beldie, A. (1967). *Flora şi vegetaţia munţilor Bucegi*. Bucureşti: Edit. Acad. Române.

- **For serials**

1. Tutin, T.G., Burges, N.A, Chater, A.O., Edmonson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M. & Webb, D.A. (eds, assist. by J.R. Akeroyd & M.E. Newton; appendices ed. by R.R. Mill). (1996). *Flora Europaea*. 2nd ed., 1993, reprinted 1996. Vol. 1. *Psilotaceae to Platanaceae*. Cambridge: Cambridge University Press.

- **For chapter in serials**

1. Beldie, A. (1955). *Leontice*. In T. Săvulescu (Ed.). *Flora României*. Vol. 3. (p. 33). Bucureşti: Edit. Academiei Române.

2. Borza, A. (1931). Die Vegetation und Flora Rumänien:. In A. Borza (Ed.). *Guide de la Sixième Excursion Phytogeographique Internationale Roumanie* (pp. 1-55). Cluj: Institutul de literatură şi Tipografie Minerva S.A.

- **For chapter in occasional volumes**

1. Boşcaiu, N. (1976). Semnificaţia documentară a florei dobrogene şi necesitatea conservării sale. In Anonymous, *Ocrotirea Naturii Dobrogene* (pp. 121-132). Cluj-Napoca.

- **For proceedings from a conference**

Field, G. (2001). Rethinking reference rethought. In Revelling in Reference: Reference and Information Services Section Symposium, 12-14 October 2001 (pp. 59-64). Melbourne, Victoria, Australia: Australian Library and Information Association.

- **For a thesis**

Coldea, G. (1972). Flora şi vegetaţia Munţilor Plopiş. Unpublished doctoral dissertation, Universitatea "Babeş-Bolyai", Cluj.

- **For a web page**

The Plant List 2010. *Version 1*. Retrieved October 25, 2012, from: http://www.theplantlist.org/

Kuo, M. (May 2007). *MushroomExpert.Com*. Retrieved 15 May 2013, from http://www.mushroomexpert.com/peziza_badioconfusa.html

The scientific reviewers analyse every paper and those not conforming to the journal's requirements will not be published.

The corresponding author will be supplied with one free volume.

Tiparul s-a efectuat sub c-da nr. 4033 / 2016
la Tipografia Editurii Universității din București
B-dul Iuliu Maniu, 1-3, Complex Leu
Tel.: 021.315 25 10 / 0788 249 545, E-mail: tipografia_unibuc@yahoo.com