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

STRAT Daniela¹

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

Keywords: *Enteromorpha intestinalis* (L.) Nees 1820, water courses, salt diapir, salt karst, Meledic Plateau, Slănic River

Received 13 October 2015

Accepted 11 December 2015

Introduction

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

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

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

¹ University of Bucharest, Faculty of Geography, 1 Nicolae Bălcescu Blvd., Sector 1, Bucharest, Romania, e-mail: danielastrat@gmail.com

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

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

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

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

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

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

Material and methods

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

Results and discussion

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

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

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

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

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

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

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

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

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

water with manure. Geographical coordinates of the site (Fig. 1) are: 45°30'13"N, 26°37' 65"E, 488 m.a.s.l.

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

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

Conclusions

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

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

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

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

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

References

- Black, D. & Weeks, D. (1972). Ionic relationships of *Enteromorpha intestinalis*. *New Phytologist*, 71, 119-127.

- Burkholder, J.M. (2010). Harmful algal blooms. In G.E. Likens (ed.), *Plankton of Inland Waters* (pp. 360-370). Amsterdam: Academic Press.
- Cojocaru, I. (2012). Qualitative and quantitative analysis of the aquatic beetles cenosis in the Cîrlic lake (Iași) in 2012. *Analele Științifice ale Universității „Alexandru Ioan Cuza” din Iași, s. Biologie animală*, 58, 43-52.
- Conner, D., Huddleston, D., Pfister, L. A. & Thompson, S. (1978). A third species of *Enteromorpha* (a marine chlorophycean) for Oklahoma. *Proceedings of the Oklahoma Academy of Science*, 58, 110. Retrieved September 10, 2015, from http://digital.library.okstate.edu/oas/oas_pdf/v58/p110.pdf.
- Eimanifar, A. & Mohebbi, F. (2007). Urmia Lake (Northwest Iran): a brief review. *Saline Systems*, 3(5). Doi: 10.1186/1746-1448-3-5.
- Gheorghievici, L. Gheorghievici, G., & Tanase, I. (2015). The phytoplankton composition features of five romanian pelogenous ecosystems. *Environmental Engineering and Management Journal*, 14(5), 975-984. Retrieved September 11, 2015, from http://omicron.ch.tuiasi.ro/EEMJ/pdfs/vol14/no5/2_28_Gheorghievici_12.pdf.
- Giurgiu, I. (2010). Cea mai mare peșteră în sare din lume. *Natura României*, 3(21). Retrieved September 10, 2015, from www.romania-natura.ro
- Hammer, T.U. (1981). Primary Production in Saline Lakes: A review. In W. Williams (ed.), *Developments in Hydrobiology: Salt Lakes* (pp. 47-58). Hague, the Netherlands: Dr. D.W. Junk Publishers.
- Hammer, T.U. & Heseltine, M. (1988). Aquatic macrophytes in saline lakes of Canadian prairies. In J.M. Melack (Ed.), *Saline Lakes: Proceedings of the Third International Symposium on Inland Saline Lakes, held at Nairobi, Kenya, August 1985* (pp. 101-116). Dordrecht: Dr. W. Junk Publishers.
- Hayden, H.S., Blomster, J., Maggs, C.A., Silva, P.C., Stanhope, M.J. & Waaland, J.R. (2003). Linneus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *European Journal of Phycology*, 38, 277-294.
- Horincar, V.-B., Parfene, G. & Bahrin, G. (2011). Evaluation of bioactive compounds in extracts obtained from three romanian marine algae species. *ACRomanian Biotechnological Letters*, 16(6), 71-78. Retrieved September 10, 2015, from <http://www.rombio.eu/rbl6vol16Supplement/10%20HORINCAR%20VICENTIU.pdf>
- Horotan, A. (2010). Studiul hidrobiologic pentru lacurile exterioare de la Ocna Sibiului. *Bruckenthal Acta Mvsei*, 3, 543-554.
- Irimia, D.-N. & Irimuş, I. (2012). The touristic area Lopătari-Mînzălești. *The role of tourism in territorial development: V. International Conference: October 10, 2012, Gheorgheni, Romania* (pp. 134-140). Cluj-Napoca: Presa Universitară Clujeană.
- John, D.M., Whitton, B.A. & Brook, A.J. (2002). *The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae* (Vol. I). Cambridge University Press.
- John, D. & Rindi, F. (2015). Filamentous (Nonconjugating) and Plantlike Green Algae. In J. Wehr, R. Sheath & J. Kociolek (eds.), *Freshwater Algae of North America: Ecology and Classification*. Second edition, Volume 8 (pp. 375-422). Academic Press, Elsevier.

- Kaštovský, J., Hauer, T., Mareš, J., Krautová, M., Bešta, T., Komárek, J., Zapomělov, E. (2010). A review of the alien and expansive species of freshwater cyanobacteria and algae in the Czech Republic. *Biological Invasion*, 12, 3599-3625.
- Legea 5/06.03.2000 privind aprobarea Planului de amenajare a teritoriului național - Secțiunea a III-a, Zone protejate. *Monitorul Oficial al României*, nr. 152/12.04.2000.
- Leskinen, E. & Pamilo, P. (1997). Evolution of the ITS Sequences of Ribosomal DNA in *Enteromorpha* (Chlorophyceae). *Hereditas*, 126(1), 17-23.
- Leskinen, E., Alström-Rapaport, C. & Pamilo, P. (2004). Phytogeographical structure, distribution and genetic variation of the green algae *Ulva intestinalis* and *U. compressa* (Chlorophyta) in the Baltic Sea. *Molecular Ecology*, 13, 2257-2265.
- Mareš, J., Leskinen, E., Sitkovska, M., Skacelova, O. & Blomster, J. (2011). True identity of the European freshwater *Ulva* (Chlorophyta, Ulvophyceae) revealed by a combined molecular and morphological approach. *Journal of Phycology*, 47(5), 1177-1192.
- Mărunțeanu, C. & Ioane, D. (2010). Salt Karst in Manzalesi - Romania. In N. Evelpidou, T. de Figueiredo, F. Mauro, V. Tecim, & A. Vassilopoulos (Eds.), *Natural Heritage from East to West: Case studies from 6 EU countries* (pp. 105-110). Berlin Heidelberg: Springer & Business Media,.
- Messyasz, B. (2009). *Enteromorpha* (Chlorophyta) populations in the Nielba River and Lake Laskownickie. *Oceanological and Hydrobiological Studies, International Journal of Oceanography and Hydrobiology*, 38(Supplement), 1-9.
- Messyasz, B., & Rybak, A. (2008). Macroalga *Ulva intestinalis* L. occurrence in freshwater of Poland: a new locality in Wielkopolska. *Teka Kom. Ochr. Kszt. Środ. Przyr. – OL PAN*, 5, 126–135. Retrieved July 12, 2015, from <http://www.pan-ol.lublin.pl/wydawnictwa/TOchr5/Messyasz.pdf>.
- Messyasz, B., & Rybak, A. (2009). The distribution of green algae species from the *Ulva* genera (syn. *Enteromorpha*; Chlorophyta) in Polish inland waters. *Oceanological and Hydrobiological Studies, International Journal of Oceanography and Hydrobiology*, 38(1), 121-138.
- Messyasz, B. & Rybak, A. (2011). Abiotic factors affecting the development of *Ulva* sp. (Ulvophyceae; Chlorophyta) in freshwater ecosystems. *Aquatic Ecology*, 45, 75-87.
- Messyasz, B., Czerwi-Marcinkowska, J., Massalski, A., Uher, B., Rybak, A., Szendzina, L. & Pikosz, M. (2013). Morphological and ultrastructural studies on *Ulva flexuosa* subsp. *pilifera* (Chlorophyta) from Poland. *Acta Societatis Botanicorum Poloniae*, 82(2), 157-163.
- Messyasz, B., Pikosz, M., Schroder, G., Leska, B. & Fabrowska, J. (2015). Identification and ecology of macroalgae species existing in Poland. In S.-K. Kim, & K. Chojnacka (Eds.), *Marine Algae Extracts: Processes, Products, and Applications*. Volume 1 (pp. 17-40). Weinheim, Germany: John Wiley & Sons.
- Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D. & Abellán, P. (2011). Mediterranean saline streams in southeast Spain: What do we know? *Journal of Arid Environment*, 75, 1352-1359.

- Moore, K.A. (2009). Submerged Aquatic Vegetation of the York River. *Journal of Coastal Research, Special Issue* (57), 50-58.
- Nastac, M., Gheorghiu, K. & Miha, I. (2014). Characteristics of macro-algae biomass from Romanian Black Sea Coast. In W.M. Petre Gătescu (ed.), *2nd International Conference-Water Resources and Wetlands* (pp. 383-387). Tulcea. Retrieved September 10, 2015, from http://www.limnology.ro/water2014/proceedings/52_Nastac.pdf
- OM MMDD nr. 1964/2007 (Ordinul Ministrului Mediului și Dezvoltării Durabile nr. 1964/2007 privind regimul de arie naturală protejată a siturilor de importanță comunitară. ROSCI0199 Platoul Meledic, Formularul Standard NATURA 2000. *Monitorul Oficial al României nr. 98 bis/ 07.02.2008*, pg. 1058-1060.
- Pfester, L.A., Felkner, O.P. & Felkner, W.O. (1976). *Enteromorpha*, a marine alga in oklahoma. *Proc. Okla. Acad. Sci.*, 56. Retrieved September 11, 2015, from http://digital.library.okstate.edu/oas/oas_pdf/v56/p66.pdf
- Reinke, D.C. (1981). *Enteromorpha*, a marine alga in Kansas. *Transactions of the Kansas Academy of Sciences*, 84(4), 228-230.
- Rybak, A., Messyas, B., Szendzina, L., Szendzina, L., Pikosz, M. & Koperski, M. (2011). A new locality of the freshwater population of of *Ulva flexuosa* subsp. *pilifera* (Chlorophyta, Ulvophyceae) in Poznań (Wielkopolska). *Teka Kom. Ochr. Kszt. Środ. Przyr. – OL PAN*, 8, 131-144.
- Sava, D., Arcuș, M. & Doroftei, E. (2010). Preliminary data on Meledic-Mânzălești Natural Reserve (Buzau county, Romania). *Analele Universității Ovidius, Seria Biologie-Ecologie*, 14, 61- 66.
- Savage, C. (1964). Lake Rezaieyeh: a specialised summer habitat for Shelduck and Flamingos. *Wildfowl & Wetlands Trust, Fifteenth Annual Report*, pp. 108-113.
- Taft, C.E. (1964). The occurrence of *Monostroma* and *Enteromorpha* in Ohio. *The Ohio Journal of Science*, 64(4), 272-274. Retrieved September 30, 2014, from <http://hdl.handle.net/1811/5018>.
- Tan, I., Blomster, J., Hansen, G., Leskinen, E., Maggs, C., Mann, D., Stanhope, M. (1999). Molecular Phylogenetic Evidence for a Reversible Morphogenetic Switch Controlling the Gross Morphology of Two Common Genera of Green Seaweeds, *Ulva* and *Enteromorpha*. *Molecular Biology and Evolution*, 16(8), 1011-1018.
- Velasco, J., Millán, A., Hernández, J., Gutiérrez, C., Abellán, P., Sánchez, D. & Ruiz, M. (2006). Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. *Saline Systems*, 2(12), 1-15.
- Vladimirescu, A. (2007). Optical microscopy, TEM and molecular investigation on freshwater *Enteromorpha* in Romania. *European Journal of Phycology*, 42(sup 1), 85-171.
- Zimmermann-Timm, H. (2007). Salinisation of inland waters. *Water uses and human impacts on the water budget*. Potsdam, Germany. Retrieved September 12, 2015, from <https://www.pik-potsdam.de/members/heike/zimmermann-timm2007.pdf>.