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MARSILEA QUADRIFOLIA L. IN THE PROTECTED WETLANDS FROM ROMANIA

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Abstract

The Romanian Pteridophyta flora has only one species that belongs to Marsiliaceae family of aquatic ferns: *Marsilea quadrifolia* L. According to the map of European naturally occurrence of *M. quadrifolia*, this fern species is spread especially between 45° and 50° North latitude, which correspond to the lowlands and flood plains of the main rivers, and hygrophilous grasslands. Its habitats are heavy clay to sandy substrates of permanent lakes and seasonal ponds. Also, man made habitats (ditches and rice fields) can be populated by this semi aquatic fern. As a hyrophyte, *M. quatrifolia* grows in mesotrofic to eutrofic waters. The *M. quatrifolia* become rare taxa throughout its range from Europe because of the lost of natural its habitats (wetland habitats) mainly due to modifications of river courses such as channelisation and embankment, changes of agricultural practice, floodplain drainage and others wetlands, high fertilization. According to assessment of World Conservation Monitoring Centre (1994), *M. quadrifolia* is threatened in 21 European countries, "Indeterminate" or "Vulnerable" in eastern Palaearctic ecozone, and known to be "Extinct" in Germany, Poland and Switzerland. In European Union this fern is protected by law starting with Bern Convention and followed by Habitat Directive 92/43/EEC – Annex IIb, and Natura 2000 Network. In Romania *M. quadrifolia* became protected since 1993 after Bern Convention was accepted by Law 13/11.03.1993. According to IUCN Plant Red List for Romania, the species is listed as vulnerable. Populations of M. quadrifolia are present in several categories of protected areas in Romania that are important in terms of local, regional, European and international level. However, according to EUNIS database (http://eunis.eea.europa.eu/species/150005/sites), of the 74 Nature 2000 sites with *M. quadrifolia* in the European Union, 11 sites are in Romania, which are mainly spread in west and south of Romania. One of them is Danube Delta Biosphere Reservation that is also Ramsar site. Despite the apparently suitable habitats, *M. quadrifolia* is sparsely spread in Danube Delta. The literature mentions four localities: Sulina, Gârla Magearu, Canalul Rusca, and Sfântu Gheorghe village. In the last locality there is a small population whose habitat is a drainage ditch.

Keywords: *Marsilea quadrifolia* L., aquatic fern, protected species, protected area, Nature 2000 sites, wetland

1. INTRODUCTION

Pteridophytes are fragile and vulnerable to anthropic disturbances and climate changes due to their microclimatic dependence (Pausas&Sáez, 2000). Beside, they have a strong dependence on high moisture for sexual reproduction because they have flagellate gamets and external fertilization.

Marsilea quadrifolia L. (water clover, four leaf clover, water shamrock), with its unusual and remarkable leaves for their simplicity, is an aquatic and amphibious fern that belongs to Marsileaceae family. *M. quadrifolia* is considered the lectotype of the generic name (Grolle, 1988). It is the unique representative of Marsiliaceae family of the flora cormophytes in Romanian (Ciocârlan, 2000). *Marsilea* species are easily recognized by their clover-like leaves, which are composed of four leaflets on a long petiole. *Marsilea* is found primarily in seasonally wet habitats, where it grows in shallow water and at the edges of ponds, lakes, or rivers (Johnson, 1986). Plants may be submerged, except for the floating leaflets, or emergent (Kornas, 1988). As a hyrophyte, *M. quatrifolia* grows in mesotrofic to eutrofic waters (Dubin, 1981).

In its natural characteristic habitats it has the ability to develop heterophyllous, aerial and submergent leaves, on same plant, in response to changes in natural environment (Hsu&colab., 2001; Lin., 2002; Lin&colab., 2007; Kao&Lin, 2010). In a completely submergeded condition, rhizomes of this fern produces fork-like submergent leaves with four elongated pinnae expanding parallel to the long axis of the petiole. Then, when the leaflets are raised above the surface of water, they expand on a plane perpendicular to the petiole, resembling a four-leaf clover – two pairs of pinnae in a cruciform arrangement. These are aerial or land leaves that have circadian movements and phototropic movements (Kao&Lin, 2010). The leaves arise solitary from the nodes on a long creeping rhizome rooting at the nodes, also.

In contrast to submerged leaves, aerial leaves have trichomes on the adaxial and abaxial surfaces. Experimental observations revealed that the presence of trichomes is of more importance in reducing water loss than in reflecting light and protecting *M. quadrifolia* against the potentially damaging effect of photoinhibition in aerial environments (Wu&Kao, 2009).

M. quadrifolia is spread in Central and Southern Europe, the Caucasus, Central Asia as well as Japan, Siberia, India. In North America it has been established in the northeastern United States for over 100 years (Johnson, 1993). It is invasive in natural and disturbed wetland habitats from New England to Florida (Jacono&Johnson, 2006). Campaigns against the spread of this species in natural aquatic habitats are taken in the U.S.A because in New World, *M. quadrifolia* is evaluated as an aggressive and invasive non-indigenous species (Thiébaut, 2007; Serviss&Peck, 2008; Campbell&colab., 2010).

M. quadrifolia thrives in man-made habitats like rice fields, were it is a weed, or survive in secondary habitats such as water ditches, standing water bodies, temporary waters and fresh water curses (Hulina, 1998; Kiem, 2002; Zemmer, 2006). This fern is important not only in terms of biodiversity but also because it is sensitive to aquatic environmental toxicant exposure, so it was chosen for ecotoxicogenomics studies (Snape&colab., 2004). *M. quadrifolia* is a high bioaccumulator of heavy metals, with maximum bioconcentration factor for Cd and Cr, and in these circumstance could be used for phytosequestration of these metals from contaminated sites (Ahmad&colab., 2010). *M. quadrifolia* presents symbiotic relationship with fungi. Mycorrhizal fungi have been found in roots. It is about the presence of vesicular colonization with species belonging *Glomus* genera (Radhika&Rodrigues, 2007).

2. DISTRIBUTION OF MARSILEA QUADRIFOLIA IN EUROPA

According to the map of European naturally occurrence of *M. quadrifolia* (Jalas&Suominen, 1972), this fern species is spread between 40° and 50° North latitude but most sites are between 45° and 50° North latitude, which correspond to the lowlands and flood plains of the main rivers, and hygrophilous grasslands. Upper Rhine in Germany, Czech Republic and Poland is the northern limit of the area of distribution in Europe. In many European countries, the species *M. quadrifolia* was once a very common and frequent plant (Hulina, 1998) but in the second half of last century it has become rare.

In the last two decades, according to literature, there are populations of *Marsilea quadrifolia* in the following countries: Croatia (Hulina, 1998; Italy, in Adige Valley (Kiem, 2002; Marchetti, 2003; Zemmer 2006), Emilia-Romagna Region (Bonafede&colab., 2002); Spain, in Ebro Delta (Lozano&colab., 1999; Sáez&Soriano, 2000; Estrelles&colab., 2001; Estrelles&Ibars, 2002); France, in floodplain of Saône and Loire (Grolle, 1998); Romania (Oltean&colab., 1994; Ciocârlan, 2000; Tainã, 2002; Petrescu, 2004; Popescu, 2005; Sârbu&colab., 2006; Pop&Anastasiu, 2008; Dihoru&Negrean, 2009); Switzerland, in Rhon Valley (Kässermann&Moser, 1999); Nederland (Drok&Weeda, 1999; Bremer, 2003, 2007); Albania, in Vjosa Delta (Buzo, 2000) and Lake Shkondra – River Buna wetlands complex (Kashta, 2007); Montenegro (Radovic & colab,., 2008); Portugal (de Sequereira&colab., 1997); Hungary, in area called Bodrogköz that is the alluvial plain of the rivers Bodrog and Tisza (Tuba, 1995); Ukraina (Witkowski&colab., 2003); Bulgaria (Valchev&colab., 2006).

Today it is rare taxa throughout its range from Europe (Godreau&colab., 1999; Lozano&colab., 1996; Estrelles&colab., 2001). According to assessment of World Conservation Monitoring Centre (1994), M. quadrifolia is "threatened" in 21 European countries, "Indeterminate" or "Vulnerable" in eastern Palaearctic region and known to be "Extinct" in Germany, Poland and Switzerland. At the Global level, according to the newest IUCN Red List of Threatened Species (Gupta, 2011), M. quadrifolia is a "least concerned" species.

The historic decline of *M. quadrifolia* in area of its natural occupancy from Europe can be attributed to the lost of natural habitats (wetland habitats) mainly due to modifications of river courses such as channelisation and embankment, changes of agricultural practice, (Poschlod&colab., 2005), drainage of floodplain and others wetlands, high fertilization (Godreau&colab., 1999). The relationship between the transformation of wetlands by human activities and disappearance of plants and plant communities of aquatic, wet and marshy habitats, including *M. quadrifolia*, with special reference to Belgium, was highlighted by early 1970s (Miège, 1971). At European level 10% of its flora is threatened but some credited prognoses estimate that by 2080 up to 60% of the European flora might be in risk, especially due to climate change (Thuiller&colab., 2005). Nevertheless, in the case of *M. quadrifolia*, the mild winter without frost from last decades of XX century from Netherlands had a positive effect (Bremer, 2003). Thus it was reported as being new species to the flora fern from Netherlands (Drok&Weeda, 1999). It was observed first time on the bank of a sheet of water in a foreland of the river Waal, a suitable habitat created by transformation of farmland along river into wetland (Bremer, 2003; Bremer, 2007). This is a proof that *M. quadrifolia* has a good ability to colonize man-made habitats such as happens with rice fields from south Asia, where it is a noxious and undesirable weed in India (Chang, 1970; Satapathy&Singh, 1985; Luo&Ikeda 2007), and also in rice field

from Ebro Delta, in Spain (Márquez, 1998). In Asia, its proliferation is regarded as a consequence of global warming – mainly related to frequent flooding, and its control is difficult because *M. quadrifolia* is tolerant to most of the grass killer herbicides used (Kathiresan, 2006). Frequent floods do favor its perpetuations because they spread spores and rhizome fragments. Also, in its natural habitats in Europe it is threatened by some invasive aquatic invasive plants such as *Azolla filiculoides* that developing populations competing with *M. quadrifolia* (Anastasiu&colab., 2007).

3. DISTRIBUTION OF MARSILEA QUADRIFOLIA IN WETLANDS FROM ROMANIA

On the map of the European occurrence of *Marsilea quadrifolia* (Jalaas&Suominen, 1972) for the Romanian territory are marked 16 sites that are spread mainly in Banat, Crişana, Oltenia and Muntenia regions. Before 2000 year, the literature quotes 42 localities with populations of *M. quadrifolia* but in 2006 only five of them were confirmed (Sârbu, 2006 a; Sârbu &colab. 2007 a, b). These are: Cefa, Comana, Mureş Meadow at Ciobotani, Ostrovu Mare – Moldova Veche in Iron Gates Gorje, Nanov Forest, Natural Park Mureş Meadow, and Rusca Chanel in Danube Delta. For same year, Pop and Anastasiu (2008), in "Natura 2000 in Romania – Species fact sheets", report more localities.

Studies carried out after 1990 year for set up of Romanian Red List, for wetlands inventory for designation of Ramsar sites (Török, 2002), and for designation Site of Community Importance (SCI) and others type of protected areas, contributed to a better understanding of spread and population status of *M. quadrifolia.*

Also, *M. quadrifolia* is cited for several habitats from south and southeastern Romania (Popescu, 2005): Danubian communities with *Salvinia natans*, *Marsilea quadrifolia*, *Azolla caroliniana* and *A. filiculoides*; Danubian communities with *Riccia fluitans* and *Ricciocarpus natans*; Danubian communities with *Hydrocharis morsus-ranae*, *Stratiotes aloides* and *Utricularia vulgaris*; Danubian communities with *Typha angustifolia* and *T. latifolia*, Dacian-Danubian communities with *Glyceria maxima* and *Schoenoplectus palustris*; Danubian communities with *Potamogeton perfoliatus*, *P. gramineus*, *P. lucens*, *Elodea canadensis* and *Najas marina*; Danubian communities with *Nymphaea alba*, *Trapa natans*, *Nuphar luteum* and *Potamogeton natans*.

In Dobrogea, both Dobrogea Plateau and Danube Delta, *M. quadrifolia* occurs extremely rarely. For Dobrogea Plateau, in the past century it was reported in only one station – Igliţa Lake (Ţopa, 1952) – and where it survived until nowadays. Fortunately the Igliţa Lake is part of Natura 2000 site – ROSCI 0012 Braţul Măcin and ROSPA 0040 Dunărea Veche - Braţul Măcin. The second site is Oltina Lake (Petrescu, 2004). Despite the apparently suitable habitats, *M. quadrifolia* is sparsely spread in Danube Delta. Previously 2000 year, the literature mentions only two localities: Sulina and Gârla Magearu (Panţu&colab., 1935; Ţopa, 1952; Negrean&Dihoru, 1976; Ciocârlan, 1994). In the Ukrainian part of Danube Delta it have been were quoted two sites (Klokov&Dyachenko, 1988). Recently, monitoring data confirm Sulina and Gârla Magearu (Sârbu&colab., 2006 a, b), and add two more: Sf. Gheorghe (Pop&Anastasiu, 2008) and Canal Rusca (Sârbu&colab., 2007 b). The last site, Canal Rusca, is an Important Plant Area with "the largest population of *M. quadrifolia* in the Danube Delta" (Sârbu&colab., 2007 b). After the Natura 2000 Network was set up, *M. quadrifolia* is quoted in 16 wetlands: 15 are SCIs and one is Special Protection Area (SPA), according to Bird Directive (Council Directive 2009/147/EC), and Important Bird Area, according to Bird Life International (Table 1, Figure 1). Some of them are protected areas al national and international level. For example, Danube Delta, Comana Natural Park, Iron Gates Natural Park, and Mureş Floodplain are Ramsar Sites (Wetlands of International Importance). Two localities with population of *M. quadrifolia* (Ciobotani from Mureş Floodplain and Nanov Forest from Vedea Floodplain) are not part of any protected area (Fig. 1).

Table 1. The protected wetlands from Natura 2000 Network with populations of Marsilea quadrifolia L.

Nr. crt.	Name of Protected Area from Natura 2000 Network	Cod Natura 2000	Coordonates	Administrative regions - County
1	Braţul Măcin (Măcin Arm –(Igliţa Lake)	ROSCI 0012	N 45° 00' 29" E 28° 7' 52"	Brăila, Constanţa, Tulcea
2	Câmpia Ierului (Ieru Plain)	ROSCI 0021	N 47° 27' 29" E 22° 15" 2"	Satu-Mare, Bihor
3	Coridorul Jiului (Jiu Corridor)	ROSCI 0045	N 44° 1' 0" E 23° 55' 32"	Gorj, Dolj
4	Diosig	ROSCI 0068	N 47° 20' 22" E 21° 56' 41"	Bihor

5	Lunca Timişului (Timiş Floodplain)	ROSCI 0109	N 45° 35' 40" E 21° 5' 22"	Timiş
6	Porţile de Fier (Iron Gates)	ROSCI 0206	N 44° 39' 57" E 21° 58' 47"	Caraş-Severin, Mehedinţi
7	Valea Călmăţuiului (Călmăţui Valley)	ROSCI 0259	N 45° 0' 24" E 27° 2' 42"	Buzău, Brăila
8	Lunca Teuzului* (Teuz Meadow)	ROSCI 0350	N 46° 34' 8" E 21° 41' 49"	Arad
9	Delta Dunării (Danube Delta)	ROSCI 0065	N 44º 54' 6" E 28º 55' 19"	Tulcea, Constanţa
10	Comana	ROSCI 0043	N 44° 8' 27" E 26° 6' 26"	Giurgiu
11	Lunca Mureşului Inferior (Mureş Lower Floodplain)	ROSCI 0108	N 46° 8' 45" E 21° 7' 31"	Arad, Timiş
12	Scroviştea	ROSCI 0224	N 44° 42' 51" E 26° 3' 46"	Ilfov
13	Lacul Oltina (Oltina Lake)	ROSPA0056	N 44° 8' 96" E 27° 38' 11"	Constanţa
14	Ciuperceni-Desa	ROSCI0039	N 43° 53' 58" E 23° 5' 38"	Dolj
15	Cefa	ROSCI 0026	N 46° 52' 53" E 21° 39' 5"	Bihor
16	Câmpia Careiului (Carei Plain)	ROSCI 0020	N 47° 37' 8" E 22° 11' 59"	Satu Mare, Bihor

- Site proposed in 2011, not confirmed yet.

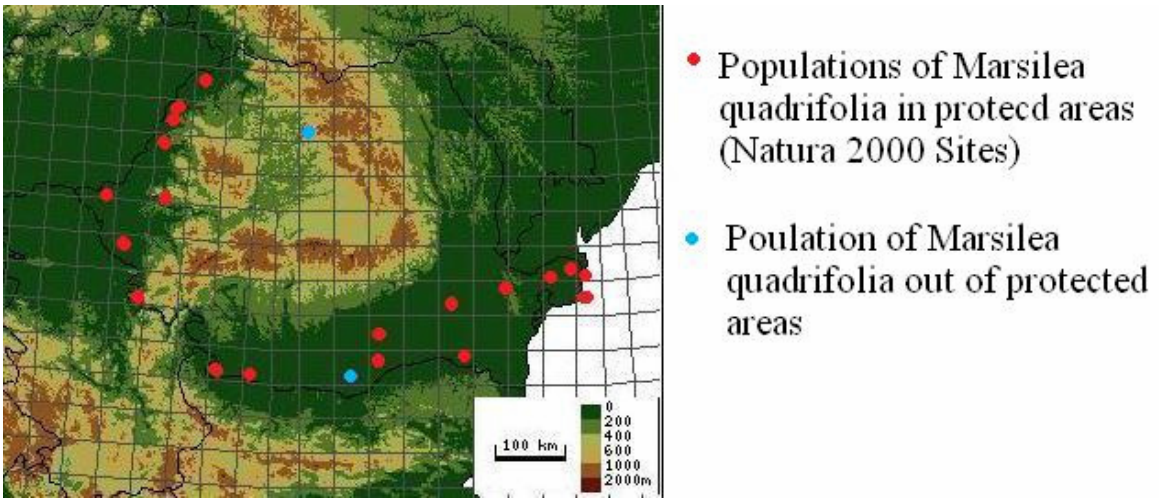

Figure 1. Map of the occurrence of *Marsilea quadrifolia L.* in Romania

4. THE PROTECTION AND CONSERVATION OF MARSILEA QUADRIFOLIA

In European Union, this fern is strictly protected by law from Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats, Council Directive 79/409/EEC of 2 April 1979, Annex I, 19.IX.1979), replaced them by Council Directive 2009/147/EC. Then followed the Habitats Directive of European Union (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora Annex II) and finally, setting up Natura 2000 Network.

The conservation of the aquatic fern *M. quadrifolia* is undertaken both *in situ* and *ex situ. According to* Heywood&Dulloo (2006), *in situ* conservation of species is a task that has proved difficult to implement even though it is explicitly mandated by the Convention on Biological Diversity in Article 8: "*…the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties."*

However, according to EUNIS database (http://eunis.eea.europa.eu/species/150005/sites) in European Union there are 74 sites with *M. quadrifolia* that are Special Areas of Conservation based on

Habitats Directive. Of these, 11are located in Romania. It is also legal protected in countries outside the European Union like Albania (Buzo, 2000; Xhulaj&Mullaj, 2002; Kashta, 2007), Croatia (Hulina, 1998), Montenegro (Radovic&colab., 2008), and Ukraina (Witkowski&colab., 2003). The *ex situ* conservation includes classical micropropagation (Bonafede&colab. 1999; Banciu&colab., 2007; Banciu&colab., 2009; Brezeanu&Banciu, 2009; Rolli&colab., 2009) and establishment of populations in botanical gardens, and reintroduction of *M. quadrifolia* in some place that became extinct. Reintroduction, which means "the deliberate establishment of individuals of a species into an area and/or habitat where it has become extirpated with the specific aim of establishing a viable self-sustaining population for conservation purposes" (Maunder, 1992), may involve the establishment of an extirpated species into a relatively intact habitat or it can be part of the restoration of a degraded habitat (Heywood&Culham, 2009). Reintroduction can be considered an ideal follow up activity for *ex situ* conservation initiatives because *in situ* and *ex situ* techniques are integrated in a complementary way. Furthermore, *ex situ* activities can play a pivotal role if they are used to support *in situ* conservation, contributing to the long term survival of the natural populations of threatened plants, reinforcing the diminishing ones and reintroducing the extinct ones (Guerrant&colab., 2004; Rossi&Bonomi, 2007). Some recovery and reintroduction programs of *M. quadrifolia* are successfully developed in countries like Spain (Estrelles&colab., 2001, Estrelles&Ibars, 2002), Portugal (de Sequereira&colab., 1999) Italy (Bonafede&colab., 1999 Bonafede&colab., 2002; Pistoja&colab., 2003; Prette&colab., 2006), Poland (Schweitzer&Polakowski, 1994), and Switzerland (Käsermann&Moser, 1999; Nöel&colab., 2011). A very good example is the reintroduction of M. quadrifolia in old rice field inside the land of the Natural Park in the Ebro's Delta, as a conservation measure ex situ applied on this species that traditionally is part of the communities of Orizetea sativae (Estelles&colab., 2001). The assessment of conservative status at the European level for M. quadrifolia covering the period 2001-2006 revealed that this species is doing remarkably well in Italy, but bad in most other countries, particularly in France, Hungary and Germany. But the anthropogenic habitats are often alternative or temporary shelters rather than mainstays, from which the plant can expands further (Nowak&Nowak, 2006), because these habitats are analogous habitats, similar to natural ones. Establishment of *M. quadrifolia* in new sites within the natural area was also reported (Pistoja&colab., 2003). Also its reappearance in place where it was declared extinct can be attributed, *inter alia*, to the high resistance at desiccation of the sporocarp, which can "germinate" even after 100 years of dormancy (Nagalingum&colab., 2007) if the suitable conditions are restored. Another explication could be that ducks and other waterfowl also help to move this species between of different bodies of water (http://nbii-nin.ciesin.columbia.edu/ipane/icat/browse.do?SpecieId=73).

4.1. Conservative status in Romania

In Romania *M. quadrifolia* became protected since 1993, after Bern Convention was ratified by Law 13/11.03.1993. Also, Romanian legislation provides protection of this species and its habitats: Law 462/2001, Law 195/2005. The Habitats Directive is transposed in the Romanian legislation through Government Urgent Ordonnance no. 57/2007 (GUO). The Article 28 (2) of the GUO 57/2007 considering the regime of natural protected areas, conservation of natural habitats, flora and fauna transposes Article 6(3) of the Habitats Directive (92/43/EEC). According to IUCN criteria, on the Romanian Red List, this plant is listed as vulnerable (Oltean&colab., 1994; Tainã, 2002; Dihoru&Negrean, 2009).

5. CONCLUSION

Using data from the literature, this paper presents geographical range of *Marsilea quadrifolia* in Europe and its conservative status. Results indicate that this fern is threatened in all Europe and it is encountered in the list of community interest. Because of that, in Romania it requires strict protection according to OUG No. 57/20.06/2007. In Romania it occurs in 19 localities, meaning significant presence in comparison to what happens in other European countries where this fern is rare or extinct in wild and where are developed reintroduction programs (Italy, Spain, Portugal, Switzerland) aiming to establish new populations of *M. quadrifolia* and others rare wetlands species. In Romania also, most of population there are in wetlands that are included in protected areas that are part of Natura 2000 Network. However, compared with the situation in the first half of the 20th century, mentioned in literature, the beginning of 21st century the sites with *M. quadrifolia* population halved. Maintaining populations of *M. quadrifolia* is dependent primarily on maintaining and preserving its natural habitats – freshwater wetlands – because the entire reproductive biology occurs in water.

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