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FIRST RECORDS OF MYXOMYCETES IN THE HUTSULSHCHINA NATIONAL NATURE PARK (UKRAINIAN CARPATHIANS)

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Mycological observations in beech, oak, hornbeam and mixed forests of Hutsulshchina National nature park, carried out in 2012, 2013 and 2016, allow to reveal 53 taxa of the Myxomycetes (48 species, 4 varieties and 1 form), including Arcyria cinerea (Bull.) Pers., Arcyria denudata (L.) Wettst., Arcyria incarnata (Pers. ex J.F Gmel.) Pers., A. insignis Kalchbr. & Cooke, Arcyria minuta Buchet in Patouliar, Arcyria oersdtedtii Rostaf., Badhamia panicea (Fr.) Rostaf., Calomyxa metallica (Berk.) Nieuwl., Ceratiomyxa fruticulosa (O.F. Müll.) T. Macbr., Collaria arcyrionema (Rostaf.) Nann.-Bremek. ex Lado, Craterium minutum (Leers) Fr., Craterium sp., Cribraria cancellata (Batsch) Nann.- Bremek., Cribraria violacea Rex, Dictydaethalium plumbeum (Schum.) Rostaf., Echinostelium minutum, de Bary, Fuligo candida Pers., Fuligo septica (L.) F.H. Wigg., Fuligo septica (L.) F.H. Wigg. f. flava (Pers.) Y. Yamam., Hemitrichia calyculata (Speg.) M.L. Farr, Hemitrichia clavata (Pers.) Rostaf., Hemitrichia serpula (Scop.) Rostaf. ex Lister, Leocarpus fragilis (Dicks.) Rostaf., Licea operculata (Wingate) G.W. Martin, Lycogala epidendrum (L.) Fr. sensu B. Ing, Lycogala exiguum Morgan, Lycogala terrestre Fr. sensu B. Ing, Metatrichia vesparia (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop., Mucilago crustacea P. Micheli ex F.H. Wigg., Paradiacheopsis fimbriata (G. Lister & Cran) Hertel ex Nann.-Bremek., Physarum album (Bull.) Chevall., Physarum cinereum, (Batsch) Pers., Physarum flavicomum Berk., Physarum leucopus Link, Physarum psittacinum, Ditmar, Physarum sp., Physarum viride (Bull.) Pers., Reticularia lycoperdon Bull., Stemonitis axifera (Bull.) T. Macbr., Stemonitis flavogenita E. Jahn, Stemonitis fusca Roth, Stemonitis fusca Roth var. nigrescens (Rex) Torrend, Stemonitis splendens Rostaf., Stemonitis splendens Rostaf. var.webberi (Rex) Lister, Stemonitopsis amoena (Nann.-Bremek.) Nann.-Bremek., Stemonitopsis hyperopta (Meyl.) Nann.-Bremek., Stemonitopsis typhina (F.H. Wigg.) Nann.-Bremek., Trichia affinis de Bary, Trichia contorta (Ditmar) Rostaf., Trichia decipiens (Pers.) T. Macbr., Trichia favoginea (Batsch) Pers. Among substrates, occupied by myxomycete fruiting bodies, the dead wood appeared to be the richest regarding to the abundance and diversity of myxomycetes. Thirty species of myxomycetes were found on the dead wood, originated from Fagus sylvatica, Picea abies, Abies alba, Larix deciduas and Pinus sylvestris. Besides the wood, myxomycetes were also found on fallen and living leaves of trees, on grasses, mosses, soil and vegetative or reproductive structure of fungi, including Armillaria australis, Hypoxylon fuscum and Rosellinia sp.

Key words: *biodiversity, Eumycetozoa, forest communities, slime molds, substrate preferences.*

INTRODUCTION

Myxomycetes are fungi-like members of the super-group Amoebozoa, in which they form a separate class within the clade Eumycetozoa [16]. The life cycle of these organisms includes uninuclear amoebae and flagellate cells, the giant multinuctelar plasmodia and the fruit bodies (sporophores), where millions of spores are formed [15, 17]. Myxomycetes inhabit soil and plant substrates, including dead wood, bark of living trees and leaf-litter, where they feed with bacteria, which are common on these substrata. They may form fructifications on the same piece of substrate where they feed, but in many cases they prefer to develop their fruit bodies on other substrata, then those used for feeding: green parts of plants, mosses, fungal fruit bodies etc. This must be taken into account then substrate preferences of myxomycetes are analyzed [15, 17].

Wood of stumps and logs on various stages of decomposition is known to be the most favorable substrate for the development of myxomycetes in temperate forest communities [4]. At least 300 species of myxomycetes are associated with this type of substrate among which 168 species are recorded in Ukraine, representing 60.4% of the total number of myxomycetes in the country so far.

The Hutsulshchina National Nature Park (NNP) is situated on the territory of Kosiv district, Ivano-Frankivska oblast. The park is situated in the Marmarosh-Chornohora-Svydovets district of oak, hornbeam-oak, beech, spruce and fir forests of Ukrainian Carpathians [1]. The area of the park is 32271 ha, from which 7606 ha are used for the conservation purposes. The forest vegetation covers more than 95% (30741 ha) of the park area. More than half of this territory (62%) is represented by communities of the European beech (*Fagus sylvatica* L.), situated at the altitudes 500–800 m a. s. l. In the low-mountain landscape they cover peaks of mountain ridges, while in middle-mountain landscape they usually occupy slopes. Pedunculate oak (*Quercus robur* L.), European hornbeam (*Carpinus betulus* L.), Norway spruce (*Picea abies* (L.) H. Karst.) and European silver fir (*Abies alba* Mill.) occupy together 33.3% of the park area [1].

MATERIALS AND METHODS

Material was collected during several expeditions to the Hutsulshchina NNP, held in 2012, 2013 and 2016. Seven locations were studied (Fig. 1): (1) Kosivske forestry, boarding-house «Baika», beech forest, collectors I.O. Dudka, D.V. Leontyev, A.V. Kochergina, august 2012; (2) Kosivske forestry, environs of village Pystyn', ridge Kamenystyi, mixed spruce-beech forest, collectors I.O. Dudka, D.V. Leontyev, A.V. Kochergina, august 2012; (3) Kosivske forestry, environs of Kosiv, ridge Holytsia, mixed forest, collectors I.O. Dudka, D.V. Leontyev, A.V. Kochergina, august 2012; (4) Starokutske forestry, environs of village Cherhanivka, hornbeam-oak forest, collectors I.O. Dudka, D.V. Leontyev, A.V. Kochergina, august 2012; (5) Sheshorske forestry, environs of village Sheshory, mixed spruce-beech forest, collectors I.O. Dudka, D.V. Leontyev, A.V. Kochergina, august 2012; collector O.Y. Akulov, august 2013; (6) Sheshorske forestry, ridge Karmatura near the Mertve lake, beech forest, collector I.O. Dudka, august 2016; (7) Sheshorske forestry, vicinities of the "St. Mikola Homestead", oak forest, collector I.O. Dudka, august 2016.

Fragments of various substrates (fallen tree trunks and branches, standing trees, old stumps, dead tree bark, fallen leaves, leaf litter, dried and leaving grasses, fruit bodies of mushrooms, etc.) were studied directly in the field using ×10 lens. Found fruit bodies of myxomycetes were immediately transferred into paper boxes and fixed with glue. In addition, samples of the bark of living trees with no visible myxomycete fructifications were collected. In the laboratory these substrates were put into the Petri dishes with the moist filter paper and incubated for 14 days at room temperature. This technique, known as the moist chamber method, allows to reveal species with very small fruiting bodies, ca. 10–50 µm [17].

Specimens were identified using specialized manuals [4, 10, 12, 13] using the light microscope GS M 27257 and the stereomicroscope Olympus VT-II 205816. Nomenclature of myxomycetes was specified according to Lado [6]. Nomenclature of the vascular plants is given according to Mosyakin et al. [11].

RESULTS

As a result of the study, 53 taxa of species and subspecies level, including 48 species, 4 varieties and 1 form of myxomycetes, were recorded from the Hutsulshchina NNP. The list of taxa, supplied by the substrate and location data, is given in the table 1.

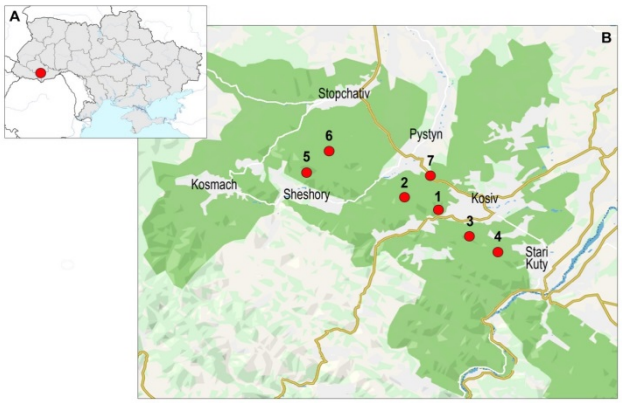

Fig. 1. The study area: A – Location of the Hutsulshchina Nature National Park; B – Plan of the Hutsulshchina National Nature Park. Dots indicate collection sites, numbered in the same order, as in the text.

According to the classification of Poulain et al. [13] all collected species belong the orders Trichiales (15 species; 15 infraspecies taxa), Physarales (14; 17), Stemonitales (9; 11), Liceales (8; 8), Echinosteliales (1; 1) and Ceratiomyxales (1; 1). The families, richest in number of species, were Physaraceae (13 species), Stemonitaceae (9), Trichiaceae (8), Arcyriaceae (6) and Reticulariaceae (5); another families were presented in our collection by 1 or 2 species. Among genera, the richest were *Physarum* Pers. (7 species), *Arcyria* Wigg. (6), *Trichia* Haller (4), *Stemonitis* Roth (4), *Hemitrichia* Rostaf. (3), *Lycogala* Adans. (3) and *Stemonitopsis* Nann.-Bremek. (3); another genera were represented by 1 or 2 species each.

Prevalence of species, evaluated by the number of collecting sites, was the highest for *Arcyria cinerea, A. denudata*, *Metatrichia vesparia* and *Stemonitis fusca*, which were revealed in five collecting areas, in beech, oak, hornbeam-oak and mixed forests. *Stemonitis axifera* and *Lycogala epidendrum* were found in four locations, in beech, oak and mixed forests. *Ceratiomyxa fruticulosa* was observed on three collection sites, in beech, hornbeam-oak and mixed forests (table 1).

Table 1.

Species composition and substrate preferences of myxomycetes recorded in NNP «Hutsulshchina»

№	Names of taxa	Locations						
		1	2	3	4	5	6	7
1	*Arcyria cinerea* (Bull.) Pers.			wPA, b+CB	l+QR	bCer (mc)	2wFS br+FS	br+QR

2	*Arcyria denudata* (L.) Wettst.	s	s		fRos	s		wQR
3	*Arcyria incarnata* (Pers. ex J.F Gmel.) Pers.			wBP	wBP			
4	*A. insignis* Kalchbr. & Cooke							wQR
5	*Arcyria minuta* Buchet in Patouliar				bCer (mc)			
6	*Arcyria oersdtedtii* Rostaf.			wFS				
7	*Badhamia panicea* (Fr.) Rostaf.		wFS					
8	*Calomyxa metallica* (Berk.) Nieuwl.					bBP (mc)		
9	*Ceratiomyxa fruticulosa* (O.F. Müll.) T. Macbr.				wBP wCB w	2wFS		wQR
10	*Collaria arcyrionema* (Rostaf.) Nann.-Bremek. ex Lado		wAA					
11	*Craterium minutum* (Leers) Fr.				bCAv+ fHyp l+CB			
12	*Craterium* sp.					l+FS?		
13	*Cribraria cancellata* (Batsch) Nann.-Bremek.				wPS, wQR+m			
14	*Cribraria violacea* Rex				wCB+m (mc) soc. ARCmin			
15	*Dictydaethalium plumbeum* (Schum.) Rostaf.			wPA?				
16	*Echinostelium minutum* de Bary					4bPS (mc)		
17	*Fuligo candida* Pers.			wPA	br+PA			
18	*Fuligo septica* (L.) F.H. Wigg.		m				br+FS wFS	
19	*Fuligo septica* (L.) F.H. Wigg. f. *flava* (Pers.) Y. Yamam.				m		wFS l+FS	
20	*Hemitrichia calyculata* (Speg.) M.L. Farr						w	br+QR
21	*Hemitrichia clavata* (Pers.) Rostaf.					wFS	2wFS	
22	*Hemitrichia serpula* (Scop.) Rostaf. ex Lister			w+fArm	wQR			
23	*Leocarpus fragilis* (Dicks.) Rostaf.				l+, b+, br+QR		wFS, 2wPA	
24	*Licea operculata* (Wingate) G.W. Martin				bCer (mc)			
25	*Lycogala epidendrum* (L.) Fr. sensu B. Ing	wFS	wAA?			wPA wFS	wPA+m	
26	*Lycogala exiguum* Morgan					w		
27	*Lycogala terrestre* Fr. sensu B. Ing			wPA				
28	*Metatrichia vesparia* (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop.	wAA			w	wFS	wFS	br+QR
29	*Mucilago crustacea* P. Micheli ex F.H. Wigg.				br			gr
30	*Paradiacheopsis fimbriata* (G. Lister & Cran) Hertel ex Nann.-Bremek.					bLD (mc)		
31	*Physarum album* (Bull.) Chevall.					wQR wFS	wFS	
32	*Physarum cinereum* (Batsch) Pers.				b+QR			
33	*Physarum flavicomum* Berk.			bPS				

34	*Physarum leucopus* Link					wFS?		
35	*Physarum psittacinum* Ditmar		wAA, wFS					
36	*Physarum* sp.					bCra (mc)		
37	*Physarum viride* (Bull.) Pers.	wFS, wPA						
38	*Reticularia lycoperdon* Bull.						wAA	
39	*Stemonitis axifera* (Bull.) T. Macbr.	wPA		wPA			wFS, br+AA	wQR
40	*Stemonitis flavogenita* E. Jahn						2wFS	
41	*Stemonitis fusca* Roth	wPA			wQR		br+FS wFS br+FS l+FS, w	wQR
42	*Stemonitis fusca* Roth var. *nigrescens* (Rex) Torrend	w		lFS		wFS		
43	*Stemonitis splendens* Rostaf.					wFS?		
44	*Stemonitis splendens* Rostaf. var.*webberi* (Rex) Lister		b+FS		wBP			
45	*Stemonitopsis amoena* (Nann.-Bremek.) Nann.-Bremek.					wFS		
46	*Stemonitopsis hyperopta* (Meyl.) Nann.-Bremek.				wBP			
47	*Stemonitopsis typhina* (F.H. Wigg.) Nann.-Bremek.				wQR	wFS		
48	*Trichia affinis* de Bary				wLD	wFS		
49	*Trichia contorta* (Ditmar) Rostaf.						wFS	
50	*Trichia decipiens* (Pers.) T. Macbr.	wFS?	wFS?			wFS?		
51	*Trichia favoginea* (Batsch) Pers.					wFS? wFS	wFS	
Number of species and intraspecies taxa / specimens		**8/11**	**8/9**	**10/11**	**22/31**	**21/31**	**15/28**	**9/9**

Legend. Substrate-forming plants: **AA** – *Abies alba* Mill., **BP** – *Betula pendula* Roth, **CAv** – *Corylus avellana* L., **CB** – *Carpinus betulus* L., **Cer** – C*erasus avium* (L.) Moench, **Cra** – *Crataegus* sp. **FS** – *Fagus sylvatica* L., **LD** – *Larix decidua* Mill., **PA** – *Picea abies* (L.) H. Karst., **PS** – *Pinus sylvestris* L., **QR** – *Quercus robur* L. Substrate-forming fungi: **fArm** – *Armillaria australis*, **fHyp** – *Hypoxylon* sp., **fRos** – *Rosellinia* sp. Myxomycetes in close association with another species: **ARCm** – *Arcyria minuta.* Substrate types: **b** – bark of living tree, **b+** – dead bark, **br** – branches of living tree, **br+** – fallen branches, **gr** – living grasses, **l** – leaves of living tree, **l+** – fallen leaves, **m** – living mosses, **+m** – wooden substrate covered with mosses, **s** – soil, **soc** – association with another myxomycetes species, **w** – dead decaying wood. Other marks: **(mc)** – myxomycete species was grown on the indicated substrate in moist chamber; **?** – substrate species cannot be accurately identified.

DISCUSSION

Among the collected species and infraspecies taxa, 23 were represented by one specimen (so called *singletons*) and 14 by two specimens (*doubletons*). These data allow to extrapolate the total number of myxomycete species, inhabiting forests of Hutsulshchina NNP, by using the bias-corrected Chao1 estimator [3]. The calculation reveals the total number of myxomycete species in Hutsulshchina NNP to be not less than 69.9. As the alternative source of the species richness data we used the Chao2 estimator [3] collection plots. The bias-corrected Chao2 estimator has extrapolated the total number of species as 74.1. Therefore, two independent ways of extrapolation have revealed that the total number of myxomycete species in Hutsulshchina NNP varies approximately from 70 to 75. For the deciduous forest communities of Ukraine such number may be considered as relatively low. For instance, in the Homilsha Forests NNP (Kharkiv region) the 168 myxomycete species were found [14], while in the Slobozhanskiy NNP (Kharkiv region) the estimated number of species is 117 [18]. Such a result may be explained by the fact, that most of the territory of the park is covered by the monodominant beech forest.

Most of the myxomycete species, recorded in the Hutsulshchina NNP (40 taxa of specific and intraspecies level), are *xylophilic* – it means that they prefer to form their fruit bodies on a dead wood [17]. Among them, 28 species were collected only on woody substrates (fallen trunks, stumps, fallen branches, solitary pieces of

decaying wood), while 13 were also found on other substrata.

Second substrate group of myxomycetes, called *corticophylic*, is formed by species, preferring to fructify on the bark of living trees [17]. Among them, 9 species were growing on bark of living trees, including *Pinus sylvestris*, *Betula pendula*, *Larix deciduas* and even such an unusual substrata as *Cerasus avium* (L.) Moench and *Crataegus* sp. Three more species were collected on bark of dead tree trunks: *Leocarpus fragilis* and *Physarum cinereum* on *Quercus robur* and *Stemonitis splendens* – on *Fagus sylvatica*.

The small fallen branches of trees are often considered as a specific type of substrate [12]. In our collection 8 species of myxomycetes were collected on this substrate, but all of them, with no exception, were also found on the dead wood (Table 1).

Less favorable types of substrate, used by myxomycetes, are represented in the Hutsulshchina NNP by fallen leaves, mosses, grass and soil. On the first type of substrate 6 species were collected, including *Craterium* sp., found only on beech leaves. The second type is represented by five species, four of which have formed their fructifications on the xylophylic mosses. In contrast, *Fuligo septica* was recorded twice on epigeous mosses.

Mucilago crustacea was the only species, found on grassy parts of vascular plants. *Arcyria denudata* was the only species, associated with forest soil.

Myxomycetes may accidentally or selectively fructify on vegetative and reproductive structures of true fungi [8, 9]. Among them, *Craterium minutum* was found in Hutsulshchina NNP on stromata of *Hypoxylon* sp. (Ascomycota), *Arcyria denudata* – on stroma of *Rosellinia* sp. (Ascomycota), and *Hemitrichia serpula* was collected on the dead wood, covered by rhizomorphs of *Armillaria australis* (Basidiomycota).

Some species are capable to form fruit bodies on several different types of substrata. For example, *Arcyria cinerea* was recorded on beech and oak wood, on hornbeam and cherry bark and on fallen oak leaves. *Leocarpus fragilis* developed its sporophores on beech and spruce wood and on the leaf debris of unidentified tree. *Stemonitis fusca* was repeatedly noted on beech, oak and spruce wood and once on fallen beech leaves. The same is typical for *Stemonitis fusca* var. *nigrescens*, which is associated with beech wood and fallen leaves. Such a wide range of used substrates is generally typical for myxomycetes, because these organisms do not feed with a plant substrate, but use it only for the formation of fructifications [15].

Species of the substrate-forming plants are associated with different number of myxomycete species. The most favorable for studied organisms in the Hutsulshchina NNP are substrates, originated from *Fagus sylvatica* (23 species; 48 specimens) and *Quercus robur* (13; 17). The same result was shown in forest communities of central [2] and southern [7] Poland. On other deciduous trees and shrubs, like *Betula pendula* Roth, *Carpinus betulus*, *Cerasus avium*, *Corylus avellana* L. and *Crataegus* sp., only 1–4 species of myxomycetes were recorded.

The coniferous trees, known to be the most favorable substrate-forming plants for the myxomycetes in Ukrainian Carpathians [9], in the "Hutsulshchina" have shown much less number of associated myxomycetes species. The substrates of *Picea abies* (8 species; 11 specimens), *Abies alba* (6; 7), *Larix decidua* Mill. (2; 2) and *Pinus sylvestris* (2; 2) appeared to be most rich in myxomycete species. Such a result may be easily explained by fact that communities of these plants occupy insignificant area in the Hutsulshchina NNP.

A significant number of myxomycete species were associated with several species of plants each. For example, *Arcyria cinerea* was found on beech, oak, spruce, hornbeam and cherry, *Ceratiomyxa fruticulosa* – on beech, birch, hornbeam and oak, *Stemonitis axifera* – on beech, oak, fir and spruce, *Lycogala epidendrum* – on beech, fir and spruce, *Metatrichia vesparia* – on beech, oak and fir, *Leocarpus fragilis* and *Stemonitis fusca* – on beech, oak and spruce. Seven species were found on two different species of trees.

All the myxomycete species, collected in the Hutsulshchina NNP, are found on the territory of the park for the first time. However, most of these species are known to be widely distributed on the territory of Ukraine [5]. Their absence in the park checklists can be explained by the fact that the specialized study of the myxomycetes at the Hutsulshchina NNP was never done before. Only three species, *Cribraria violacea*, *Fuligo candida* and *Lycogala terrestre* were found for the first time in the Ukrainian Carpathians.

CONCLUSON

Our study allowed to reveal near 75% of the myxomycete taxonomic diversity in the Hutsulshchina NNP. The predominance of relatively low mountains covered with deciduous forest causes the unusual species composition of myxomycetes of this park, in comparison with other reserves of the Ukrainian Carpathians. The identification of three species, new to the Ukrainian Carpathians, can be partially explained by this. Further studies, carried out in other seasons of the year, are necessary for better understanding of the species composition and ecological preferences of myxomycetes in this national park.

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ПЕРШІ ЗНАХІДКИ МІКСОМІЦЕТІВ У НАЦІОНАЛЬНОМУ ПРИРОДНОМУ ПАРКУ «ГУЦУЛЬЩИНА» (УКРАЇНСЬКІ КАРПАТИ)

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Мікологічні спостереження в букових, дубових, грабових та мішаних лісах Національного природного парку «Гуцульщина», здійснені в 2012, 2013 та 2016 р., дали змогу виявити 53 видові та внутрішньовидові таксони міксоміцетів (48 видів, 4 різновиди та 1 форма), серед яких до рідкісних належать Badhamia panacea, Arcyria oersdtedtii, Calomyxa metallica та Physarum psittacinum. Серед субстратів, на яких міксоміцети сформували плодові тіла, мертва деревина виявилася найбагатшою як щодо кількості плодових тіл міксоміцетів, так і щодо їхнього видового різноманіття. На деревині Fagus sylvatica, Picea abies, Abies alba, Larix deciduas, Pinus sylvestris та деяких інших деревних рослин були виявлені 30 видів міксоміцетів. Також міксоміцети були виявлені на опалому та живому листі дерев, на живих частинах трав'янистих рослинах, мохоподібних, ґрунті та вегетативних або репродуктивних структурах грибів, у тому числі Armillaria australis, Hypoxylon fuscum та Rosellinia sp.

Ключові слова: *біорізноманіття, Eumycetozoa, лісові екосистеми, слизовики, субстратні уподобання*.

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