

Fungi and Straminipilous Organisms Growing in the Narew River and its Chosen Tributaries in NE Poland

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Abstract

The authors analyzed the occurrence of zoosporic fungi and straminipilous organisms in relation to environmental factors in the main bed of the Narew River and its three tributaries (Awissa, Turośnianka, and Kurówka) in two vegetative seasons: spring and autumn. The bait method used to isolate microorganisms helped to distinguish various physiological groups, such as phytosaprotrophs, zoosaprotrophs, and parasites of plants, animals, and fungi. Forty taxons that were determined were more frequently found in the main current of the Narew River than in its tributaries. The major factors limiting the species composition of zoosporic fungi and straminipilous organisms in the sampling locations included hydrochemical parameters, such as CO₂, nitrites, nitrates, ammonium nitrogen, and phosphates that showed negative correlation, pH, and O₂ being positively correlated with the number of the microorganisms studied.

Keywords: stramenopiles, hydrochemical study, rivers

Introduction

Fungi and straminipilous organisms constitute an active group of microorganisms involved in organic matter decomposition in surface waters.

They secrete enzymes that degrade macromolecular complexes. At the initial stages of this degradation, aquatic fungi are more active than bacteria and are an important component of trophic networks in water basins [1]. Some of them play a role of pollution and water purity bio-indicators [2, 3].

Thus, the study objective was to determine and compare fungus species composition in the River Narew and its three short tributaries, namely the Awissa, Turośnianka, and

Kurówka, based on the classical culture method, and to examine the effect of seasonality and hydrochemistry of the waters on fungal growth.

Material and Methods

The Narew River is a right tributary of the Vistula River. On Polish territory, it is 455 km long (total length 484 km). It rises from the swampy area of the Białowieśka Forest in Belarus. The basin of the Upper Narew lies in a relatively poorly industrialized region, being typically agricultural and with huge forests. The Narew River flows through Narwiański National Park and, due to its natural values, is called “the Polish Amazon.” The River belongs to the unique anastomizing river system in Europe.

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Aquatic conditions in the valley of this river result from natural factors and man's actions within the Park, and in the whole Narew River basin [4].

Water for the experiment was collected from bathing sites situated in the upper part of the Narew in Suraż (site I), Uhowo (site II), and Łapy (site III), and its three small tributaries, i.e. Awissa (site IV), Turośnianki (site V), and Kurówka (site VI) (53°2'51"N 22°52'34"E).

Water samples for mycological and physicochemical analyses were collected from the respective sites in two seasons: spring (March, April, May) and autumn (September, October, November) 2011. The samples, 2.0 dm³ volume, were collected for physicochemical analyses approximately 0.20 m under the surface. The hydrochemical analysis of water involved 19 parameters (1 to 0.0001 accuracy) and was performed according to the Standard Methods [5]. Hydrochemical parameters of water samples were determined by means of pH/ion METER-CPI-502, pH-metr-Inolab level 1 and Nanocolor spectrophotometer.

Qualitative investigations of fungi and straminipilous in the main river-bed and in its tributaries were performed with the baiting method used in a mycology laboratory [6, 7]. Three water samples were collected to sterile containers from each of the designated sites in the spring (March, April, May) and autumn months (September, October, November). Next, every month the containers were transported to the mycology laboratory, where the water was poured into 18 beakers (500 ml), to which sterile baits were added. Since substrates used as baits should be highly attractive to aquatic fungi and frequently colonized by them, the material of plant origin (clover, hemp seeds, onion skin, cellophane) and of animal origin (snake skin *Natrix natrix* and freshwater shrimp *Gammarus* sp.) was applied. Water samples from the bathing sites were stored at room temperature (22-24°C). The fungi that appeared were successively examined under an optic microscope every few days, starting from the third day of the culture. Several microscopic preparations were made from each sample. An optic microscope NIKON ECLIPSE 50i at magnification of 100x and 400x was used for observations. At the same time, the respective developmental stages of a fungus were measured using an ocular micrometer. Fungus species were identified taking into consideration vegetative organs – shape and size of the hyphae, organs of asexual reproduction – shape of sporangium and spores, and generative organs – oogonia with oospores and antheridia [7-11]. The systematics of the straminipilous organisms was defined according to Dick [8].

Within six months of research tested 108 samples and each of them drawn from a few to several microscopic preparations.

In statistical analysis, the nonparametric U Mann-Whitney test was used to compare quantitative variables not normally distributed for two groups. Spearman's rank-order correlation coefficient was determined.

The results were considered statistically significant at $p \leq 0.05$. For calculations, Statistica 10.0 (StatSoft) and PASW Statistics 17.0 (Predictive Solutions) were used.

Results

A total of 40 fungal and straminipilous organism species were found in water samples collected for mycological analysis from three select locations of the main bed of the Narew River. They belonged to two kingdoms: Fungi (15) and Stramenopila (25), and four classes: Ascomycota (2), Blastocladiomycota (6), Chytridiomycota (5), Zygomycota (1), and Oomycota (21) (Table 1).

At the significance level $p=0.009$, statistically significant differences were found in the number of fungi noted in the respective sampling locations in the spring season between the Narew River and its tributaries. In the Narew River the values were higher $Me=9$, ($Q_1=8$, $Q_3=10$), being lower in the tributaries $Me=7$, ($Q_1=6$, $Q_3=7$) (Fig. 1). Likewise, in autumn, at $p<0.001$ statistically significant differences were observed in the number of species found in the respective sampling sites between the Narew and its tributaries. In the Narew the values were higher $Me=8$ ($Q_1=8$, $Q_2=10$), and lower in the tributaries $Me=4$ ($Q_1=3$, $Q_2=5$) (Fig. 2).

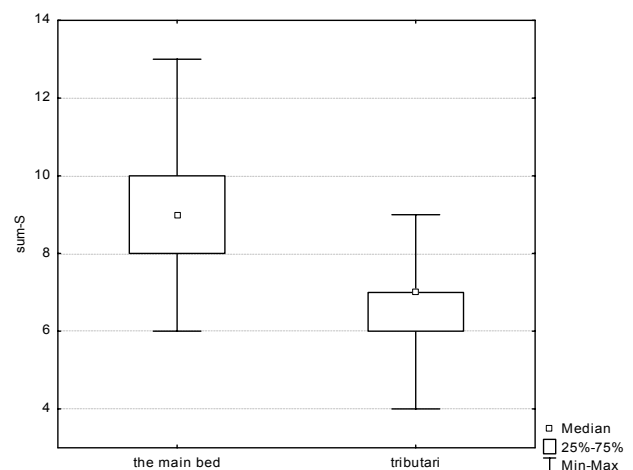


Fig. 1. Relationship between the number of fungi species found in the Narew River and its tributaries in spring.

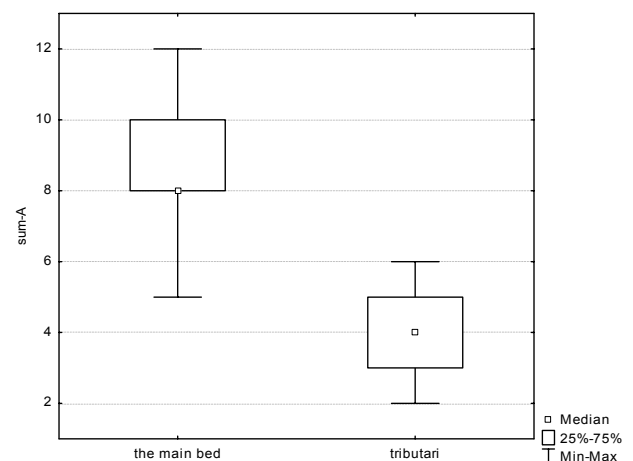


Fig. 2. Relationship between the number of fungi species found in the Narew River and its tributaries in autumn.

Table 1. Fungi and straminipilous organisms found in water from rivers in spring and autumn.

Kingdom, class, order, and species	In water from rivers					
	Narew-main bed (N _b)			Narew-tributaries (N _t)		
	N _b I	N _b II	N _b III	N _t IV	N _t V	N _t VI
Fungi						
Ascomycota						
Pleosporales						
1. <i>Alternaria alternata</i> (Fr.) Keissl	s					
Eurotiales						
2. <i>Aspergillus flavus</i> Link			a		s	
Saccharomycetales						
3. <i>Candida albicans</i> C.P. Robin, Berkhout Castellani	s				s	s
Blastocladiomycota						
Blastocladales						
4. <i>Achlyogeton entophyllum</i> Schenk	s					
5. <i>Blastocladiopsis parva</i> (Whiffen) Sparrow	a	a	s, a	a		s, a
6. <i>Catenaria verrucosa</i> Karling	a		a	a		
7. <i>C. spherocarpa</i> Karling	s				s	
8. <i>Catenophlyctis variabilis</i> (Karling) Karling				s	s	s
9. <i>Micromycopsis cristata</i> Scherffel				a		
Chytridiomycota						
Chytridiales						
10. <i>Chytridium sphaerocarpum</i> Dangeard	s					
Spizellomycetales						
11. <i>Rhizophlyctis rosea</i> (de Bary et Woronin) A. Fisch				s		
Cladochytriales						
12. <i>Nowakowskiella elegans</i> (Nowakowski) Schröter	s,a	a	a			a
13. <i>Polyphagus euglenae</i> Nowakowski			a	s	s, a	
14. <i>Rhizophyidium globosum</i> (Braun) Rabenhorst	s	s	s			s
Zygomycota						
Zoopagales						
15. <i>Zoopagus insidians</i> Sammerstorff	a	a	s,a		s	s
Stramenopila						
Oomycota						
Monoblepharidiales						
16. <i>Monoblepharis hypogyna</i> Perrot	s,a	s,a	s,a			s,a
17. <i>M. macrandra</i> (Lagerheim) Woronin	s,a	s,a	s,a			s,a
Leptomitales						
18. <i>Leptomitus lacteus</i> (Roth) Agardh			s			
Pythiales						
19. <i>Pythium conidioforum</i> Jokl.		a	a		a	
20. <i>Py. debaryanum</i> Hesse	a	s	s			a
21. <i>Py. pulchrum</i> Minden				a		
22. <i>Py. rostratum</i> Butler		s,a	s,a			

Table 1. Continued.

Kingdom, class, order, and species	In water from rivers					
	Narew-main bed (N _k)			Narew-tributaries (N _t)		
	N _b I	N _b II	N _b III	N _t IV	N _t V	N _t VI
Rhipidiales						
23. <i>Rhipidium americanum</i> Thaxter				s		
24. <i>R. interruptum</i> Cornu	a	a				
Saprolegniales						
25. <i>Achlya americana</i> Humphrey	s			s		s
26. <i>Ac. caroliniana</i> Coker		a	s		a	s
27. <i>Ac. debaryana</i> Humphrey	a	s, a	a			a
28. <i>Ac. dubia</i> Coker			s	s, a	s	s
29. <i>Ac. glomerata</i> Coker				s, a		
30. <i>Ac. orion</i> Coker and Couch		s			a	
31. <i>Ac. racemosa</i> Hildebrandt		s	s			s
32. <i>Aphanomyces irregularis</i> Scott	s, a	s, a	s, a	a	a	a
33. <i>Saprolegnia anisospora</i> de Bary		s				
34. <i>S. crustacea</i> Maurizio	s				s	s
35. <i>S. diclina</i> Coker			s			
36. <i>S. litoralis</i> Coker	a	a	a			a
37. <i>S. megasperma</i> Coker	s, a	s, a	a			a
38. <i>S. mixta</i> de Bary	s					
39. <i>S. monoica</i> Pringsheim	s		s	s, a		a
40. <i>S. unisporea</i> (Coker et Couch) M.W. Dick	a					
Total number	15s/13a	11s/13a	12s/14a	8s/8a	9s/5a	13s/10a

a – autumn, s – spring

The diversity of the microorganisms studied was slightly higher in the spring season than in autumn. Such taxons as: *Alternaria alternata*, *Achlyogeton entophyllum*, *Candida albicans*, *Catenaria spherocarpa*, *Karlingia rosea*, *Nowakowskiella makrospora*, *Monoblepharis macrandra*, and *Rhizophyidium globosum* were found only in the spring season. Those identified only in autumn included *Catenaria verrucosa*, *Micromycopsis cristata*, *Monoblepharis hypogyna*, *Pythium marsipium*, *Py. multisporum*, *Py. pulchrum*, and *Rhipidium interruptum* (Table 1).

The most effective baits to isolate micromycetes were clover seeds *Trifolium pratense* L. (23 species) and crustacean *Gammarus pulex* (12 species), whereas the least effective was cellophane (2 species) (Table 2).

The hydrochemical investigations conducted at the respective sites showed higher oxygenation of the main water current and higher chloride content as compared to the tributaries in both study seasons. The main current, however, demonstrated lower content of biogenic compounds (three forms of nitrogen and phosphates) as well as calcium and magnesium ions than in the tributaries (Tables 3 and 4).

Statistical analysis confirmed that the number of taxons in the sampling locations both in the spring and autumn was most affected by such hydrochemical parameters as pH, and the content of oxygen, carbon dioxide, and biogenic compounds.

The pH values in the spring season ranged from 7.13 to 7.64. At $p=0.03$ a strong positive correlation was noted between the number of fungi in spring and pH. In autumn, the parameter had similar values (7.14-7.74). At $p=0.006$ a strong positive correlation was observed between the sum of fungi in autumn and pH.

Oxygen content ranged from 12.68 mg·L⁻¹ to 15.69 mg·L⁻¹, showing a strong positive correlation with the sum of the taxons found ($p=0.0004$). In autumn, oxygen content was slightly lower (10.67 mg·L⁻¹-15.67 mg·L⁻¹), but also presented a strong positive correlation with the number of taxons noted ($p=0.0001$).

The results indicate that the amount of CO₂ in the water samples collected for analysis ranged from 11.0 mg·L⁻¹ to 13.0 mg·L⁻¹ in the spring season and showed a very strong negative correlation with the number of fungus-like organisms found ($p=0.00$). In autumn, the level of CO₂ was sim-

Table 2. Occurrence of aquatic fungi and straminipilous organisms on the investigated baits.

Type of bait	Fungi and fungus-like organisms (see Table 1)	Total number of species
Freshwater shrimp <i>Gammarus pulex</i> L.	4, 7, 11, 13, 28, 30, 33, 35, 36, 38, 39, 40	12
Grass snake skin <i>Natrix natrix</i> L.	5, 8, 28, 32	4
Clover seeds <i>Trifolium pratense</i> L.	3, 5, 6, 7, 11, 12, 14, 16, 17, 19, 20, 22, 25, 26, 27, 29, 30, 32, 33, 34, 36, 38, 39	23
Hemp seeds <i>Cannabis sativa</i> L.	14, 20, 22, 23, 25, 27, 31	7
Onion skin <i>Allium cepa</i> L.	11, 13, 17, 20, 25, 26, 31	7
Cellophane	8, 32	2

Table 3. Physicochemical composition of water samples from different rivers in spring (n=3).

Specification	In water from rivers					
	I	II	III	IV	V	VI
Temperature (°C)	18	16	19	18	19	20
pH	7.60	7.64	7.64	7.10	7.21	7.13
O ₂ (mg·L ⁻¹)	15.69	15.45	15.68	12.68	12.69	13.69
BOD ₅ (mg·L ⁻¹)	7.81	7.22	9.23	9.23	7.22	7.22
COD (mg·L ⁻¹)	9.02	7.00	9.05	9.05	14.64	14.64
CO ₂ (mg·L ⁻¹)	11.0	11.3	11.0	13.0	12.0	12.0
Alkalinity in						
CaCO ₃ (mval·L ⁻¹)	4.4	4.1	4.5	4.5	4.3	4.2
N-NH ₃ (mg·L ⁻¹)	0.770	0.770	0.970	1.970	1.12	1.54
N-NO ₂ (mg·L ⁻¹)	0.0236	0.0262	0.0236	0.0336	0.0336	0.0420
N-NO ₃ (mg·L ⁻¹)	0.220	0.460	0.460	0.660	0.660	0.660
P-PO ₄ (mg·L ⁻¹)	0.520	0.760	0.560	0.860	0.700	0.730
Sulphates (mg·L ⁻¹)	0.830	19.33	20.15	20.15	38.26	38.26
Chlorides (mg·L ⁻¹)	65	58	54	54	53	53
Total hardness						
Ca (mg·L ⁻¹)	72.00	72.00	69.84	101.40	109.6	108.8
Mg (mg·L ⁻¹)	3.44	9.03	8.32	10.32	10.74	10.74
Fe (mg·L ⁻¹)	0.90	0.40	0.95	1.90	2.40	2.40
Dry residue (mg·L ⁻¹)	294	256	256	272	295	295
Dissolved solids (mg·L ⁻¹)	281	247	240	256	283	283
Suspended solids (mg·L ⁻¹)	13	9	16	16	12	12

ilar (10.0 mg·L⁻¹-13.0 mg·L⁻¹) and correlated strongly negatively with the number of fungal species identified ($p=0.00$).

Water samples collected at the sampling sites showed a relatively heavy load of biogenic compounds. In spring, the content of nitrogen forms was within the range 0.770-1.970 mg·L⁻¹ for N-NH₃, the amount of N-NO₂ was lower

(0.0236-0.0320 mg·L⁻¹), and so was the content of N-NO₃ (0.220-0.760 mg·L⁻¹). At that time water samples contained from 0.520 to 0.860 mg·L⁻¹ phosphates. In autumn, the levels of the three forms of nitrogen and phosphates were close to those noted in the spring season. At $p=0.00$, we observed a very strong negative correlation between the sum of fungal species and the content of such biogenic compounds as

Table 4. Physicochemical composition of water samples from different rivers in autumn (n=3).

Specification	In water from rivers					
	I	II	III	IV	V	VI
Temperature (°C)	6	8	8	10	10	11
pH	7.60	7.74	7.61	7.10	7.22	7.14
O ₂ (mg·L ⁻¹)	15.67	15.43	15.66	11.66	10.67	12.68
BOD ₅ (mg·L ⁻¹)	7.81	7.21	9.22	8.22	8.22	8.22
COD (mg·L ⁻¹)	9.03	7.00	9.04	9.06	14.63	14.64
CO ₂ (mg·L ⁻¹)	13.0	12.1	10.0	11.0	11.0	12.0
Alkalinity in						
CaCO ₃ (mval·L ⁻¹)	4.4	4.0	4.5	4.5	4.3	4.2
N-NH ₃ (mg·L ⁻¹)	0.790	0.770	0.970	1.870	1.220	1.640
N-NO ₂ (mg·L ⁻¹)	0.0236	0.0272	0.0336	0.0536	0.0430	0.0520
N-NO ₃ (mg·L ⁻¹)	0.220	0.460	0.430	0.750	0.680	0.790
P-PO ₄ (mg·L ⁻¹)	0.621	0.763	0.560	0.560	0.800	0.730
Sulphates (mg·L ⁻¹)	26.32	19.33	20.15	20.15	38.26	38.26
Chlorides (mg·L ⁻¹)	65	58	54	54	53	53
Total hardness						
Ca (mg·L ⁻¹)	72.00	72.00	69.84	100.30	107.50	104.70
Mg (mg·L ⁻¹)	3.44	9.03	8.32	10.25	10.16	10.22
Fe (mg·L ⁻¹)	0.90	0.40	0.95	1.80	2.30	2.20
Dry residue (mg·L ⁻¹)	292	266	258	278	298	299
Dissolved solids (mg·L ⁻¹)	279	247	240	266	286	286
Suspended solids (mg·L ⁻¹)	13	19	18	12	12	13

N-NH₃, N-NO₂, and P-PO₄, as well as a strong negative correlation between the amount of NNO₃ and a number of fungus-like organisms (p=0.01) found in selected sampling sites in the spring and autumn seasons.

Discussion

Fungi constitute a common and important element of almost every trophic level of any aquatic ecosystem. Reduced fungal species diversity may suggest progressing anthropoppression and contributes to ecological imbalance [1, 2, 12, 13].

The aquatic environment of rivers is characterized by the presence of autochthonic and allochthonic organic matter, decomposed by e.g. aquatic fungi [14, 15]. Besides bacteria, they are an important component of the microbiological loop. In the trophic network of rivers, some taxons function not only as saprotrophs but also as parasites of aquatic algae, plants, and animals, contributing to the maintenance of the ecological balance of river ecosystems [16, 17].

At the study sites of the River Narew and its tributaries, the most common were the taxons described by other researchers as phytosaprotrophs [18]. They included *Achlya americana*, *Ac. caroliniana*, *Ac. glomerata*, *Ac. racemosa*, *Monoblepharis macrandra*, *Nowakowskiella elegans*, genus *Pythium*, and *Rhipidium americanum*, being among the very few microorganisms that secrete polymeric substances such as hemicellulose, cellulose, starch, pectin, and lignin contained in the organic matter of plant origin [19]. A study conducted by Marano et al. [1] confirms that zoosporic fungal species of the genus *Nowakowskiella* and *Pythium* substantially decompose fallen leaves in surface waters.

In our study, *Achlya dubia*, *Saprolegnia megasperma*, and *S. anisospora* were found to grow on animal baits (*Gammarus pulex*) (Table 2). These taxons produce zoospores which through chemotactic activity search for a suitable substrate and in a short time cause its decomposition [20, 21].

We also identified a predacious fungus – *Zoophagus insidians*, which generates hyphae with short processes that

catch invertebrates such as amoebas, rotifers, nematodes and small arthropods (mites). The hyphae penetrate and destroy the victim, functioning to some extent as endoparasites [20, 21].

We noted such taxons as *Polyphagus euglenae* and *Rhizophyidium globosum*, belonging to the class Chytridiomycota. A study conducted by Rasconi et al. [22] indicates that zoosporic fungi of the genus Chytridiomycetes cause approximately 20% of all infections in phytoplankton. Our observations show that these species grow on dead plant and animal substrates (Table 2).

On plant baits we observed *Achlya racemosa*, *Pythium debaryanum*, and *Py. conidiophorum* (Table 2), which according to Sparrow [20] belong to plant parasites. The life cycle of parasitic fungi is nearly identical to that of saprotrophs, except that host cells are still alive. However, with time, parasitic fungi attenuate the host cells and in some cases cause their death. Also known are fungi that parasite on various living organisms, including fungi such as *Achlya*, – *Chytridium sphaerocarpum* detected in the current study [20, 23].

We also isolated some taxons that had been earlier described as fish parasites. These were: *Achlya debaryana*, *A. orion*, *Saprolegnia diclina*, *S. mixta*, and *S. monica*. A study conducted by Czczuga et al. on fish eggs revealed that *Achlya debaryana* and *A. dubia* can cause fish mycosis, which markedly reduces the fish population [24, 25]. The biocenotic interactions with the involvement of fungi described from the sampling sites in the Narew River waters and its tributaries were associated with the type of substrate. This has been confirmed by other authors [26-32].

We found the presence of *Leptomitius lacteus*, the species characteristic of polluted waters, in the main bed of the Narew River. Riethmüller et al. [33], investigating this fungus in various types of waters in Germany, reported that its growth preference is in rivers rather than stagnant waters.

Species diversity of the microorganisms studied also depends on the physicochemical properties of water. The soluble oxygen concentration has a substantial effect. We found a greater number of microbiota species in the waters of the main bed of the Narew River, which were better oxygenated than in its tributaries, which also has been confirmed by other authors [31, 33]. Another factor limiting the composition of aquatic fungi and straminipilous organisms in the waters studied was the content of biogenic compounds. Their higher content stimulated the growth of fungal biota to a certain limit. However, the excess over this limit inhibited growth [15]. Probably because of that the Narew River waters showed much greater species diversity than its tributaries, which had a more substantial load of biogenic compounds (Table 1). The results seem to be confirmed by those reported by Czczuga et al. [15], who noted a negative correlation between the number of fungi species and the organic matter in Narew water and its tributaries.

The current mycological study carried out in two seasons (spring and autumn) showed a greater diversity of the microorganisms in the main current of the Narew than in its tributaries. It is commonly known that the actual role of zoosporic fungi and straminipilous organisms in hydro-

cenoses depends on the whole range of ecological interactions in a respective water basin. In small reservoirs, such as tributaries, their own primary production may be smaller than allochthonic biomass, and is decomposed by non-aquatic fungi migrating to the reservoir together with allochthonic material [7].

Conclusions

The number of taxons found at sampling locations both in spring and autumn depended largely on such hydrochemical parameters as CO₂, nitrites, nitrates, ammonium nitrogen, and phosphates that showed a negative correlation, and pH and O₂ correlating positively with the number of the microorganisms studied.

The entire range of ecological interactions in the main bed of the Narew River creates more favorable conditions for the development of zoosporic fungi than its tributaries.

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