

Original Research

# Rare Aquatic Fungus–Like Organisms of the Order Leptomitales (Chromista) in Waters of North-Eastern Poland

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## Abstract

The authors described and illustrated 8 rare aquatic fungus–like organisms of the order Leptomitales (Chromista), which were isolated from the water of nine water bodies of different trophy of north-eastern Poland: *Apodachlya punctata*, *Apodachlyella completa*, *Araiospora spinosa*, *Mindeniella spinospora*, *Nellymyces megaceros*, *Rhipidium europaeum*, *Sapromyces androgynus*, and *Sapromyces elongatus*.

**Keywords:** Chromista, Leptomitales, rare aquatic fungus–like organisms, distribution in north-eastern Poland

## Introduction

The order Leptomitales contains 9 genera of fungus–like organisms, including several species which all have thallus built up of the thick-wall basal cell, layered or ramified at the apex [1]. *Leptomitus lacteus*, a known sewage species and *Aqualinderella fermentans*, a very rare fungus–like organism usually encountered in the waters of subtropical or tropical countries belong to this order [2, 3]. These are aquatic phytosaprophytes, more seldom zoosaprophytes, growing on branches or fruits in water.

When studying aquatic fungus–like organisms growing on fruits of various plant species in the water of various reservoirs we observed the growth of thalli of species belonging to the Leptomitales, which are regarded as rare [1, 4-6] and of which most have been never before encountered in Polish waters.

## Material and Methods

The water for experiments was collected from nine different water bodies:

- Spring Cypisek, localized in the north part of Białystok. Limnokrenic type, width 0.41 m, depth 0.17 m, discharge 0.6 l/s. The spring is surrounded by single pine trees, around the spring are cultivated fields. The bed is covered with sand.
- Spring Jaroszówka, localized in the north part of Białystok. Limnokrenic type, width 0.65 m, depth 0.12 m, discharge 2.4 l/s, surrounding without trees. The spring is surrounded by cultivated fields. The bed is covered with sand.
- River Biała, length 9.8 km, a left–bank tributary of the Supraśl river flowing through Białystok City. The samples were collected in the upper course of the Biała river, the water was the least polluted.
- River Supraśl, right-bank tributary of the middle part of the Narew river flowing through the Knyszyńska Forest. Length 106.6 km. The samples were collected from the site above the municipal swimming pool at

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- the sluice of an arm of the Supraśl river flowing just through the town Supraśl. The sampling site is surrounded by meadows. The bed is muddy.
- Pond Akcent, area 0.45 ha, max. depth 1.5 m, localized in the Municipal Park, is habitat of wild ducks and breeding swans. The sampling site is surrounded by single trees. The bed is muddy.
  - Pond Dojlidy, localized near Białystok: Area 34.2 ha, max. depth 2.85 m, its south shore bordered by coniferous woods and its western part with the town of Białystok. The samples were collected from the western end of this pond, which is used by -the inhabitants of the town as a beach.
  - Pond Fosa, localized in the Palace Park of Białystok. Area 2.5 ha, max. depth 1.75 m. –Pond with wild ducks and breeding swans as well as crucian carp and tench bred, – used by anglers. The pond is surrounded by meadows with linden (*Tilia cordata* Mill.) and elm (*Ulmus carpiniifolia* Gled.).
  - Lake Komosa, localized in the Knyszyńska Forest.

Area 12.1 ha, max. depth 2.25 m. the lake is surrounded by extensive coniferous woods.

- Lake Necko, area 518 ha, max. depth 25 m; the northern shores of the lake adjoin Augustów Forest while the south-western shores border the town of Augustów. For this reason most of the municipal and industrial wastes of the town are drained into the lake. The sampling site was on the eastern side of the lake next to the Polish Tourist Country Lovers' Association Centre; the shore is sandy for 1.5 m.

Geographical localized of lake Necko – 53°52'N, 22°58'E and other of the water bodies 53°02'N, 23°05'E.

Samples of water were collected in summer (August) 2000 for hydrochemical analysis and the order to determine fungal species. Samples of water were collected from the rivers, the ponds and the lakes with 2 l Ruttner sampler, approximately 2 m from the shore and 50 cm under the surface. In the spring, samples were taken from surface. Nineteen water parameters were determined in each water body (Table 1) according to the generally accepted methods [7].

Table 1. Chemical properties of water in particular water bodies (n=5).

Specyfification	Spring		River		Pond			Lake	
	Cypisek	Jaroszówka	Biała	Supraśl	Akcent	Dojlidy	Fosa	Komosa	Necko
Temperature (°C)	11.0	12.0	18.5	18.0	17.5	19.5	17.0	11.5	10.5
pH	7.78	7.86	7.61	7.88	7.77	7.42	7.61	7.93	7.53
O <sub>2</sub> (mg dm <sup>3</sup> )	8.20	9.40	9.60	9.20	2.20	7.80	3.65	12.16	14.86
BOD <sub>5</sub> (mg dm <sup>3</sup> )	3.20	5.60	3.60	5.80	1.80	2.50	0.50	4.18	3.91
COD (mg dm <sup>3</sup> )	4.30	5.58	10.98	7.84	12.54	12.35	22.97	3.12	5.15
CO <sub>2</sub> (mg dm <sup>3</sup> )	15.40	12.20	19.80	11.95	24.20	11.15	18.80	13.20	12.12
Alkalinity in CaCO <sub>3</sub> (mval dm <sup>3</sup> )	5.20	2.30	5.00	5.10	7.40	3.70	4.50	5.40	3.85
N-NH <sub>3</sub> (mg dm <sup>3</sup> )	1.280	0.290	0.590	0.250	3.530	0.280	0.500	0.235	0.320
N-NO <sub>2</sub> (mg dm <sup>3</sup> )	0.014	0.020	0.042	0.005	0.012	0.005	0.007	0.021	0.008
N-NO <sub>3</sub> (mg dm <sup>3</sup> )	0.080	0.010	0.050	0.070	0.090	0.060	0.900	0.040	0.105
P-PO <sub>4</sub> (mg dm <sup>3</sup> )	1.530	2.680	2.160	1.530	12.720	0.120	1.670	0.105	0.080
Sulphates (mg dm <sup>3</sup> )	85.54	49.33	32.50	20.16	89.27	30.86	69.08	58.42	21.85
Chlorides (mg dm <sup>3</sup> )	38.00	15.00	42.00	36.05	49.15	43.05	52.15	25.05	30.55
Total hardness Ca (mg dm <sup>3</sup> )	105.80	110.16	80.64	72.25	137.52	60.48	56.16	113.04	66.96
Total hardness Mg (mg dm <sup>3</sup> )	21.07	15.19	27.52	15.91	21.93	11.61	11.50	17.63	21.82
Fe (mg dm <sup>3</sup> )	0.700	0.250	0.050	0.650	0.525	0.350	0.450	0.258	0.160
Dry residue (mg dm <sup>3</sup> )	473.0	465.0	369.0	242.0	640.0	280.0	444.0	425.0	218.7
Dissolved solids (mg dm <sup>3</sup> )	461.0	354.0	360.0	222.0	606.0	261.0	433.0	398.0	181.9
Suspended solids (mg dm <sup>3</sup> )	12.0	111.0	9.0	20.0	34.0	19.0	11.0	27.0	36.7

Water for analysis was placed in 3 containers for each water body. Water from each water body was transferred to three 1.0 litre vessels and added to the substrata as bait (see Table 2) and placed in the laboratory at ambient temperature. Fruits and seeds were used as bait during exposure in the laboratory. The fruits and seed baits before adding to waters were rewashed three times with distilled water. The methods of the experiments are described in detail by Fuller and Jaworski [8].

The following procedures for the determination of the presence of fungus-like organisms on the baits were employed: during one month of exposure the baits were examined under a light microscope (once or twice a week) and mycelium of aquatic fungi-like organisms growing on the baits was recorded. Identification of these fungus-like organisms was based on morphology and biometric data of thallus, zoosporangium, zoospores, oogonium, oospore, and basal cells which are different for particular species of the order Leptomitales [1, 9-11].

The systematic of Leptomitales species was used according to McLaughlin et al. [11].

## Results and Discussion

The aquatic reservoirs involved in the study on the occurrence of the order Leptomitales fungi differ in morphology and chemical parameters of water (Table 1). The content of nutrients (all forms of nitrogen and phosphates) was the highest in pond Akcent and the lowest in lakes Komosa and Necko. The concentrations of sulphates and chlorides as inorganic pollution indices differed in the respective reservoirs. Pond Akcent was the most abundant in sulphates (89.27), the Supraśl River being the poorest (20.16 mg/dm<sup>3</sup>). The highest content of chlorides was noted in Fosa pond (52.12), the lowest in Jaroszkówka spring (15.0 mg/dm<sup>3</sup>).

In the water of the aquatic reservoirs 8 species of the order Leptomitales were identified, most being new to Polish waters. These are (Table 2, Fig. 1):

- *Apodachlya punctata* Minden – was found to grow on the fruits of *Juniperus communis* L. in autumn in pond Fosa. This species was first described by Minden [12] and was later encountered in Japanese waters [13].

Table 2. Aquatic fungi found on particular fruits in particular water bodies and seasons.

Fungus	Substrata (fruits)	Water bodies	Season
<i>Apodachlya punctata</i> Minden	<i>Juniperus communis</i> L.	pond Fosa	autumn
<i>Apodachlyella completa</i> (Humph.) Indoh	<i>Juniperus phoenicea</i> Nakai	pond Fosa	spring
<i>Araiospora spinosa</i> (Cornu) Thaxter	<i>Berberis thunbergii</i> Thunb.	spring Cypisek	autumn
	<i>Crataegus azarolus</i> Borkh	river Supraśl	spring
	<i>Limonium multifforme</i> Pignatti	river Supraśl	spring
<i>Mindeniella spinospora</i> Kausse	<i>Berberis vulgaris</i> L.	river Biała	autumn
<i>Nellymyces megaceros</i> Batko	<i>Arctostaphylos uva-ursi</i> L.	river Biała	autumn
	<i>Berberis aggregata</i> Schn.	pond Fosa, lake Komosa	autumn
	<i>Berberis vulgaris</i> L.	lake Necko	spring
	<i>Cotoneaster divaricanta</i> Redh. et Wils.	river Biała	spring
	<i>Sorbus intermedia</i> Pers.	river Biała	spring
<i>Rhipidium europaeum</i> (Cornu) Minden	<i>Juniperus virginiana</i> L.	pond Dojlidy	spring
	<i>Leonicera periclymenum</i> Hantz	pond Akcent	spring
	<i>Prunus mahaleb</i> L.	river Biała	spring
	<i>Rosa spinosissima</i> L.	river Biała	autumn
	<i>Sorbus aucuparia</i> L.	lake Komosa	spring
	<i>Vitis labrusca</i> L.	spring Jaroszkówka	spring
	<i>Vitis vinifera silvestris</i> (Gmel) Willd	river Supraśl	spring
<i>Sapromyces androgynus</i> Thaxter	<i>Juniperus procumbens</i> Miq.	pond Dojlidy	spring
	<i>Juniperus virginiana</i> L.	pond Dojlidy	spring
<i>Sapromyces elongatus</i> (Cornu) Coker	<i>Vitis vinifera apiifolia</i> Lodn	lake Komosa	autumn

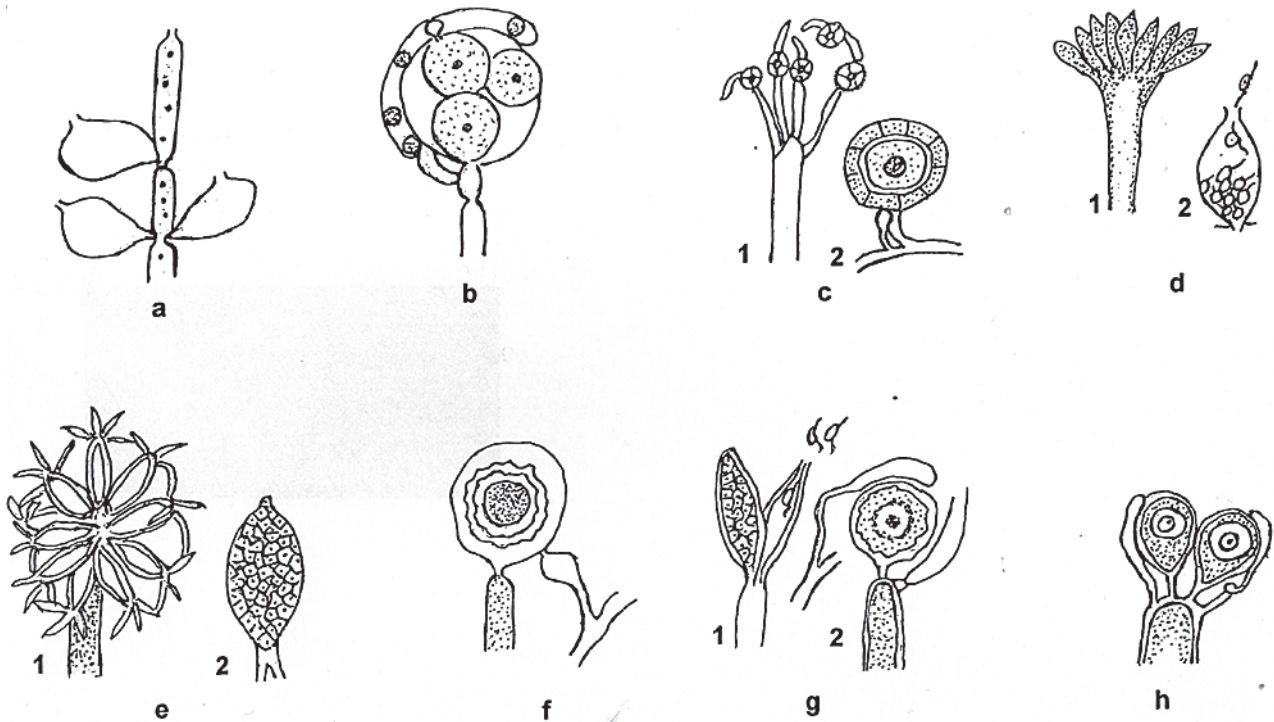


Fig. 1. Fungus – like organisms found in waters of north-eastern Poland

a – *Apodachlya punctata*: part of thallus and sporangia with an exit canals; b – *Apodachlyella completa*: oogonium (23-50µm) with antheridium (with 5 spermatozoidal cells); c – *Araiopora spinosa*: 1 – top of thallus, 2 – gametangium; d – *Mindeniella spinospora*: 1 – top of thallus, 2 – sporangium with discharging of zoospores; e – *Nellymyces megaceros*: 1 – top of thallus and sporangia with the spines, 2 – sporangium without the spines; f – *Rhipidium europaeum*: oogonium with antheridium; g – *Sapromyces elongatus*: 1 – sporangia, 2 – oogonium with antheridium; h – *Sapromyces androgynus*: oogonia with antheridia

- *Apodachlyella completa* (Humphrey) Indoh – described by Humphrey [14], then observed in Japanese waters [15]. We observed its growth in spring in pond Fosa on the fruits of *Juniperus phoenicea* Nakai.
- *Araiopora spinosa* (Cornu) Thaxter – was observed on the fruits of *Berberis thunbergii* Thunberg, *Crataegus azarolus* Borkh and *Limonium multifforme* Pignatti in spring and autumn in Cypisek spring and Supraśl River. It was already described in the 19<sup>th</sup> century [16], its systematics were investigated by Thaxter [17]. In the 1930s, another species of this genus, *Araiopora streptandra* [18], which is more frequently encountered in the aquatic environment, was described [19, 20].
- *Mindeniella spinospora* Kanouse – was first described in the State of Michigan [21]. In our study, it was found to grow on the fruits of *Berberis vulgaris* L. only in the Biała River. Its biology was studied by Sparrow and Cutter [22].
- *Nellymyces megaceros* Batko – this species was first reported from Polish waters [23], where it was found to grow on branches fallen to water. Batko [24] also described another fungus *Rozellopsis uliginosa* Batko parasiting on the mycelium of *Nellymyces megaceros*. In our study, *Nellymyces megaceros* was found to grow in the Biała River, Fosa pond and lakes Komosa

and Necko on the fruits of such plants as *Arcostaphylos uva-ursi* L., *Berberis aggregata* Schn., *Berberis vulgaris* L., *Cotoneaster divaricanta* Rehd. et Wils. and *Sorbus intermedia* Pers.

- *Rhipidium europaeum* (Cornu) Minden – was found to grow in spring and autumn in the water of Jaroszówka spring, the Biała and Supraśl Rivers, ponds Akcent and Dojlidy, and Lake Komosa on the fruits of *Juniperus virginiana* L., *Leonicera periclymenum* Hantz, *Prunus mahaleb* L., *Rosa spinosissima* L., *Sorbus aucuparia* L., *Vitis labrusca* L. and *Vitis vinifera silvestris* (Gmel) Willd.
- *Sapromyces androgynus* Thaxter – in our study it was found only in spring months in pond Dojlidy on the fruits of *Juniperus procumbens* Miq. and *Juniperus virginiana* L. It was described by Thaxter [17]. In Europe this fungus-like organism was first identified by Cejpa [25].
- *Sapromyces elongatus* (Cornu) Coker – was first described in the second half of the 19<sup>th</sup> century [16]. We found this fungus-like organism in Lake Komosa only on the fruits of *Vitis vinifera apiifolia* Lodn. This species has already been identified in the Kampinoski National Park near Warsaw [23].

8 Leptomitales species studied, five were present in the water of only one reservoir, *Araiopora spinosa* was

identified in two, *Nellymyces megaceros* in four and *Rhipidium europaeum* in as many as six out of the eight examined. This would indicate that *Nellymyces megaceros* and *Rhipidium europaeum* show a wide range of tolerance of the respective environmental factors, as the level of hydrochemical parameters in the reservoirs where these two species were found was high. For instance, phosphate content ranged from 0.050 (lake Necko) to 12.720 mg/dm<sup>3</sup> (pond Akcent). The water of the river Biała, Fosa pond and Lake Komosa, with the abundance of biogenes indicating the eutrophic type, contained 3 species of the fungus-like organisms each. This would suggest that this type of water favours the Leptomitales representatives most. In pond Akcent, showing a polytrophic nature, only one species *Rhipidium europaeum* was detected.

The chemical composition of water in every reservoir is a dynamic phenomenon, and environmental factors in significant ways influence fungus-like organism choice compositions, they undergo changes also. Therefore the water fungus-like organisms choice composition in data the reservoir in this and the representatives Leptomitales it is the product resultant of mutual reports among individual environmental factors.

The order Leptomitales also includes such two species as *Leptomitus lacteus* and *Aqualinderella fermentans*, whose interesting biology is described in mycological monographs. *Leptomitus lacteus* is called a sewage fungus-like organism as it has been reported from waters heavily polluted with municipal effluents. It also grows in water containing sugar industry and brewing sewage and is considered to be a nitrogenophilic species [1]. According to Batko [1] it is one of the few aquatic fungi which in natural conditions are able to grow in liquid substrates with no contact with a solid substrate. Further studies have confirmed that these fungus-like organisms frequently grow on various substrates of animal origin, e.g. crustacean carapaces and insect wings [26, 27], dead leeches [28], spawn of amphibians [29] and on eggs of many fish species [30] and fish body [31, 32], as well as on the hair of many mammalian species found in water [33], and on feathers of birds [34].

*Aqualinderella fermentans* is an anaerobe which tolerates oxygen but requires a relatively high carbon dioxide concentration. It was supposed to occur in warm waters of the tropical and subtropical zones, where it was found to grow on juicy fruits [1, 3, 8]. In recent studies [2, 3, 35] this fungus-like organism has been identified in European waters. It has also been established that *Aqualinderella fermentans* may not only grow on juicy fruits fallen into water but also on dry fruits and seeds of certain plants [3].

All the eight species of the order Leptomitales involved in the present study and *Aqualinderella fermentans* have been sporadically identified at various latitudes and are therefore regarded as rare species. It seems that such rare occurrences should be explained by stenotypic environmental requirements. Both zoosporic and conidial species are cosmopolitan [1, 2, 5, 36-38] and thus the lim-

ited occurrence of certain fungi may be due to ecological and environmental factors and not geographous. Spores of certain fungus species carried by air motion, currents [39] or waterfowl during spring and autumn passages [40] begin to develop when they encounter favourable conditions. Environmental requirements of rare species usually refer to one environmental factor. For instance, *Aqualinderella fermentans* grows in the carbon dioxide-rich aquatic environment. Perhaps for the growth of other rare aquatic fungus-like organisms, proportional correlations between the respective physico-chemical factors are required. However, as the physico-chemical parameters are not permanent and are subject to constant dynamic changes, the occurrence of a particular rare fungus in one year does not mean its presence in the following years.

The literature of the subject mentions only *Mindeniella spinospora* and the two species of the genus *Sapromyces* to grow on fruits [5, 24]. The other five species included in our present study are described to grow on branches fallen to water [41]. The present research also supplements our knowledge on their substrates.

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