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Letter to Editor

Zoosporic Fungi Growing on Leeches (*Hirudinea*)

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Abstract

The authors investigated zoosporic fungi developing on 17 species of leeches (*Hirudinea*) in six bodies of water of various trophic states. A total of 101 zoosporic fungus species were noted, with predominance by the *Saprolegniales* (65) and *Peronosporales* (21). Of 101 fungus species found, 38 have been fish parasites or necrotrophs. Four fungus species were recorded for the first time in Polish waters.

Keywords: zoosporic fungi, leeches, *Hirudinea*, hydrochemical study

Introduction

Among numerous aquatic zoosporic fungus species involved in the mineralization of the organic matter some lead a parasitic mode of life on plant or animal organisms. Animal parasites constitute a large group and are found on the spawn, fish fry or grown up fish species, including those economically valuable [1,2]. As shown by our studies in recent years, these dangerous to fish fungi also grow on specimens of many invertebrates that inhibit the respective water reservoirs [3], and are thus vectors of mycotic infections for fish.

The aim of the present study was to investigate which of the zoosporic fungi which are fish parasities can grow on leeches in Polish waters.

Material and Methods

Seventeen leech species collected in water bodies of northeastern Poland *(Trocheta bykowskii* from Barani stream in Magurski National Park, Low Beskid) were subjected to investigation (Table 2).

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

(I) Cypisek Spring, limnokrenic type; width 0.41 m, depth 0.17m, discharge 0.6 1/s, is in the southern part of Knyszyńska Forest.

(II) Jaroszówka Spring, limnokrenic type, width 0.65 m, depth 0.12 m, discharge 2.4 1/s, is in the northern part of Białystok, without trees.

(III) Supraśl River, length 106.6 km, is the right-bank tributary of the middle part of the Narew River, flowing through Knyszyńska Forest.

(IV) Akcent Pond, 0.45 ha, max. depth 1.50 m, contains wild ducks and breeding swans.

(V) Fosa Pond, 2.5 ha, max. depth 1.75 m, is in the Palace Park, and contains wild ducks and breeding swans, as well as crucian carp and tench bred for anglers.

(VI) Komosa Lake, 12.1 ha, max. depth 2.25m, is surrounded by the extensive coniferous woods of Knyszyńska Forest.

Nineteen parameters of these samples were determined (Table 1) according to the methods of Greenberg et al. [4].

To determine the presence of aquatic fungi on the leeches, the following procedure was employed: 10-15 small fragments of each species of leech were each transfered to two samples for each water basin in a 1-litre vessel (altogether twelve vessels for each species) and placed in a glass tank (50 x 75 x 75 cm) at ambient temperature in the laboratory. Some pieces of leeches from each vessel were observed under a microscope and the mycelium

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(zoosporic, oogonia, antheridia and for *Saprolegnia parasitica* secondary cysts) of aquatic fungi growing on the leeches was recorded. The methods are described in detail by Fuller and Jaworski [5]. The pieces of the various leech species were observed under a microscope once a week, and each experiment lasted three weeks. To identify the fungi, keys by Johnson [6], Sparrow [7], Seymour [8], Batko [9], Karling [10], Dick [11] and Pystina [12] were used.

Results

The water of six reservoirs used for experiments differed in the respective hydrochemical parameters (Table 1). Oxidability as an index of the organic matter content was the lowest in Cypisek Spring and the highest in Fosa Pond. The water in Akcent Pond was the richest in ammonium nitrogen and phosphates, while in Fosa in nitrite nitrogen. The highest content of phosphates was found in Akcent and of chlorides in Fosa. The lowest indices of sulphates and chlorides were revealed in Jaroszówka Spring.

One hundred and one aquatic zoosporic fungus species (Table 3, Fig. 1) were isolated from 17 species of leeches (Table 2). The most fungi were found to grow on *Trocheta bykowskii* specimens (37), the fewest on *Erpobdella octoculata* (12). The most common were *Saprolegnia ferax* and *Saprolegnia parasitica,* found on specimens of all leeches examined, and *Dictyuchus sterile,* observed on 13 species. Of 101 fungus species found, 38 have been fish parasites or necrotrophs. The most fungi developed on leeches in Cypisek (56), the fewest in Akcent and Komosa Lake (38 and 39). Some fungus species were observed on leeches only in one of the six reservoirs included in the study (Table 4). Nine such species were isolated from Jaroszówka, while only 4 from the Supraśl River.

Discussion

The present study has revealed a great number of aquatic fungus species known so far as parasites or necrotrophs of various fish species [13,14] on the specimens of leech species examined.

Worth noting is the fact that two such dangerous fish parasites as *Saprolegnia ferax* and *Saprolegnia parasitica* were found to grow on all the leech species examined. *Saprolegnia ferax* has been known from literature as a fish parasite since the second half of the 19 th century, when first on the British Isles and then in European rivers it caused mass death of the Atlantic salmon *Salmon salar* [15]. Since

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

Specification	Cypisek Spring	Jaroszówka Spring	Supraśl River	Akcent Pond	Fosa Pond	Komosa Lake
Temperature (° C)	11.0	12.0	18.0	17.5	17.0	19.5
pH	7.78	7.86	7.88	7.77	7.61	7.42
O_2 (mg L^{-1})	8.20	9.40	9.20	2.20	3.65	7.80
BOD_5 (mg L^{-1})	3.20	5.60	5.80	1.80	0.50	2.50
COD (mg L^{-1})	4.30	5.58	7.84	12.54	22.97	12.35
CO_2 (mg L^{-1})	15.40	12.20	11.95	24.20	18.80	11.15
Alkalinity in $CaCO_3$ (mval L^{-1})	5.20	2.30	5.10	7.40	4.50	3.70
N-NH_3 (mg L^{-1})	0.280	0.290	0.250	3.530	0.500	0.280
N-NO_2 (mg L^{-1})	0.014	0.012	0.005	0.012	0.017	0.005
N-NO_3 (mg L^{-1})	0.080	0.010	0.070	0.090	0.900	0.060
P-$P0_4$ (mg L^{-1})	0.530	0.680	1.530	12.720	0.670	0.120
Sulphates (mg L^{-1})	55.54	19.33	20.16	89.27	39.08	30.86
Chlorides (mg L^{-1})	28.00	15.00	36.05	49.15	52.15	43.05
Total hardness (mg Ca L^{-1})	105.80	110.16	72.25	137.52	56.16	60.48
Total hardness (mg Mg L^{-1})	21.07	15.19	15.91	21.93	11.50	11.61
Fe (mg L^{-1})	0.700	0.250	0.650	0.525	0.450	0.350
Dry residue (mg L^{-1})	473.0	465.0	242.0	640.0	444.0	280.0
Dissolved solids (mg L^{-1})	461.0	354.0	222.0	606.0	433.0	261.0
Suspended solids (mg L^{-1})	12.0	111.0	20.0	34.0	11.0	19.0

that time mass deaths of various fish species have been reported from time to time on different continents [14], with the contribution of *Saprolegnia parasitica* and species of the genus *Achlya. Saprolegnia parasitica* causes great losses in hatcheries [16], while species of the genus *Achlya* are involved in hatcheries and pond-breeding of consumption species [17], and attack lake fish species as well.

Of the genus *Saprolegnia* species found on a few leech species two are worth noting - *Saprolegnia australis* and *Saprolegnia shikotsuensis*. *Saprolegnia australis* paralyses mainly cyprinidae fish species [18] and salmonids in pond fish-breeding [19], while *Saprolegnia shikotsuensis* attacks wild forms of certain salmon of the Pacific as well as those bred in ponds [20].

Aphanomyces laevis was found to grow on 8 leech species, *Dictyuchus sterile* on 13. These species are very common in the waters of various trophicity and frequently cause the death of different fish species. Known are mass deaths of salmons caused by *Aphanomyces laevis* during reproduction on the Taiwan [21] and of other species by *Dictyuchus sterile* [22].

Also interesting is the finding of the so-called sewage fungus *Leptomitus lacteus* on three leech species. For a long time this fungus was considered to be a nitrogen-loving species growing on liquid substrates, and not on a solid substrate [9]. However, with increased water pollution, this fungus can be also found on a solid substrate, where it attacks both lake fish species [23] and the eggs of various species incubated in hatcheries [24].

A number of zoosporic fungus species of the genus *Pythium* parasite on fish eggs and adult specimens [25]. Only two species of that group, namely *Pythium artotrogus* and *Pythium undulatum* were found to grow on the leech species examined. However, worth noting is the finding *of Pythium fluminum* on *Hirudo medicinalis* specimens. This fungus, up to now referred to as typically cellulosophilic [26], in the present study was isolated only from Jaroszówka.

Also worth noting is the growth of a rare fungus, new to Polish waters - *Amoebochytrium rhizidioides* on *Piscicola geometra* in Cypisek. It was first described as a saprophyte in the gelatinous sheath of *Chaetophora elegans* [27]. Then it was isolated by Harder [28] from soil in Germany. Another new species to Polish hydromycology. *Rhizophydium elyensis,* first isolated from snake skin from soil in New Zealand [29], was found on *Theromyzon maculosa* in Akcent. *Achlya intricata,* also new species to Polish waters, was first described in America as saprophitic [30], in our study was found on *Trocheta bykowskii* in Cypisek.

Table 2. Leeches species and fungi found on the investigated specimens.

Taxa		Fungi (see Table 3)	Number of species
1.	*Boreobdella verrucata* (O.F. Müller)	16,20,23,39,41,44,48,54,55,60,61,63,66,68,69,70,73,74	18
2.	*Dina apathyi* (Gedr.)	20,28,36,39,40,43,48,50,52,58,60,63,64,65,68,70,77	17
3.	*Dina lineata* (O.F. Müller)	15,20,33,36,39,45,48,49,56,60,61,62,68,69,70,73,78	17
4.	*Erpobdella nigricollis* (Brand.)	16,21,32,34,36,39,43,57,60,61,63,65,66,70,72,75	16
5.	*Erpobdella octoculata* (L.)	2,12,16,33,42,43,48,51,56,60,70,72	12
6.	*Erpobdella testacea* (Sav.)	15,23,26,28,32,34,36,42,49,50,52,56,60,63,69,70,72,85,87,99	20
7.	*Glossiphonia complanata* (L.)	19,20,36.39,40,41,48,60,61,66,70,86,94	13
8.	*Glossiphonia heteroclita* (L.)	13,15,23,36,38,39,48,49,56,60,62,70,73,96,98	15
9.	*Haementeria costata* (O.F. Müller)	16,21,26,32,34,39.42,43,48,49,50,52,54,56,60,70	16
10.	*Haemopis sanguisuga* (L.)	2,24,28,33,36,42,43,48,51,53,58,60,68,70,72,79,80,88	18
11.	*Helobdella stagnalis* (L.)	15,23,32,39,44,48,49,50,52,56,57,60,63,67,68,70,96	17
12.	*Hemiclepis marginata* (O.F. Müller)	7,10,11,17,18,19.21,24,28,29,30,32,35,37,40,46,50,55,57,59, 60,63,65,67,70,71,73,84,92,95,96,97,100	33
13.	*Hirudo medicinalis* (L.)	8,16,18,24,40,48,50,60,65,70,72,80,91	13
14.	*Piscicola geometra* (L.)	2,3,4,5,6,10,34,36,40,42,48,56,60,70,72,81	16
15.	*Theromyzon maculosa* (Rathke)	5,7,9,11,16,21,22,28,30,32,33,34,39,40,48,50,54,57,58,60,61, 62,63, 65,66,69,70,71,77,80,90,92	32
16.	*Theromyzon tesselata* (O.F. Müller)	16,30,33,36,39,40,48,49,52,56,60,61,70,73,74	15
17.	*Trocheta bykowskii* Gedr.	1,16,20,21,23,27,28,30,31,32,33,35,40,43,46,47,49,52,54,58, 59,60,63,68,69,70,75,76,79,82,83,85,88,89,93,97,101	37

Table 3. Aquatic fungi found on the leeches.

Taxa fungi	Leeches (See Tab. 2)	Number of species
Chytridiomycetes		
Olpidiales		
1. Myiophagus ucrainica (Wize) Sparrow	17	1
2. Rozella septigena Cornu	5,10,14	3
Chytridiales		
3. Amoebochytrium rhizidioides Zopf	14	1
4. Chytriomyces aureus Karling	14	1
5. Phlyctochytriutn aureliae Ajello	14,15	2
6. Polyphagus euglenae Nowakowski	14	1
7. Rhizophydium elyensis Sparrow	12,15	2
Blastocladiales		
8. Allomyces anomalus Emerson	13	1
9. Allomyces arbuscula Butler	15	1
10.Blactocladiopsis parva (Whiffen) Sparrow	12,14	2
11. Catenaria anguillulae Sorokin	12,15	2
Hyphochytriomycetes		
Hyphochytriales		
12. Hyphochytrium catenoides Karling	5	1
Plasmodiophoromycetes		
Plasmodiophorales		
13. Woronina polycystis Cornu	8	1
Oomycetes		
Lagenidiales		
14. Lagenidium giganteum Couch	17	1
Saprolegniales		
*15. *Achlya ambisexualis* Raper	3,6,8,11	4
*16. *Achlya americana* Humphrey	1,4,5,9,13,15,16,17	8
17. Achlya apiculata de Bary	12	1
*18. *Achlya bisexualis* Coker et Couch	12,13	2
*19. *Achlya caroliniana* Coker	7,12	2
20. Achlya colorata Pringsheim	1,2,3,7,17	5
21. Achlya crenulata Zeigler	4,9,12,15,17	5
22. Achlya debaryana Humphrey	12,15	2
*23. *Achlya diffusa* Harvey ex Johnson	1,6,8,11,17	5
*24. *Achlya dubia* Coker	10,12,13	3
*25. *Achlya flagellata* Coker	14	1
26. Achlya hypogyna Coker et Pemberton	6,9	2
*27. *Achlya intricata* Beneke	17	1

Table 3 continues on next page

*28. *Achlya klebsiana* Pieters	2,6,10,12,15,17	6
29. Achlya megasperma Humphrey	12	1
30. Achlya oblongata de Bary	12,15,16,17	4
31. Achlya oligocantha de Bary	17	1
*32. *Achlya orion* Coker et Couch	4,6,9,11,12,15,17	7
*33. * Achlya polyandra* Hildebrand	3,5,10,12,15,16,17	7
*34. *Achlya prolifera* Nees	4,6,9,14,15	5
*35. *Achlya proliferoides* Coker	12,17	2
*36. *Achlya racemosa* Hildebrand	2,3,4,6,7,8,10,14,16	9
37. Achlya treleaseana (Humphrey) Kauffman	12	1
38. Aphanomyces helicoides Minden	8	1
39. Aphanomyces irregularis Scott	1,2,3,4,7,8,9,11,15,16	10
*40. *Aphanomyces laevis* de Bary	2,7,9,12,13,14,15,16,17	9
41. Aphanomyces parasiticus Coker	1,7	2
*42. *Aphanomyces stellatus* de Bary	5,6,9,10,14	5
43. Aplanes androgynus (Arches) Humphrey	2,4,5,9,10,17	6
*44. *Calyptralegnia achlyoides* (Coker et Couch) Coker	1,11	2
45. Calyptralegnia basraensis Muhsin	3	1
46. Cladolegnia unispora (Coker et Couch) Johannes	12,17	2
*47. *Dictyuchus monosporus* Leitgeb	17	1
*48. *Dictyuchus sterile* Coker	1,2,3,5,7,8,9,10,11,13,14, 15,16	13
*49. *Isoachlya monilifera* (de Bary) Kauffman	3,6,8,9,11,16,17	7
*50. *Leptolegnia caudata* de Bary	2,6,9,11,12,13,15	7
51. Olpidiopsis saprolegniae (Braun) Cornu	5,10	2
*52. *Protoachlya paradoxa* (Coker) Coker	2,6,9,11,16,17	6
*53. *Pythiopsis cymosa* de Bary	10	1
54. Saprolegnia anisospora de Bary	1,9,15,17	4
55. Saprolegnia asterophora de Bary	1,12	2
*56. *Saprolegnia australis* Elliott	3,5,6,8,9,11,14,16	8
*57. *Saprolegnia delica* Coker	4,11,12,15	4
*58. *Saprolegnia diclina* Humphrey	2,10,15,17	4
59. Saprolegnia eccentrica (Coker) Seymour	12,17	2
*60. *Saprolegnia ferax (Gruith.) Thuret*	1,2,3,4,5,6,7,8,9,10,11,12,13, 14,15,16,17	17
61. Saprolegnia furcata Maurizio	1,3,4,7,15,16	6
62. Saprolegnia glomerata (Tiesenhausen) Lund	3,8,15	3
63. Saprolegnia hypogyna (Pringsheim) de Bary	1,2,4,6,11,12,15,17	8
64. Saprolegnia invaderis Davis et Lazar	2	1
65. Saprolegnia latvica Apinis	2,4,12,13,15	5

Table 3 continues on next page

66. Saprolegnia litoralis Coker	1,4,7,15	4
*67. *Saprolegnia megasperma* Coker	11,12	2
*68. *Saprolegnia mixta* de Bary	1,2,3,10,11,17	6
*69. *Saprolegnia monoica* Pringsheim	1,3,6,15,17	5
*70. *Saprolegnia parasitica* Coker	1,2,3,4,5,6,7,8,9,10,11,12,13, 14,15,16,17	17
71. Saprolegnia pseudocrustosa Lund	12,15	2
*72. *Saprolegnia shikotsuensis* Hatai et al.	4,5,6,10,13,14	6
*73. *Saprolegnia subterranea* Dissmann	1,3,8,12,16	5
74. Saproiegnia terrestris Cookson ex Seymour	1,16	2
75. Saproiegnia torulosa de Bary	4,17	2
76. Saproiegnia turfosa (Minden) Gaumarm	17	1
77. Saproiegnia uliginosa Johannes	2,15	2
*78. *Saproiegnia unispora* Coker et Couch	3	1
*79. *Thraustotheca clavata* (de Bary) Humphrey	10,17	2
Leptomitales		
*80. *Leptomitus lacteus* (Roth) Agardh	10,13,15	3
Peronosporales		
81. Pythiogeton utriforme Minden	14	1
82. Pythium afertile Kanouse et Humphrey	17	1
83. Pythium aquatile Hohnk	17	1
84. Pythium arrhenomanes Drechsler	12	1
*85.*Pythium artotrogus* de Bary	6,17	2
86. Pythium butleri Subramaniam	7	1
87. Pythium capillosum Paul	4,6	2
88. Pythium debaryanum Hesse	10,17	2
89. Pythium deliense Meurs	17	1
90. Pythium dissotocum Drechsler	15	1
91. Pythium fluminum Park	13	1
92. Pythium helicandrum Drechsler	12,15	2
93. Pythium intermedium de Bary	17	1
94. Pythium middletonii Sparrow	7	1
95. Pythium oedochilum Drechsler	12	1
96. Pythium rostratum Butler	8,11,12	3
97. Pythium tenue Gobi	12,17	2
98. Pythium torulosum Coker et Patterson	8	1
99. Pythium ultimum Trow	6	1
*100. * Pythium undulatum* Petersen	12	1
101. Pythium zingiberis Takahashi	17	1

* Known in literature as parasites or necrotrophs of fish

Pythium zingiberis, also new to Polish waters, has been known as a parasite of a rotten root of *Zingiber officinale.* It was first described in Japan in Osaka by Takahashi [31]. In our study it was observed also on *Trocheta bykowskii* specimens in the water of Jaroszowka.

Myiophagus ucrainica was found on *Trocheta bykowskii* in Jaroszówka. This interesting fungus species was first described by Wize [32] as an insecticidal parasite of the beetroot pest *Cleonus punctiventris* in Ukraine. Further studies have revealed that it parasites on the body of other insect species on different continents. It has been encountered in Florida [33], Bermuda [34] and in northern Canada [10]. This would be the second example of its growth on animal substrate in the aquatic environment. We observed the growth of *Myiophagus ucrainica* on fragments *of Chironomus anthracinus* larvae in Jaroszówka [3].

Although water samples collected for analysis from 6 reservoirs showed different parameters, the total number of fungi growing on leeches was practically similar. More substantial differences occurred in the fungus

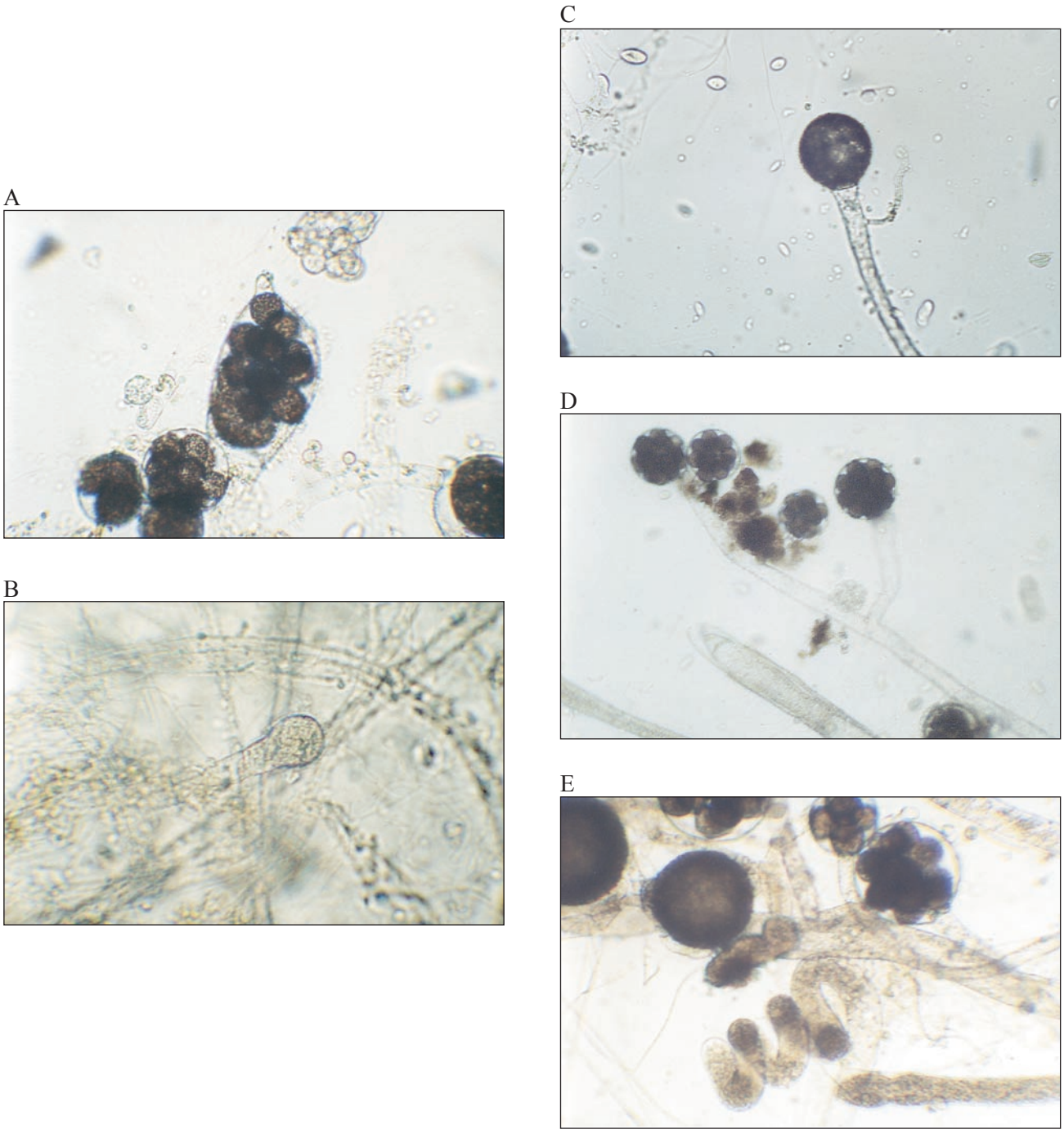

Fig. 1. Some zoosporic fungus species growing on leeches (x 200): A - *Achlya apiculata* - oogonia (60-80 x 50-60 µm); B - *Achlya intricata* - formed oogonium; C - *Saprolegnia furcata* - oogonia (25-42 µm); D - *Saprolegnia litoralis* - oogonium (20-40 µm) and antheridium; E - *Saprolegnia uliginosa* - oogonia (60-65 µm).

Table 4. Aquatic fungi found on leech specimens in different waters.

Water from	Fungi (see Table 3)	Only in one water	Total number
Cypisek Spring	2,3,10,16,19,20,21,22,23,25,27,28,30,31,32,33,34,35,36,37,39,40,46,47,48,49, 50,51, 52,53,54,56,57,58,59,60,61,63,65,66,68,70,72,73,74,75,77,78,79,81,83,87, 88,92,96, 97	3,25,27,37,53,81, 83	56
Jaroszówka Spring	1,2,6,7,9,10,16,19,22,23,24,28,32,36,39,40,41,43,45,48,49,50,55,56,57,60,61,63, 65, 66,69,70,72,73,77,79,82,87,91,92,94,96,97,99,100,101	1,6,9,45,91,94,99, 100,101	46
Supraśl River	2,10,12,15,16,18,19,21,23,24,28,31,32,33,34,36,39,40,42,43,44,46,47,48,49,.50, 51, 52,54,56,57,58,59,60,61,63,64,66,67,68,69,70,71,73,75,76,79,80,82,85,97	12,18,76,85	51
Akcent Pond	4,7,11,13,15,16,20,21,22,30,32,36,38,39,40,42,43,44,48,50,52,54,55,56,60,61,62 ,63, 64,66,69,70,73,78,80,84,90,97	4,13,38,84,90	38
Fosa Pond	11,14,17,20,21,22,28,30,32,34,35,36,39,40,41,42,43,46,48,50,51,57,58,60,61,62 ,63, 65,66,67,68,69,70,71,73,75,87,89,95,96,97,98	14,17,35,89,95,98	42
Komosa Lake	2,5,8,15,16,20,22,26,28,29,32,33,34,36,40,41,42,43.44,48,50,52,57,59,60,61,62, 63, 65,68,70,72,74,75,80,86,87,88,93	5,8,26,29,59,86,88	39

species found on leeches only in the water of one out of six reservoirs. The fewest species (4) were found on leeches in the Supraśl, the most (9) in Jaroszówka. It can therefore be assumed that among fungus species growing on leeches some are characterised by specific environmental requirements. As revealed by chemical investigations, water in the Supraśl, compared to other reservoirs, was the poorest in ammonium nitrogen and nitrite nitrogen but had the highest BOD index, while water in Jaroszówka contained the smallest amounts of nitrate nitrogen, sulphates, chlorides and had the lowest alkalinity index.

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