Pol. J. Environ. Stud. Vol. 21, No. 4 (2012), 923-927

Original Research

Frequency and Distribution of Zoosporic True Fungi and Heterotrophic Straminipiles from River Springs

Bożena Kiziewicz*

Department of General Biology, Medical University,
Mickiewicza 2C, 15-222 Białystok, Poland

Received: 7 January 2011
Accepted: 31 January 2012

Abstract

In this paper I studied the occurrence and distribution of zoosporic true fungi (Blastocladiomycota) and heterotrophic straminipiles (Hyphochytriomycota and Oomycota) from the water of three springs of the Biała River (Sobolewo, Dojlidy Górne, and Kurjany springs) in the vicinity of Białystok. Twenty-one species of zoosporic true fungi and heterotrophic straminipiles belonging to 10 genera of the orders Blastocladiales, Hyphochytriales, Pythiales, and Saprolegniales were isolated using hemp seeds and snake skin as baits. The most commonly encountered species were: *Achlya apiculata*, *Aphanomyces laevis*, *Catenophlyctis variabilis*, *Dictyuchus monosporus*, *Pythium debaryanyum*, and *Saprolegnia ferax*. The highest species richness (S), the largest, and relative frequency (RF) were noted from springs Dojlidy Górne (S=16, RF=76.19%) and Kurjany (S=13, RF=61.90%), whereas the lowest richness and frequency were obtained from Sobolewo (S=9, RF=42.85%).

Keywords: Białystok, diversity, heterotrophic straminipiles, springs, zoosporic true fungi

Introduction

"Fungi" comprise a heterogeneous and diverse group of eukaryotic organisms. Assemblages living in aquatic environments represent more than 1.000 species [1-4]. Members of the phyla Blastocladiomycota, Chytridiomycota, Hyphochytriomycota, Labirynthulomycota, and the classes Oomycetes and Phytomyxea are mostly aquatic and commonly known as zoosporic true fungi, heterotrophic straminipiles and plasmodiophorids [2, 5]. These organisms are an ecologically similar but phylogenetically unrelated group of eukaryotes that belong to the kingdoms Fungi, Straminipila, and Protozoa [6-8].

They are widely distributed and ubiquitous, being primarily saprotrophic in nature. As saprotrophs they play an important role in decomposing plant and animal debris [9-13]. Some species are parasitic on plants, aquatic animals and even other fungi [14-23]. They have a diverse array of enzymes that hydrolyze chitin, keratin, lignin, and cellulose, and thus they contribute to the release of carbon and inorganic compounds in food webs [8].

The occurrence of zoosporic true fungi and heterotrophic straminipiles in different aquatic habitats from various geographical regions worldwide have been intensively studied [2, 8, 24, 25]. Preliminary studies in several springs have demonstrated the occurrence of interesting taxa and a high number of uncommon species [26, 27].

The aim of this study was to assess distribution of zoosporic true fungi and heterotrophic straminipiles and compare the frequency of the genera and species recovered from three limnokrenic springs situated in the vicinity of Białystok, Podlasie Province, Poland.

*e-mail: bozena.kiziewicz@umwb.edu.pl

Materials and Methods

The experiments were performed in March and November 2007 and 2008. Three limnokrenic springs of the Biała River in the vicinity of Białystok (Podlasie Province, Poland) were investigated: Sobolewo (53º70'N, 23º17'E), Dojlidy Górne (53º06'N, 23º12'E), and Kurjany (53º08'N, 23º27'E). The springs are mostly of limnokrenic type and vary in the rate of discharge from less than 0.1 to 2.5 $dcm^3 \cdot s^{-1}$ [28-30]. The Biała River flows through Białystok and the Knyszyńska Forest and represents a left-bank tributary of the Supraśl River with a length of 29.9 km.

Surface water samples with organic matter (twigs, leaves etc.) were collected back to the laboratory in sterile plastic bottles.

Samples were processed in the laboratory by routine methods commonly used to isolate these organisms [31]. Water samples (100 ml) of each site were homogenized and four aliquots of 25 ml were placed in Petri dishes of 9 cm diameter with sterile baits (a total of four dishes). The baits used were halves of hemp-seeds, and small pieces of snake skin (3-6 mm in diameter). Dishes were left overnight at room temperature (20-23ºC) for 4-5 days. The colonized seeds were transferred to new Petri dishes that contained sterilized filtered spring or distilled water and crystalline penicillin (2000 units per liter of water) to inhibit bacterial growth [32]. Dishes were examined weekly for up to three weeks in order to identify the species.

Identification was performed based on morphological characteristics, including qualitative and quantitative measurements of vegetative (hyphae, gemmae), asexual (zoosporangia and zoospores), and sexual structures (oogonia, oospores, and antheridia). Taxonomic identifications were made according to Batko [1], Scott [33], Karling [34], Dick [35], and Johnson [36]. The systematics of straminipiles was used according to Dick [2], and of zoosporic true fungi according to James et al. [37].

The occurrence of each species in each water sample was recorded. At each sampling site the total number of species (richness), total frequency (TF%), and relative frequency (RF%) for each genera and species were determined [38].

Sorensen's similarity index (SI) was calculated between the springs analyzed, according to Müeller et al. [12], Iqual [39], Sarma and Hyde [40]: $SI = [2c/a+b] \times 100$, where a is the number of genera in spring a, b is the number of genera in the spring b, and c is the number of genera common to both springs.

The results were subjected to statistical analysis using t-test to determine the significance of differences ($p \leq 0.05$) between sampling sites (springs) in a) total number of species (richness) and b) total frequency.

Results

Twenty-one species of aquatic fungi belonging to 10 genera in the orders Blastocladiales Hyphochytriales, Pythiales and Saprolegniales were identified. Seven of these genera belong to the class Oomycetes/Peronosporomycetes: *Achlya*, *Aphanomyces*, *Dictyuchus*, *Myzocytium*, *Pythium*, *Saprolegnia*, and *Thraustotheca*. Only *Catenaria* and *Catenophlyctis* were found within the Blastocladiomycetes, and *Rhizidiomyces* within the Hyphochytriomycetes. *Saprolegnia* showed the highest diversity (six species) followed by *Achlya* (five species), while most of the genera were represented by single species. The largest number of species (S) and the highest total frequency were obtained from the springs Dojlidy Górne and Kurjany, whereas the lowest number of species and total frequency were recorded from Sobolewo Spring. Differences in the total number of species and in total frequency were found between the springs Sobolewo and Dojlidy Górne (Table 1; $p \leq 0.05$). The distribution of total frequencies between the genera was different and also the species composition and the relative frequency of each genera varied between springs (Figs. 1 and 2 and Table 1).

The most commonly encountered species were: *Achlya apiculata*, *Aphanomyces laevis*, *Catenophlyctis variabilis*, *Dictyuchus monosporus*, *Pythium debaryanyum*, and *Saprolegnia ferax*. The species in the genera *Saprolegnia* (TF=28.57%) and *Achlya* (TF=23.81%) were the most frequent, whereas the less common species were from the genera *Aphanomyces* (TF=9.52%) and *Pythium* (TF=9.52%). Similar relations were observed in all examined spring sites. The similarity index (SI) showed a higher similarity of genera between the springs Dojlidy Górne and Kurjany (SI=67%), followed by Sobolewo and Kurjany (SI=61%), and finally a less similar composition between Sobolewo and Dojlidy Górne (SI=14%).

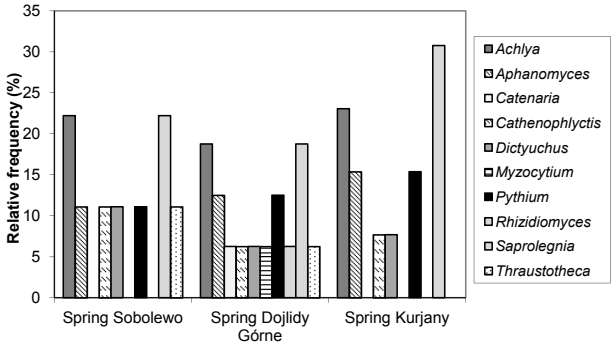

Fig. 1. Relative frequency (RF%) of the genera in each of the analyzed springs.

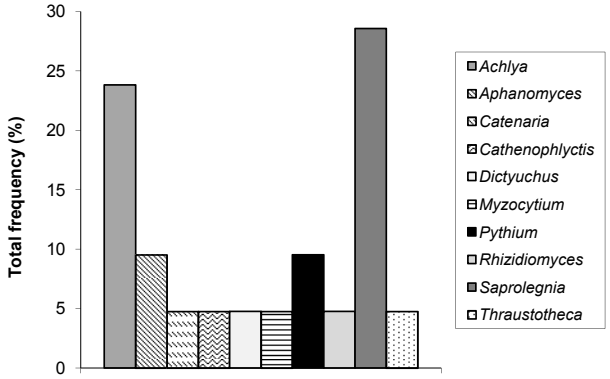

Fig. 2. Total frequency (TF%) of the genera recovered in the study.

Table 1. Species found at the analyzed springs.

Taxa (Kingdom, Class, Order, species)	Spring Sobolewo	Spring Dojlidy Górne	Spring Kurjany
Straminipila			
Hyphochytriomycetes			
Hyphochytriales			
Rhizidiomyces apophysatus Zopf		x	
Oomycetes (=Peronosporomycetes)			
Pythiales			
Myzocytium zoophthorum Sparrow		x	
Pythium debaryanum R. Hesse#	x	x	x
P. rostratum Butler		x	x
Saprolegniales			
Achlya apiculata de Bary#	x	x	x
Ac. colorata Pringhs.		x	
Ac. crenulata Ziegler			x
Ac. polyandra Hildebr.	x	x	
Ac. treleaseana (Humphrey) Kauffman			x
Aphanomyces irregularis W.W. Scott		x	x
Ap. laevis de Bary#	x	x	x
Dictyuchus monosporus Leitg.#	x	x	x
Saprolegnia delica Coker		x	
S. eccentrica (Coker) R. L. Seymur		x	
S. ferax (Gruith.) Thur.#	x	x	x
S. glomerata (Tiesenh.) A. Lund			x
S. parasitica Coker			x
S. unispora (Coker et Couch) R.L Seymur	x		x
Thraustotheca clavata (de Bary) Humphrey	x	x	
Fungi			
Blastocladiomycota			
Blastocladiales			
Catenaria anguillulae Sorokin		x	
Catenophlyctis variabilis (Karling) Karling#	x	x	x
S	9	16	13
TF (%)	42.85a	76.19b	61.90a,b

S – total number of species (richness); TF(%) – total frequency; # – species found in all springs.
The different letters indicate significant differences ($p \leq 0.05$).

Discussion

Most of the species recovered in the present study were commonly found worldwide [2] and have been previously isolated from Polish waters [13, 41-44] such as lakes [45], springs [26, 27], streams running through forests [46], and the rivers Supraśl, Narew, and Bug, which are the biggest rivers of the region [43, 44, 47].

In our study the most frequent genera were: *Achlya*, *Aphanomyces*, *Catenophlyctis*, *Dictyuchus*, *Pythium*, and *Saprolegnia*. Similar results were obtained by El-Hissy et al. [48], El-Hissy and Khallil [49], El-Nagdy and Nasser [50], Marano and Steciow [51], and Paliwal and Sati [24], who reported that zoosporic assemblages in freshwater habitats are mainly composed of *Achlya*, *Dictyuchus*, *Pythium*, *Saprolegnia*, and *Thraustotheca*.

Dojlidy Górne and Kurjany showed highest species richness and total frequency of zoosporic true fungi and heterotrophic straminipiles, and also the highest similarity index (SI) in the genera composition. The lowest similarity was found between the springs Sobolewo and Dojlidy Górne. According to Christensen [52], a similarity index greater than 70% indicates similar assemblages. Even Sobolewo Spring is an affluent of the springs Dojlidy Górne and Kurjany, the Sobolewo Spring is eutrophic. Therefore at and the content of pollutants have exceeded the range of tolerance of some species, and delimited their occurrence [53]. This appeared to be the probable reason for finding the lowest number of species and total frequency at Sobolewo.

Investigations of conidial fungi from the Class Hyphomycetes in the Springs of Knyszyńska Forest revealed a number of uncommon species [54]. The presented study demonstrated that springs are not only rich in Hyphomycetes species, but also provide favorable conditions for the growth of numerous zoosporic true fungi and heterotrophic straminipiles. Far less work has been carried out on the ecology of these organisms in rivers and streams than in lentic habitats [36]. Thus, it is important that studies in lentic habitats continue to increase.

Conclusions

The obtained results are in most cases consistent with previous studies recently made in fresh waters from Podlasie Province of Poland. The species diversity of zoosporic true assemblages at the investigated springs of Biała River appeared to depended on ecosystem characteristics. The most eutrophic was Sobolewo, which contained the lowest number of zoosporic true fungi and heterotrophic staminipiles species (9) compared to Dojlidy Górne and Kurjany, where I found a larger number of species (Dojlidy Górne 16, Kurjany 13). Most likely, therefore, at Sobolewo the content of pollutants have exceeded the range of tolerance of some species, and delimited their occurrence.

References

1. BATKO A. Hydromycology-an overview. PWN, Warszawa, pp. 478, **1975** [In Polish].
2. DICK M.W. The Peronosporomycetes. In: D.J. MCLAUGLIN, E.G. MCLAUGLIN, P.A. LEMKE (Eds), Systematics and Evolution. The Mycota VII Part A, Springer-Verlag, Berlin, Heidelberg, **2001**.
3. HAWKSWORTH D.L. Fungal diversity and its implications for genetic resource collections. Stud. Mycol. **50**, 9, **2004**.
4. SHEARER C.A., DESCALS E., KOHLMEYER B., KOHLMEYR J., MARVANOVÁ L., PADGETT D., PORTER D., RAJA H.A., SCHMIT J.P., THORNTON H.A., VOGLYMAYR H. Fungal Biodiversity in Aquatic Habitats. In: D.L HAWKSWORTH, E.G. MÜELLER (Eds), Biodivers. Conserv. **16**, 49, **2007**.
5. PATTERSON D.J. Stramenopiles: chromophytes from a protistan perspective. In: P. GREEN, B.S.C. LEADBEATER, W.C. DIVER, (Eds), The chromophyte algae: problems and perspectives. Oxford, UK: Clarendon Press, pp. 357, **1989**.
6. SHEARER C.A., LANGSAM D.M., LONGCORE J.E. Fungi in freshwater habitats. In: G.M. MÜELLER, G.F. BILLS, M.S. FOSTER (Eds), Biodiversity of fungi, inventorying and monitoring methods. Elsevier Academic Press, pp. 513, **2004**.
7. MILANEZ A.I., PIRES-ZOTTARELLI C.L.A., GOMEZ LENK A. Brazilian zoosporic fungi. Vinnegraph Press, São Pablo. **2007**.
8. KIRK P., CANNON P.F., MINTER D.W., STALPERS J.A. Dictionary of the Fungi. 10th ed. Wallingford, U K: CABI, pp. 340, **2008**.
9. CARROLL G.C., WICKLOW D.T. The fungal community: its organization and role in the ecosystem. 2nd Edn. M. Dekker, Inc. New York. **1999**.
10. WETZEL R.G., LIKENS G.E. Limnological analyses. 3rd ed. Springer-Verlag, New York, **2000**.
11. CZECZUGA B., GODLEWSKA A., KIZIEWICZ B. Aquatic fungi growing on feathers of wild and domestic bird species in limnologically different water bodies. Pol. J. Environ. Stud. **13**, 21, **2004**.
12. MÜELLER G.M., BILL G.F., FOSTER M.S. Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier Academic Press, Burlington, UK. **2004**.
13. KIZIEWICZ B. Aquatic fungi growing on seeds of plants in various types of water bodies of Podlasie Province. Pol. J. Environ. Stud. **14**, 49, **2005**.
14. SPARROW F.K. Aquatic Phycomycetes. 2nd ed. Ann Arbor. The University of Michigan, Press, **1960**.
15. UNESTAM T. Fungal diseases of Crustacea. Rev. Med. Vet. Mycology **8**, 1, **1973**.
16. BRUNO D.W., WOOD B.P. Saprolegnia and other Oomycetes. In: P.T.K WOO, D.W. BRUNO (Eds), Fish diseases and disorders: Viral, bacterial and fungal infections. Vol. 3. CABI Publishing, Wallingford, UK, pp. 599, **1994**.
17. BARRON G.L. Fungal parasites and predators of rotifers, nematodes, and other invertebrates. In: G.M. MÜELLER, G.F. BILLS, M.S. FOSTER (Eds), Biodiversity of Fungi. Elsevier, Amsterdam, pp. 435, **2004**.
18. AL-REKABI S.A.W., AL-JUBORI S.S., SHNESHIL S.S. A study of the pathogenicity of two aquatic fungi on carp eggs. Egypt. J. Aquatic Res. **31**, 149, **2005**.
19. ROSSETTI G. Fungal parasitism in freshwater calanoid population: ecological consequences and possible mechanisms involved in infection process. Hydrobiologia **548**, 167, **2005**.
20. RAMAIAH N. A review on fungal diseases of algae, marine fishes, shrimps and corals. Ind. J. Mar. Sci. **35**, 380, **2006**.
21. KIZIEWICZ B., NALEPA T. F. Some fungi and water molds in waters of Lake Michigan with emphasis on those associated with the benthic amphipod *Diporeia* spp. J. Great Lakes Res. **34**, 774, **2008**.
22. WOLINSKA J., KING K.C., VIGNEUX F., LIVELY C.M. Virulence, cultivating conditions, and phylogenetic analyses of oomycete parasites in *Daphnia*. Parasitology **135**, 1667, **2008**.
23. SHAHBAZIAN N., EBRAHIMZADEH MOUSAVI H.A., SOLTANI M., KHOSRAVI A.R., MIRZARGAR S., SHARIFPOUR I. Fungal contamination in rainbow trout eggs in Kermanshah province propagations with emphasis on Saprolegniaceae. Iran J. Fish Sci. **9**, 151, **2010**.

24. PALIWAL P.C., SATI S.C. Distribution of aquatic fungi in relation to factors of Kosi River in Kumaun Himalaya. Nat. Sci. **79**, 70, **2009**.
25. SAJAL SAJU D. Occurrence of fungi in pond water (Dumaratarai Talab) of Raipur City, C.G., India. J. Phytol. **3**, (4), 30, **2011**.
26. CZECZUGA B., WORONOWICZ L., BRZOZOWSKA K., CHOMUTOWSKA H. Mycoflora of different types of springs. Acta Hydrobiol. **31**, 273, **1989**.
27. CZECZUGA B., KIZIEWICZ B., WYKOWSKA E. Zoosporic fungi n springs in the vicinity of Białystok. Acta Mycol. **34**, 55, **1999**.
28. PAZDRO Z. General Hydrobiology. Wydawnictwo geologiczne, Warszawa, **1977** [In Polish].
29. ŁOSZEWSKI H. Natural outflows of underground waters, basins of Supraśl River. Nauka i praktyka – studia, ekspertyzy, informacje. **4**, 111, **1984** [In Polish].
30. ŁOSZEWSKI H. Sources in the area of Bialystok and requirement of their protection. Białostocczyzna **4**, 140, **1995** [In Polish].
31. SEYMOUR R.F., FULLER M.S. Collection and isolation of water molds (Saprolegniaceae) from water and soil. In: M.S. FULLER, A. JAWORSKI (Eds). Zoosporic fungi in teaching and research, Southeastern Publishing, Athens, pp. 125, **1987**.
32. ROBERTS R.E. A study of the distribution of certain members of the Saprolegniales. Trans. Brit. mycol. Soc. **46**, 213, **1963**.
33. SCOTT W.W. A monograph of the genus *Aphanomyces*. Tech. Bull. **151**, 1, **1961**.
34. KARLING J.S. Chytridiomycetarum Iconographia. An Illustrated and Brie Descriptive Guide to the Chytridiomycetous Genera with Suplement of the Hyphochytriomycetes. Lubrecht and Cramer, Vaduz, pp. 414, **1977**.
35. DICK M.W. Key to *Pythium*. College of Estate Management Whiteknights, Reading, UK. **1990**.
36. JOHNSON T.W.JR., SEYMOUR. R.L., PADGET D.E. Biology and Systematics of the Saprolegniaceae. Online publication: http://www. ilumina-dlib.org. **2002**.
37. JAMES T.Y., LETCHER P.M., LONGCORE J.E., MOZLEY-STANDRIDGE S.E., PORTER D., POWELL M.J., GRIFFITH G.W., VILGALYS R. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia **98**, 860, **2006**.
38. LETCHEL P.M., POWELL M.J. Distribution of zoosporic fungi in forest soils of the Bluc Ridge and Appalachian Mountains of Virginia. Mycologia **93**, 1029, **2001**.
39. IQUAL S.H. Species diversity of freshwater Hyphomycetes in some streams of Pakistan. Comparison of sampling techniques. Mycoscience **35**, 331, **1994**.
40. SARMA V.V., HYDE K.D. A review on frequently occurring fungi in mangroves. Fungal Diversity **8**, 1, **2001**.
41. CZECZUGA B., KIZIEWICZ B. Zoosporic fungi growing on the eggs of *Carrasius carrasius* (L.) in oligo- and eutrophic water. Pol. J. Environ. Stud. **8**, 63, **1999**.
42. CZECZUGA B., KIZIEWICZ B., DANILKIEWICZ Z. Zoosporic fungi growing on the specimens of certain fish species recently introduced to Polish waters. Acta Ichthyol. Piscat. **32**, 117, **2002**.
43. CZECZUGA B., KIZIEWICZ B., ORŁOWSKA M. Zoosporic and conidial fungi within the Podlasie stretch of the river Bug. Ann. Acad. Med. Bialostocensis **47**, 40, **2002**.
44. KIZIEWICZ B. Aquatic fungi and fungus-like organisms in the baiting sites of the river Supraśl in Podlasie Province of Poland. Mycologia Balcanica **1**, 77, **2004**.
45. CZECZUGA B., KIZIEWICZ B., GODLEWSKA A. Zoosporic fungi growing on eggs of *Coregonus lavaretus holsatus* Thienemann, 1916 from Lake Wdzydze in Kaszuby. Pol. J. Environ. Stud. **13**, 355, **2004**.
46. CZECZUGA B., WORONOWICZ L., BRZOZOWSKA K. Aquatic fungi of two forest brooks. Nova Hedwigia **43**, 459, **1986**.
47. CZECZUGA B., KIZIEWICZ B., GODLEWSKA A. Further studies on aquatic fungi in the river Narew within the Narew National Park. Ann. Acad. Med. Bialostocensis **47**, 58, **2002**.
48. EL-HISSY F.T., MOUSBASHER A.H., EL-NAGDY M.A. Seasonal fluctuations of freshwater fungi in River Nile, Egypt. Zeit. Alg. Microbiol. **22**. 521, **1982**.
49. EL-HISSY F.T., KHALLIL A.M. Studies on aquatic fungi in Delta region. Egypt. Zent. Microbiol. **144**, 421, **1989**.
50. EL-NAGDY M.A., NASSER L.A. Occurrence of zoosporic fungi and terrestrial fungi in accumulated rainfall water in the Riyadh Region (Saudi Arabia). Fungal Diversity **5**, 75, **2000**.
51. MARANO A.V., STECIOV M.M. Frequency and abundance of zoosporic fungi in some lotic environments of Buenos Aires Province (Argentina). J. Agric. Tech. **2**, 17, **2006**.
52. CHRISTENSEN M. A view of fungal ecology. Mycologia **81**, 1, **1989**.
53. DOJLIDO J.R. The chemistry of surface water. Wydawnictwo Ekonomia i Środowisko, Białystok, pp. 342. **1995** [In Polish].
54. CZECZUGA B., ORŁOWSKA M. Hyphomycetes in twenty springs of the Knyszyn Białystok Forest in various seasons. Int. Rev. ges. Hydrobiol. Hydrog. **81**, 417, **1996**.