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Ecological diversity and economical importance of species from *Aphanomyces* genus

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Species from *Aphanomyces* genus were investigated in 225 limnologically and trophically different water bodies (springs, rivers, ponds and lakes) as well as in humid soils of north- eastern Poland. Distribution of particular species and their role in diverse ecosystems has been investigated. Thirty taxa, including: 2 species of cultivated parasitic plants, 3 species of parasitic animals, 6 saprotrophic species and 5 species of saprotrophic/ opportunistic species were recorded. Some of *Aphanomyces* species occurred also as parasites of algae, straminipiles and invertebrates. Among parasitic species: *A. cochlioides*, *A. euteiches* (cultivated plant parasite), *A. astaci* (crayfish parasite), *A. piscicida* (fish parasite) playing economically important role were found. Amino acid, carbohydrate and urease assimilation tests were used.

Key words: *Aphanomyces* species, ecological diversity, hydrochemistry, parasite, economical importance.

INTRODUCTION

The number of fish species bred in the control conditions increased in recent years according to Food and Agriculture Organization (FAO, 2012). On the other hand, death on a large scale of the particular fish populations may occur due to bacterial and mycotic infections (Bruno and Wood, 1988). Straminipila from the Saprolegniales order, especially *Saprolegnia*, *Achlya* and *Aphanomyces* species are responsible for eggs infection in the fish species. For example Hatai and Hoshiai (1992) dealt with saprolegniosis of *Oncorhynchus kisutch* demonstrating heavy losses caused by *Saprolegnia parasitica* in the breeding of this species, even up to 50%. *Achlya flagellata* and *A. prolifera* are known to have caused total damage to the incubated eggs of *Tor tor* Lac. in India (Sati and Khulbe, 1981). *Aphanomyces astaci* is known to cause so-called “plague” of crayfish (Schikora, 1903) and *A. laevis* known in mass deaths of rainbow trout during reproduction on the Taiwan (Chien, 1981). Since the mid- 1980s epizootic ulcerative syndrome (EUS) have been described as a disease affecting wild and farmed freshwater and estuarine fish (Chinabut, 1998). It has been reported from Australia, North America, Asia and Africa (OIE, 2007). The agent associated with this disease is the straminipiles organism belonging to *Aphanomyces* genus. In 1997 Kitancharoen and Hatai (1997) described also *A. frigidophilus* as a parasite of the Japan charr eggs.Whereas, such species of the *Aphanomyces* genus as *A. cochlioides* and *A. euteiches* because of their agriculturally importance, are specialized to parasitize roots of sugar beet and Fabaceae species,

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respectively (Bangsund and Leistritz, 1993; Sauvage et al., 2007; Dieguez-Uribeondo et al., 2009).

On account of that, authors have decided to find out which of the already known straminipiles organisms belonging to *Aphanomyces* genus is responsible for the mycotic diseases in plants and animals and can grow in different ecosystems of the north-eastern Poland.

MATERIALS AND METHODS

Description of *Aphanomyces* (de Bary, 1860) genus

Aphanomyces genus was described firstly in 1860 by de Bary (de Bary, 1860) and included initially following four species: *A. laevis*, *A. phycophilus*, *A. scaber* and *A. stellatus*. Other species from this genus have been described by Drechsler (1929) and Scott (1961). At present, according to Index Fungorum, *Aphanomyces* genus comprises 45 taxa and 40 species (David and Kirk, 1997), according to Ballesteros et al. (2006)- 30 species and according to Dieguez- Uribeondo et al. (2009)- 35-40 species. Dick (2001) classifies *Aphanomyces* together with *Leptolegnia* and *Plectospyra* to Leptolegniaceae family. Some species specialize in plant or animal parasitism, other ones are saprotrophic growing on decaying plant and animals debris (Scott, 1961; Fuller and Jaworski, 1987; Dick, 2001; Johnson et al., 2002; Dieguez-Uribeondo et al., 2009). There are also species which are primarily saprotrophic but in some instances become opportunistic pathogens (Royo et al., 2004; Patwardhan et al., 2005). Plant parasitic species include: *A. cochlioides*- pathogen of roots of sugar beet and *P. euteiches* which parasitize on Fabaceae species (Papavizas and Ayers, 1974; Levenfors and Fatehi, 2004). *A. astaci* is a parasite of a freshwater crayfish (Söderhäll and Cerenius, 1999). *A. invadans* and *A. frigidophilus* (*A. piscicida*) devastate both freshwater and estuarine fishes (Chnabut, 1998; Czeczuga et al., 2011a). *A. laevis*, *A. stellatus* and *A. helicoides* belong to opportunistic pathogens (Patwardhan et al., 2005; Dieguez-Uribeondo et al., 2009).

Occurrence environment of *Aphanomyces* species

Material was collected in north- eastern part of Poland within 1985 to 2012. Water samples were collected from 225 limnologically and trophically different water bodies-springs (31), rivers (45), ponds (21) and lakes (128). Oligotrophic, mesotrophic, eutrophic and dystrophic types of lakes were investigated. Water samples were collected at a depth of 10 (spring)- 30cm (other water bodies) and at a distance of 0.5m from the bank, at three sites. Nineteen water parameters were determined (Table 1, only as representatives for particular types of water bodies) according to generally accepted methods (Golterman and Clymo, 1969; APHA, 2005).

The soil species was collected from roots of common bean, common serradella, common vetch, pea and sugar been (Table 4) at farmed land of north-eastern Poland. The amino acid, carbohydrate and urease assimilation tests were performed according to Yuasa and Hatai (1996) and Kitancharoen and Hatai (1998).

Determination of *Aphanomyces* species

Thickness, height and branches of the hyphae were measured in *Aphanomyces* species. The diameter of zoosporangia, zoospores, cystospores, oospores, oogonia and antheridia were defined. Morphological structure of the oogonia, antheridia and their number were also studied. It was investigated, if the oogonia are mono-, diclinous or androgynous. Some parasitic *Aphanomyces* species showed the repeated zoospore emergence (RZE) as adaptation to the parasitic mode of life (Cerenius and Söderhäll, 1985). For parasitic species (plant and animal) general principles of culture procedures were used (Seymour and Fuller, 1987; Roberts et al., 1993; Willoughby and Roberts, 1994; Paterson and Bridge, 1994; Watanabe, 2002). The parasitic species of the sugar beet and of some species of the legumes (soil species) were isolated according to Dyer et al. (2004) and Sauvage et al. (2007) methods. The isolates collected from the necrotic roots of the investigated plants were transferred to the corn meal agar (CMA, Difco, Detroit, MI) supplemented with rifampicin (38 mg/l). For each isolate, a single hyphal tip was transferred to a new CMA plate to insure only one genotype per isolate. The cultures were stored in sterile distilled water and were replaced in storage by culturing on CMA with rifampicin to repeat the process for storage (Parke and Grau, 1992).The taxa of *Aphanomyces* species were identified using the keys of Scott (1961), Batko (1975), Pystina (1994), Johnson et al. (2002) and Petrini and Petrini (2013).

RESULTS

The water samples used for the analysis differed in nutrient content and other parameters (Table 1). The highest nitrogen, sulphates and chlorides content was found in Spring Cypisek, Akcent and Fosa, Komosa Pond. The lowest content of nitrogen, sulphates and chlorides was noted in Hancza Lake. 30 taxa from *Aphanomyces* genus were found in the water bodies and soil of north-eastern Poland (Table 2). Same species occurred in all types of the investigated water reservoirs. *A. astaci*, *A. laevis*, *A. irregularis*, *A. parasiticus* and *A. stellatus* belong to this group. Such species as *A. apophysii*, *A. coniger*, *A. ovidostruens*, *A. polysporus*, *A. scaber* and *A. volgensis* were found only on the plant debris, other species also on animals' substratum (Table 3). *A. cochlioides* and *A. euteiches* are the parasit on cultivated plants (Table 4). 6 *Aphanomyces* species including: *A. frigidophilus*, *A. invadans* and *A. piscicida* and others were present on the fish eggs (Table 5). Investigated *Aphanomyces* species assimilated only three amino acids: alanine, glutamine and cysteine and from carbohydrates only glucose and starch, but they did not assimilate urease (Table 6).

DISCUSSION

Thirty taxa *Aphanomyces* were found in the water bodies of north-eastern Poland (Table 2). Five species occurred in all types of investigated water bodies, two species- *A. cochloides* and *A. euteiches* occurred in humid soils. *A. cochlioides* is a parasite of roots of sugar beet, *A. euteiches* of pea and other legumes species roots. The infected roots of plants ranged from 31.8% (common bean) to 50.7% (pea) during humid years. Therefore, soil species of the *Aphanomyces* genus are economically important, especially for the food industry (pea, sugar beet) (Levenfors and Fatehi, 2004) and for some plant

Table 1. Chemical and physical properties of water in represented of particular types of the water bodies (in mg l^{-1}).

Specification	Spring Cypisek	Stream Miahka	River Narew	Pond Komosa	Lake				
					Białe W. (a)	Hańcza (b)	Wigry (c)	Blizno (d)	Suchar (e)
Temperature (°C)	13.5	19.7	16.2	17.4	16.8	16.2	16.4	13.8	19.2
pH	7.4	8.0	8.0	7.6	8.0	8.1	8.2	8.3	5.2
DO	9.0	18.2	15.0	12.8	16.8	21.4	18.8	16.4	7.6
BOD_5	1.8	3.8	3.2	7.4	1.2	1.6	3.4	2.8	2.4
COD	4.6	6.0	7.0	13.2	4.8	5.2	9.5	6.2	27.9
CO_2	11.0	10.4	6.6	8.3	15.4	6.1	10.4	5.3	6.6
Alkalinity in $CaCO_3$ (mval l^{-1})	4.9	3.1	3.5	3.9	1.8	2.4	3.1	3.2	0.4
N-NH_3	0.1	0.05	0.33	0.16	0.05	0.13	0.98	0.27	1.78
N-NO_2	0.01	0.02	0.0	0.01	0.0	0.0	0.01	0.03	0.01
N-NO_3	0.95	0.09	0.0	0.03	0.01	0.02	0.48	0.29	0.03
P-PO_4	0.78	0.48	0.59	0.26	0.08	0.18	0.94	0.15	0.01
Sulphates	56.83	42.3	11.2	42.8	17.4	15.7	10.2	14.1	6.9
Chlorides	31.0	10.5	24.5	23.5	10.2	13.3	18.6	15.2	170.1
Calcium	119.5	64.3	17.2	68.4	23.7	29.7	32.4	45.4	6.5
Magnesium	18.5	10.9	29.1	28.8	6.2	12.1	10.8	12.1	0.01
Iron	0.2	0.52	0.53	1.53	0.0	0.0	0.04	0.12	0.02
Manganese	0.0	0.04	0.03	0.02	0.0	0.01	0.10	0.01	0.0
Dry residue	478.0	286.0	402.0	375.0	86.8	175.4	208.0	196.0	80.0
Dissolved solids	316.0	264.0	256.0	312.0	32.1	161.1	180.0	158.0	8.0
Suspended solids	162.0	22.0	146.0	63.0	14.7	14.3	28.0	38.0	72.0

(a) – oligotrophic; (b) – α-mesotrophic; (c) – β-mesotrophic; (d) – eutrophic; (e) – dystrophic.

Table 2. *Aphanomyces* species founded in particular types of the environment.

Taxa	Cultivated soil	Spring	Stream	River	Pond	Lakes group				
						a	b	c	d	e
A. acinetophagus A. F. Bor. & F.T. Wolf										x
A. americanus (A. F. Bor. & F.T. Wolf) W.W. Scot									x	
A. *amphigynus* Cutter		x		x	x			x	x	
A. *apophysii* Lacy			x		x			x		
A. *astaci* Schikora		x	x	x	x	x	x	x	x	x
A. bosminae W.W. Scott		x			x				x	
A. cochlioides Drechsler	x									

Table 2. (Contd.)

A. coniger H.E. Petersen			x		x			x		
A. daphniae Prowse		x		x	x				x	
A. euteiches Drechsler	x									
A. eut. f. sp. *phaseoli* W.F. Pfen. & D.J. Hag.	x									
A. eut. f. sp. *pisi* W.F. Pfen. & D.J. Hag.	x									
A. exoparasiticus Coker et Couch			x		x		x			
A. frigidophilus Kitanch. & Hatai		x	x	x	x				x	
A. helcoides Minden		x	x							
A. hydatinae Valkanov								x		
A. invadans Willoug. et al.					x					
A. irregularis W.W. Scott		x	x	x	x	x	x	x	x	x
A. keratinophilus (Okub. & Kob.) R.L. Seym. T.W. Joh.		x		x					x	
A. laevis de Barry		x	x	x	x	x	x	x	x	x
A. norvegicus Ville		x	x							
A. ovidestruens Gickelh.				x					x	
A. parasiticus Coker		x	x	x	x	x	x	x	x	x
A. phycophilus de Bary			x		x					
A. piscicida Hatai				x	x					
A. polysporus Milovtz.					x					
A. scaber de Barry									x	
A. sparrowii Cutter									x	
A. stellatus de Bary		x	x	x	x	x	x	x	x	x
A. volgensis Domash.			x	x						
Total number of taxa	4	12	13	12	16	5	6	9	14	6

species used as animal foodstuff for domestic animals (Papavizas and Ayers, 1974; Holub et al., 1991; Brantner and Windels, 2001).

Most *Aphanomyces* species were found in such eutrophic reservoirs of the stagnant water bodies as ponds (16) and eutrophic lakes (14 species), (Table 2). Only a few *Aphanomyces* species were found in water with a small content of the biogenic substances. There are oligotrophic, α-mesotrophic and dystrophic lakes (5-6 species). In the flowing waters (springs, streams, rivers) the authors found many (12-13) *Aphanomyces* species and such species as *A. astaci*, *A. irregularis*, *A. laevis*, *A. parasiticus* and *A. stellatus* were found in all types of the investigated water bodies. According to this fact they are called the eurytrophic species and have a wide range of the ecological tolerance. There are various organic compounds of carbon, free saccharides, free amino acids, numerous enzymes and other compounds (Hoagland et al., 1993) called extracellular products (being excreted by the phytoplankton and makrophytes) which exert the influence on the mycotal species in water. They are a medium for different heterotrophic organisms including mycotal species. As it is known, in oligotrophic, α-mesotrophic and dystrophic lakes there is a little amount of biogenic compounds, the phytoplankton and the macrophytes excreting less extracellular products serving as nutrients for heterotrophic organisms.Such species as *A. apophyscii*, *A. coniger*, *A. ovidostruens*, *A. polysporus*, *A. scaber* and *A. volgensis* were

Table 3. Ecological-physiological groups of the *Aphanomyces* species founded in north-eastern Poland.

Farming plants parasite	Algal-fungal parasite	Crayfish, fish parasite	Invertebrate parasite	Saprotrophs species	Saprotroph/opportunis species
A. cochlioides	*A. apophysii*	*A. astaci*	*A. acinetophagus*	*A. amphigynus*	*A. frigidophilus*
A. euteiches	*A. exoparasiticus*	*A. invadans*	*A. americanus*	*A. coniger*	*A. irregularis*
A. eut. f. sp. *phaseoli*	*A. norvegicus*	*A. piscicida*	*A. bosminae*	*A. helicoides*	*A. laevis*
	A. phycophilus		*A. daphniae*	*A. keratinophilus*	*A. parasiticus*
A. eut. f. sp. *pisi*	*A. scaber*		*A. hydatinae*	*A. polysporus*	*A. stellatus*
	A. sparrowii		*A. ovidestruens*	*A. volgensis*	
Number 4	6	3	6	6	5

Table 4. The infection of some farming plants by two *Aphanomyces* species.

Cultivated plant	*Aphanomyces* species	% infected plants	
		Normal[1] season	Humidity[2] season
Common bean (*Phaseolus vulgaris* L.)	*A. euteiches* f. sp. *phaseoli*	11.6	31.8
Common serradella (*Ornithopus sativus* L.)	*A. euteiches*	26.1	38.4
Common vetch (*Vicia sativa* L.)	*A. euteiches*	19.8	49.2
Pea (*Pisum sativum* L.)	*A. euteiches* f. sp. *pisi*	24.2	50.7
Sugar beet (*Beta vulgaris* L.)	*A. cochlioides*	18.4	42.5

1. 4-6 clouded and rainy day in one month (P – 18 mm/month); 2. 12-18 clouded and rainy day in one month (P – 174 mm/month).

Table 5. The infection of crayfish and fish by some *Aphanomyces* species.

Aphanomyces species	Infection	Authors
A. astaci	-Plague of *Astacus* species and other crayfish	Söderhäll and Cerenius (1999)
A. frigidophilus	- 22.8% eggs of Atlantic salmon (*Salmo salar* L.)	Czeczuga et al. (2011°)
	- Mass mortality of wild and farmed fish	Lilley et al. (2003)
A. invadans	- 3.6% of eggs of Chinook salmon (*Oncorhynchus tschawytscha* Wal.)	Czeczuga et al. (2011b)
A. irregularis	- Mass mortality of rainbow trout (*Oncorhynchus mykiss* Wal.)	Chien (1981)
A. laevis	- 11.4% eggs of Chinook salmon (*Oncorhynchus tschawytscha* Wal.)	Czeczuga et al. (2011b)
A. parasiticus	- 2.5% of eggs of Atlantic salmon (*Salmo salar* L.)	Czeczuga et al. (2011a)

Table 6. Amino acid, carbohydrate and urease assimilated by two *Aphanomyces* species.

Species	Amino acid	Carbohydrate	Urease
A. *frigidophilus*	Ala, Cys, Glu	Glu Sta, Tre	-
A. *laevis*	Glu	Glu, Sta	-

Abbreviations: Amino acids: Ala – alanine; Cys – cysteine; Glu – glutamine. Carbohydrate: Glu – glucose; Sta – starch; Tre – trehalose.

found only on the plant substratum, other species also on the animal substratum. The 9 *Aphanomyces* species present in fish, *A. invadans* and *A. piscicida* occurred only on fish eggs. *A. euteiches* is the parasite of Fabaceae family (Papavizas and Ayers, 1974). *A. astaci*, *A. invadans* and *A. piscicida* are animal parasites. *A. astaci*, which has been described by Schikora (1903), infects freshwater crayfish (Söderhäll and Cerenius, 1999). Both *A. invadans* and *A. piscicida* devastated natural and cultured stocks of freshwater and estuarine fish (Chinabut, 1998; Johnson et al., 2004). Some authors confirmed this both species as synonym (Dieguez-Uribeondo et al., 2009), others- as separate species (Phadee et al., 2004a, b). *A. piscicida* was described by Hatai (1980) and causes mycotic granulomatosis in fish (MG) (Egusa and Masuda, 1971), whereas *A. invadans* was described by Willoughby et al. (1995) and is an agent of EUS (Epizootic ulcerative syndrome). Kitancharoen and Hatai (1997) described new species of *A. frigidophilus* from eggs of Japan charr. In Europe, first *A. frigidophilus* was described on eggs of some species of coregonide fishes (Czeczuga et al., 2004). Two years later, Ballesteros et al. (2006) found this species in cuticule of dead crayfish *Austropotamobius pallipes* in Spain and Kiziewicz et al. (2013) observed its occurrence in water from the some springs of north- eastern Poland. Growth of *A. frigidophilus* was also stated on eggs of some species from *Salmo* genus (Czeczuga et al., 2004, 2011a), sturgeonids, Chinook salmon (Czeczuga et al., 2011b) and African catfish (Czeczuga et al., 2013).

As shown in Table 3, such species as: *A. irregularis*, *A. laevis*, *A. parasiticus* and *A. stellatus* were found on plant debris and on fish eggs. They all belong to opportunistic pathogens, which are sapro- and necrotrophic species similar to many species belonging to *Achlya* and *Saprolegnia* genera. Accourding to our study such saprotrophic species as *A. amphiginus* and *A. helicoides* were growing on decaging invertebrate animals and plant debris. According to Dieguez-Uribeondo et al. (2009). *A. helicoides* belongs to saprotrophic- opportunistic group. The most numerous pathogens from this group on fish eggs are: *A. laevis* (41 species) and *A. stellatus* (29). *A. irregularis* was found on eggs of 8 fish species, *A. parasiticus*- on 7. Mentioned species were also present on the eggs of lamprey (Czeczuga, 1997). According to Batko (1975) and Pystina (1994) *A. irregularis* is saprotrophic species and *A. parasiticus*-parasite on other fungal species, especially from *Achlya* genus. *A. laevis* both- eggs and adult individuals of many economically valuable fish species (Lartzeva, 1986; Dudka et al., 1989). *A. bosminae* and *A. daphniae* (occurring on fish eggs) were found in some of investigated water bodies. *A. bosminae* was firstly described by Scott (1961) and first investigated fungus in America. It is a parasite of freshwater crustacean from the *Bosmina* genus. The authors found it on the eggs of *Leuciscus leuciscus* in water from River Suprasl (Czeczuga and Muszynska, 1999). *A. daphniae* is known to be a parasite of the plankton crustacean *Daphnia hyalina* (Leydig) (Prowse, 1954). In our study its growth was revealed on the eggs of *Cobitis aurata* (Filippi) in River Suprasl water (Czeczuga and Muszynska, 1997).

The occurrence of *Aphanomyces* species has been registered in water bodies of African continent by El- Sharouny and Badram (1985), Shaheen et al. (1999), El- Hissy et al. (2004) and Ali (2009). *Aphanomyces* species have been associated with skin lesions and mortality of some fish species. This phenomenon has been observed in two species of fishes from excavated earthen pond at the western shore of the Suez Canal, Egypt, during the winter of 1971 (Shaheen et al., 1999). According to Lilley et al. (2003) this invasive *Aphanomyces* fish infection has been reported by Shaheen et al. (1999) and has been caused by *A. invadans* species. It has also been observed on skin-muscles on aquarium species of *Labeo bicolour* Smith specimens and on the alevins of the Nile tilapia- *Oerochromis niloticus* L.

The assimilation of the amino acids, carbohydrate and urease by two investigated *Aphanomyces* species was very small. Specimens of *A. frigidophilus* from fish eggs have assimilated only 3 amino acids, 3 carbohydrates, whereas *A. laevis* assimilated only one amino acid and 2 carbohydrates. Whereas specimens of *Saprolegnia*, *Achlya* or *Pythium* assimilated 4 to 5 amino acids, 9 to 16 carbohydrates and urease (Kitancharoen and Hatai, 1998). The specimens

of both species from *Aphanomyces* genus did not assimilate urease.

Conclusions

All together, 30 taxa of *Aphanomyces* genus occurred in 225 water bodies and some farming land of north-eastern Poland. This straminipiles species was found in limnologically and trophically different springs, rivers, ponds and lakes. Two species were found in farming land. All 30 *Aphanomyces* taxa belonged to four groups: a) plant parasite species, b) animal parasites, c) saprotrophic/opportunistic species and d) saprotrophic species. Group a) consists of: *A. cochlioides* and *A. euteiches*, group b) *A. astaci*, *A. invadans* and *A. piscicida*. *A. astaci* are etiologically an agent of the crayfish plague. *A. invadans* and *A. piscicida* infect freshwater and estuarine fishes as well as devastation of natural and cultured stocks (epizootic ulcerative syndrome). Some species, for example: *A. acinetophagus*, *A. exoparasiticus*, *A. hydatinae* and *A. sparrowii* occurred on some algae, straminipiles and invertebrates species as their parasites. Group c) - *A. frigidophilus*, *A. irregularis*, *A. laevis*, *A. parasiticus* and *A. stellatus* belong to the group of saprotrophic/opportunistic. These species are growing on decaying plant and animal debris (saprotrophic) and on fish eggs (parasitic). Fourth group- d) includes saprotrophic species fragments which grow only on decaying plant and animal debrits. Representatives of this group are: *A. amphiginus, A. coniger*, *A. helicoides*, *A. keratinophilus*, *A. polysporus*, and *A. volgensis*. Pathogenic *Aphanomyces* species in soil and in water bodies play economically significant role.

Conflict of Interests

The authors have not declared any conflict of interests.

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