Polish Journal of Environmental Studies Vol. 14, No 5 (2005), 691-698

Letter to Editor

Zoosporic Microorganisms Isolated from *Dermacentor reticulatus* F. Ticks Found in Surface Waters of the Białowieża National Park

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Received: January 13, 2004
Accepted: May 19, 2005

Abstract

The occurrence and growth of aquatic zoosporic microorganisms on the tick *Dermacentor reticulatus* F. were investigated in laboratory conditions. Water samples were collected from sites of the Narewka River and natural ponds in Białowieża National Park. Some physico-chemical parameters were analyzed as well. The highest contents of ammonium nitrogen and phosphates were found in the water of the Narewka River. Forty-six species of zoosporic microorganisms were isolated from *Dermacentor reticulatus* F. ticks, including 8 of the Chytridiomycetes class, 1 of Plasmodiophoromycetes, 35 of Peronosporomycetes and 2 Zygomycetes. The highest number of species was identified in ponds situated in the village of Białowieża (30), the lowest (22) in the Narewka River outside the village. The most common aquatic Peronosporomycetes included *Achlya americana, Ac. prolifera, Aphanomyces laevis, Ap. irregularis, Dictyuchus monosporus* and *Saprolegnia ferax*, found on the fragments of most ticks in the river and pond water. Cluster analysis of the investigated parameters revealed that the environmental factors determining the number of aquatic zoosporic microorganisms was oxidability in the Narewka River, and nitrate and nitrite in the ponds of Białowieża village.

Keywords: zoosporic straminipiles, *Dermacentor reticulatus* F. ticks, river, ponds, hydrochemistry, Białowieża National Park

Introduction

Water microorganisms, a biological factor of the aquatic ecological system, significantly influence the environment and its modification. They decompose the organic matter in water reservoirs and colonize dead animal and plant fragments both in natural and laboratory conditions. They can also act as facultative parasites and then frequently occur on their hosts [1]. Zoosporic chitinophylic fungi growing on arthropods that fall down onto the bottom of water reservoirs play an important role in aquatic ecosystems. Fungi and straminipiles deserve special attention as they show a special proteolytic nature. First of all, they are able to decompose animal chitin, thus facilitating mineralization of animal remnants and contributing to the self-cleaning process of the aquatic environment [2-4]. Most of the ticks carry pathogenic microorganisms among animals and humans. The *Dermacentor reticulatus* F. is a three-host tick, which leaves the host after sucking the blood at each developmental stage and remains on the ground until the subsequent activity period. Under Polish climatic conditions, the feeding period of the ticks' active developmental stages lasts from spring to autumn, with distinct spring and summer-autumn peaks for the adult form and summer peak for the young form [5].

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The primary aim of the current research was:
- to isolate zoosporic microorganisms growing on the tick *Dermacentor reticulatus* F. in the Narewka River and in natural ponds of Białowieża village within the area of Białowieża National Park,
- to determine the influence of environmental parameters on the development of microorganisms.

Material and Methods

The study of the occurrence of fungi and zoosporic organisms on the *D. reticulatus* F. tick was conducted in the Narewka River and natural ponds in the village of Białowieża within Białowieża National Park.

Water samples were collected from two different reservoirs (Fig. 1):

(I) The Narewka River on the Polish side, 39.4 kilometers long, sets off from the territory of the Republic of Belorussia, a left-bank tributary of the Narew River. In the upper course, the Narewka River flows across the area of Białowieża National Park. Mycological investigations were conducted at two measurement points: the upper and lower, twice a year, in spring and autumn.

(II) Natural ponds, 2 hectares in Białowieża National Park overgrown by rushes; supplied by the Narewka River; sites of carp and crucian carp breeding for angling.

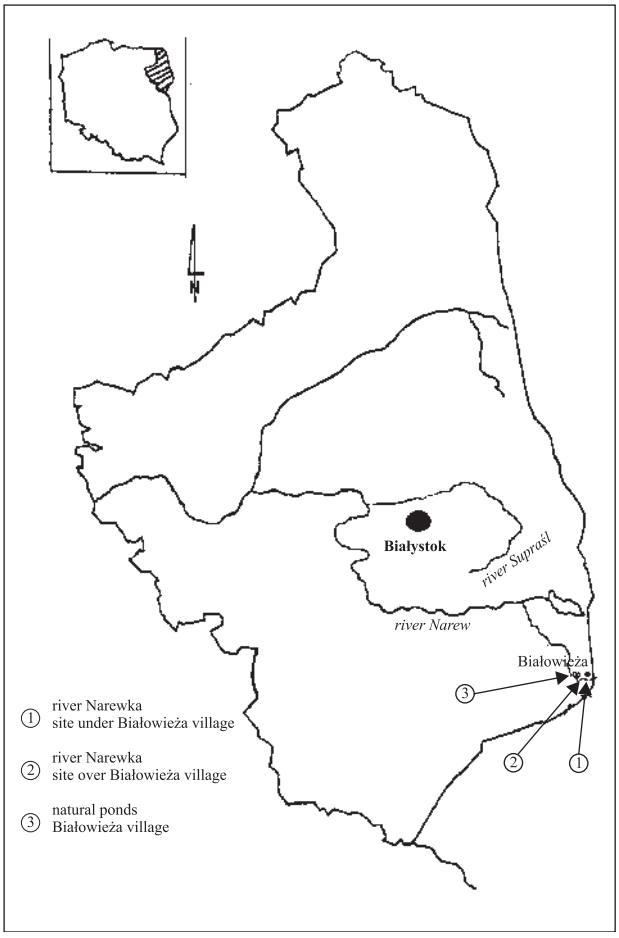

Fig. 1 – Map. A sites of the Narewka River and natural ponds in Białowieża National Park.

Eight water samples were collected from each site. Bait method using fragments of *D. reticulatus* F. placed in water samples from each reservoir was employed to isolate microorganisms.

The ticks used in the experiment were collected from free living *Bison bonasus* aurochs, which were killed by selection in December 2002-March 2003 within Białowieża National Park. Fragments of *Dermacentor reticulates* F. ticks were placed in containers with water samples collected from the respective reservoir or waterhouse. The containers were covered with glass plates to protect the water, at least partly, against bacteria. The samples were stored for four weeks in the laboratory at a temperature approximating that of water in rivers and ponds in the respective month. At that time, lighting and warming were regulated. The microscopically detected mycelia were transferred to sterilized Petri plates containing distilled water. The affected insects were observed under a light microscope every day starting from the third day of the culture. At the same time the respective developmental stages of microorganisms were determined using an ocular micrometer. The identification microorganisms were based on characteristics observed under the microscope, such as shape and size of hyphae, shape of sporangium and spores and the structure of oogonium, oospores and antheridium. The species were identified following similar methods employed by Czeczuga et al. [6].

Water samples used for physico-chemical analysis were collected approximimately from 0.20 m under water surface with a Ruttner's apparatus of 2.0 dm^3 vessel. In natural conditions and in the laboratory, the hydrochemical analysis was performed to determine temperature, pH, oxidability, ammonium nitrogen, nitrate nitrogen and phosphates. Physico-chemical investigations were performed using commonly applied hydrobiological methods [7,8].

The results were processed by cluster analysis [9].

Results

Physico-chemical parameters of water used for the experiment are presented in Table 1 and Figs. 2 and 3. The highest contents of ammonium nitrogen and phosphates were noted in the Narewka River.

Forty-six species of zoosporic microorgansims, belonging to such classes as Chytridiomycetes (8), Plasmodiophoromycetes (1), Peronosporomycetes (35) and Zygomycetes (2) were observed on *Dermacentor reticulatus* F. ticks in the water of the Narewka River and Bialowieża ponds (Table 2, Fig. 4). Thirty species (the highest number) were found in the ponds of Białowieża village, followed by 22 in the Narewka River outside the village. The most common Peronosporomycetes species including *Ac. americana*, *Ac. prolifera, Aphanomyces laevis, Ap. irregularis, Dictyuchus mono-*

Table 1. Physical and chemical charactors (in mg l^{-1}) of water from the different sites (mean from 3 samples).

Specification	Water bodies	
	Narewka River	Białowieża ponds
Temperature (°C)	8.4	12.5
pH	7.2	7.9
Oxidability	15.4	12.6
N-NH_4	0.610	0.450
N-NO_2	0.014	0.010
N-NO_3	0.17	0.22
P-PO_4	0.83	0.40

sporus and *Saprolegnia ferax* were found on the fragments of most ticks isolated from the river and natural pond water.

As shown by cluster analysis of the investigated parameters, oxidability in the River Narewka and nitrite and nitrate levels in the Białowieża ponds had the most substantial effect on the number of aquatic filamentous microorganisms.

Discussion

Forty-six species of zoosporic microorganisms were isolated on the tick *Dermacentor reticulatus*. The most common species included *Aphanomyces amfigynus, Ap. astaci, Ap. laevis, Ap. stellatus, Ac. diclina, Ac. klebsiana, Ac. oligacantha, Catenaria verrucosa, Catenophlyctis variabilis, Phlyctochytrium aureliae, Pythium artotrogus, Sommerstorffia spinosa* and *Zoophagus insidians*.

The three species of Chytridiales isolated in the study were *Karlingia chitynophila*, *Phlyctochytrium aureliae* and *Rhizidium chitinophilum. Phlyctochytrium aureliae,* found in the Narewka River in the current study, had been previously grown on dead organisms by Umphleett and Olson [10] and classified as phyto- and zoo-saprophytes. Miller [11] observed this species on shrimp exuviae, while Dogma [12] on plant fragments in the lakes of the U.S. state of Michigan.

Four species of the order Blastocladiales were isolated on *Dermacentor reticulatus* in the Narewka River, namely *Allomyces arbuscula, Catenaria anguillulae, C. verrucosa)* and *Catenophlyctis variabilis. Catenaria verrucosa*, a frequently detected species in the current study, was also reported by Gaerthner [13] from crustacean *Daphnia magna* and by Peterson [14] from snake exuviae. This fungus was frequently isolated in the waters of northeastern Poland on animal substrates by Czeczuga and Godlewska [2] and Czeczuga and Muszyńska [15].

Catenophlyctis variabilis is a well known saprobiont found on keratin- and cellulose-containing media. The species was first described by Karling [16], who isolated this fungus from ditch water, human skin, hair, fingernails, hooves, wool and from the soil in Brazil and the USA. Later, Booth and Barret [17] found this species on snake exuviae from Arctic ice water. Czeczuga and Muszyńska [15] identified this fungus on organic keratin-containing substrates from the waters of Northeastern Poland.

Representatives of the class Peronosporomycetes were the most numerous group of fungi found on *Dermacentor reticulatus*. Thirty-five taxons were isolated. *Ac. americana* was isolated from the ticks in the Narewka River and ponds of Białowieża village. Khulbe and Sati [18] found this species on fish in Asian mountain streams. In Poland, *Ac. americana* was observed on crayfish [19]. *Ac. klebsiana* was detected in the Narewka River and in natural ponds of Białowieża village. It was isolated by other authors on capped mushrooms, membraneous wings of flies and cover wings of potatoes beetles in ponds in Białystok [2]. *Ac. oligacantha* was found on ticks in natural ponds of Białowieża village. In Poland, it was found on dead flies from inland waters in Lubelskie Province [20], in peatbog waters of Podlasie Province [21] and on fish spawn [22].

Interesting is the finding of fungus-like organisms *Leptolegnia caudata* on *Dermacentor reticulatus* in the River Narewka and Białowieża village ponds. *Leptoleg-*

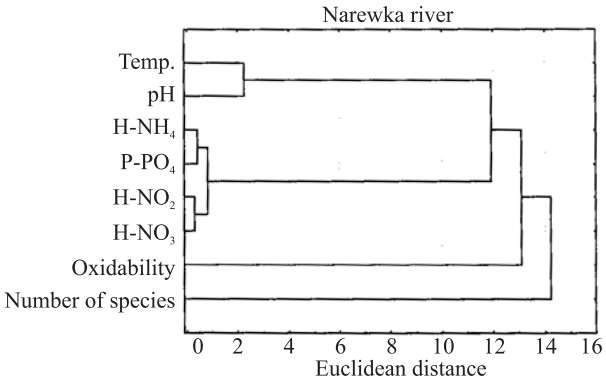

Fig. 2. Clustering of Narewka River according of water chemistry data and to number of fungus and straminipiles species.

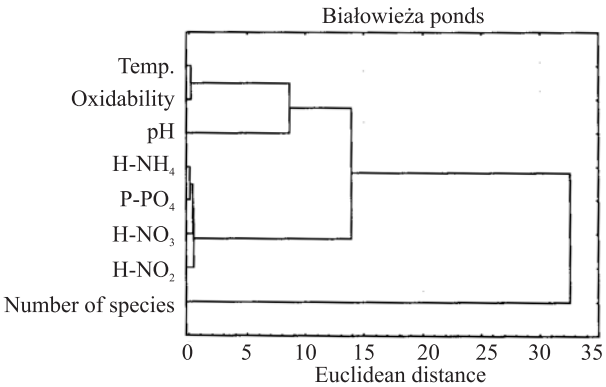

Fig. 3. Clustering of natural ponds in Białowieża according to water chemistry data and to number of fungus and straminipiles species.

Table 2. Zoosporic straminipiles found on *Dermacentor reticulatus* F. ticks in water from different places.

Class, order and species	Narewka River Site under Białowieża	Narewka River Site over Białowieża	Bialowieza Natural ponds
Chytridiomycetes			
Olpidiales			
Rozella septigena Cornu	+	-	+
Chytridiales			
Karlingia chitytwphila Karling	-	+	+
Phlyctochytrium aureliae Ajello	-	+	-
Rhizidium chitinophihim Sparrow	-	+	-
Blastocladiales			
Allomyces arbuscula Butler	+	-	+
Catenaria anguillulae Sorokin	-	+	+
C. verrucosa Karling	+	+	-
Catenophlyctis variabilis (Karling) Karling	-	+	-
Plasmodiophoromycetes			
Plasmodiophorales			
Woronina polycystis Cornu	-	-	+
Peronosporomycetes			
Saprolegniales			
Achlya americana Humphrey	+	+	+
Ac. apiculata de Bary	-	-	+
Ac. hypogyna Cocer et Pemberton	-	+	-
Ac. klebsiana Pieters	-	+	+
Ac. oligacantha de Bary	-	-	+
Ac. polyandra Hildebrandt	-	+	+
Ac. A.proliferoides	+	+	+
Ac. treleaseana (Humprey) Kauffman	-	+	-
Aphanomyces amphigynus Cutter	+	-	+
Ap. astaci Schikora	+	-	+
Ap. irregularis Scott	+	+	+
Ap. laevis de Bary	+	+	+
Ap. stellatus de Bary	+	+	+
Dictyuchus monosporus Leitgeb	+	+	+
Isoachlya unispora (Coker et Couch) Coker	+	-	+
Leptolegnia caudata de Bary	+	-	+
Saprolegnia anisospora de Bary	-	+	-
S. delica Coker	-	-	+
S. diclina Humphrey	+	-	-
S. ferax (Gruith.) Thuret	+	+	+

continued of Table 2.

S. glomerata (Tiesenh.) Lund	+	-	-
S. parasitica Coker	-	+	+
S. pseudocrnstosa Lund	-	-	+
S. terrestris Cookson ex Seymour	-	-	+
S. unispora Coker et Couch	+	+	-
Thraustotheca clavata (de Bary) Humphrey	-	-	+
Leptomitales			
Apodachlya brachynema (Hildebrand) Pringsheim	-	-	+
Leptomitus lacteus (Roth) Agardh	-	+	+
Pythiales			
Pythium afertile Kanouse et Humph.	+	-	-
Py. artotrogus de Bary	-	+	+
Py. aquatile Hohnk	-	+	-
Py. cactacearum Preti	+	-	+
Py. debaryanum Hesse	-	+	-
Py intermedium de Bary	+	-	+
Py. myriotylum Drechsler	+	-	-
Zygomycetes			
Zoopagales			
Sommerstorffia spinosa Arnaudov	-	-	+
Zoophagits imidians Sommerstorff	+	-	-
Total numbers of microorganisms	22	24	30

"+" indicates found, and "-" not found

nia caudata has been known as an aquatic and soil saprophyte found on crustaceans, dragonflies and spawn of various fish species [23, 24].

Saprolegnia anisospora, S. delica, S. diclina, S. ferax, S. glomerata, S. parasitica, S. pseudocrustosa, S. terrestris and *S. unispora* were isolated in the present experiment. These Perenosporomycetes are commonly encountered both on dead vegetation and animal material. However, in favorable conditions they parasite on weakened and mechanically damaged animals, mainly spawn and fish in their different developmental stages [25, 26, 27].

Thrausthoteca clavata, an aquatic-soil saprophyte, was found on *Dermacentor reticulatus* in Białowieża village ponds. This species has been frequently encountered in inland waters [23, 28].

Ap. laevis was found on ticks at all measurement points of the current study. Karling [29] observed this fungus in the water in New Zeland on the skin of insects and snakes. Staniak [20] isolated the species from flies in the water in Lubelskie Province. In northeastern waters of Poland, *Ap. laevis* has been detected on various insect species such as dragonflies, flies and potato beetles [2, 30]. *Ap. laevis* and *S. diclina* have been recognized by Dick [31] to be original colonizers of dead insects inhabiting the coastal zone of Marion Lake in Canada.

Ap. stellatus is an aquatic-soil microorganism. It was reported by Sparrow [32] from chitin substrates found in soil samples and by Staniak [20] and Zaborowska [33] from surface waters. *Ap. stellatus* was also detected on spawn in surface waters of northestern Poland [34]. *Ap. astaci*, which is a dangerous pathogen of crayfish, was not common to ticks in the current study. It was investigated by Söderhall and Dick [35], who reported it from the epidermis of crayfish from lakes in Turkey. *Ap. astaci* was found to inhabit plankton and benthos crustaceans and water insects by Czeczuga and Godlewska [3], and Czeczuga et al. [36, 37]. Spread of *Ap. astaci* throughout Europe has irreversibly eliminated the entire populations of the European crayfish from many river systems [38].

Two species of the order Leptomitales, namely *Apodachlya brachynema* and *Leptomitus lacteus* were detected on ticks in the Narewka River and in Białowieża ponds. *L. lacteus* is a known environmental bioindicator. Its presence in surface waters helps localize non-purified

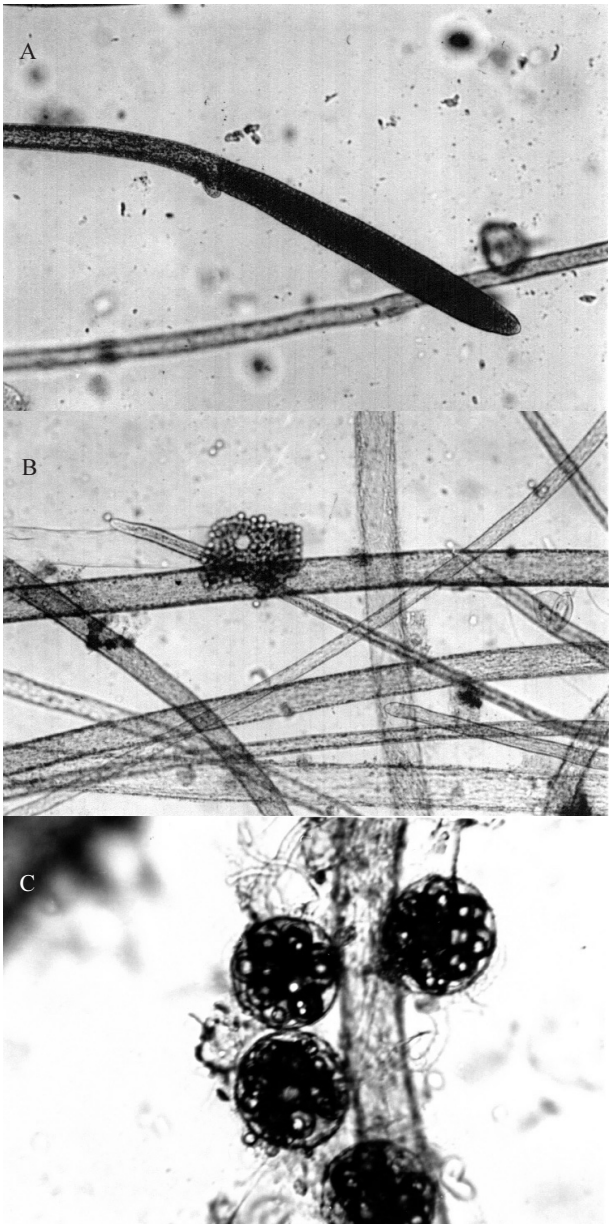

Fig. 4. *Achlya americana* growing on *Dermacentor reticulatus* F. a -hyphae from sporangium b - hyphae from sporangium with spores c - hyphae from oogonia.

sewage supplies from farms, creameries and other sources. *Leptomitus lacteus* is a pathogenic fungus of young perch [39]. The other fungus, *Apodachlya brachynema*, a rare species in Poland, occurred in Białowieża village natural ponds. The presence of species of the genus *Apodachlya* was reported from fish farm ponds in Lubelskie Province [20] and from lakes in northeastern Poland [40].

Species of the class Pythiales, such as *Py. afertile, Py, artotrogus, Py. aquatile, Py. cactacearum, Py. debaryanum, Py. intermedium* and *Py. myriotylum* were regularly found on ticks in water samples from the investigated sites. Until recently these Peronosporomycetes were regarded as aquatic-soil saprophytes or plant parasites [41]. Later studies have revealed that *Pythium* species grow in water reservoirs of varied trophicity (springs, rivers, ponds and lakes) on vegetation, dead animals or can also be animal parasites [42, 43, 44]. An example is *Py. artotrogus*, which grows as a saprotroph on plant fragments, dead flies and fish spawn [27, 45].

During the present study, we once found two predatory fungi of the class Zygomycetes, namely *Sommerstorffia spinosa* and *Zoopagus insidians*, on meadow ticks both in the River Narewka and in Białowieża village ponds. The former is a rare aquatic and soil fungus species, found to appear on rotifers and algae, like *Zoophagus insidians*. Predatory fungi have been described by Dodge [46], Sajkawa [47], Czeczuga and Próba [48], Czeczuga [49], and Kiziewicz and Czeczuga [50].

The analysis of the physical and chemical parameters characteristic of the water reservoirs involved in the present study revealed that the water of the Narewka River had the highest oxidability and the highest amounts of ammonium nitrogen and phosphates, while the water of the natural ponds of Białowieża village had the highest levels of nitrate nitrogen. The environmental parameters that determined the number of aquatic microbial species included oxidability in the Narewka River and the level of nitrite and nitrate in the natural ponds within Białowieża village.

Acknowledgements

The author are grateful to Prof. Małgorzata Krasińska and Dr. Zbigniew Krasiński of Białowieża National Park for their help in obtaining the *Dermacentor reticulatus* ticks.

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