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Parasitology

GROWTH OF ZOOSPORIC FUNGI OF THE EGGS OF NORTH PACIFIC SALMON OF THE GENUS *ONCORHYNCHUS* IN LABORATORY CONDITIONS

GRZYBY ZOOSPOROWE ROZWIJAJĄCE SIĘ NA IKRZE PÓŁNOCNO-PACYFICZNYCH ŁOSOSI Z RODZAJU *ONCORHYNCHUS* W WARUNKACH LABORATORYJNYCH

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The authors investigated of the mycoflora developing on the eggs of nine taxons of North Pacific salmon (genus *Oncor-hynchus*).

INTRODUCTION

Salmons of the genus *Oncorhynchus* which live in the waters of the North Pacific for the spawning time enter rivers or lakes of the North American and Asian continents (Bazarkin 1990). The following species are commonly found: pink salmon (Ishida 1967), chum salmon (Seno 1967), coho salmon (Smirnov 1975), masu salmon (Tsygir and Ivankov 1987), sockeye salmon (Parensky 1989) and chinook salmon (Vronskij 1972). After the spawning season most individuals die of exhaustion, which mainly refers to pink salmon and chum salmon that flow dead down the rivers and thus serve as food for birds and as culture medium for the growth of aquatic fungi.

Since these species provide valuable meat and red eggs rich in carotenoids which are the vitamin A provitamin (Czeczuga 1979), ichthyologists have become interested in the biology of these fish, reproduction in particular (Gribanov 1948; Sano 1959; Tanaka 1965; Roslyj et al. 1987; Savvaitova et al. 1989, Shuntov 1989; Chebanov 1990).

Studies on the aqatic fungi growing on species of the genus *Oncorhynchus* concern mainly young and grown-up individuals.

Kolgajev and Ivanova (1966) mentioned an infection in embryoes of autumn chum salmon, while Hatai and Egusa (1977) described saprolegniosis in the young of amago salmon (*Oncorhynchus rhodorus*). Studies on mycotic infections in adult individuals of the North Pacific salmon were prior to those in the young. Rucker (1944), in *Oncorhynchus* sp. individuals, found *Saprolegnia* sp., now included in the species *Saprolegnia parasitica*

(Neish and Hughes 1980), while in coho salmon individuals Mc Kay (1967) observed paraziting of Saprolegnia diclina. Neish (1976, 1977) investigated saprolegnosis in grown-up individuals of coho salmon and sockeye salmon, and according to the monograph data (Neish and Hughes 1980), those studies were concerned with the occurrence of Saprolegnia parasitica. Then, Hatai and Hoshiai (1992 a, b; 1993) dealt with saprolegnosis of coho salmon, demonstrating heavy losses caused by Saprolegnia parasitica in the breeding of this species, even up to 50% (Hatai 1980; Hatai and Hoshiai 1992 b). Hatai et al. (1977 a) revealed the growth of Saprolegnia australis on rainbow trout individuals and a new species – Saprolegnia shikotsuensis on kokanee salmon individuals (Hatai et al. 1977 b). Moreover, Neish and Hughes (1980) presented a series of colour photographs of saprolegnosis in the species of the genus Oncorhynchus: sockeye salmon, coho salmon, rainbow trout, amago salmon and chinook salmon. However, they did not give the names of fungus species that induced the mycosis. Marchenko (1988), investigated fungi of the Hyphomycetes that caused mycosis of pink salmo and chum salmo on fish-farms in Sakhalin.

Data concerning mycosis of the float in individuals of certain species of the genus *Oncorhynchus* can be found in the works of Hatai and Eguse (1977), Miyazaki et al. (1977), Tashiro et al. (1977) and Marchenko (1988).

The literature on saprolegnosis in the *Oncorhynchus* representatives revealed data concerning young and adult individuals, and not the eggs, except one – rainbow trout (Scott and O'Bier 1962; Czeczuga and Woronowicz 1993), Therefore, we decided te investigate the occurrence of zoosporic fungi on the eggs of certain species of the genus *Oncorhynchus* in laboratory conditions.

The literature survey shows that the only species described (four of them) belong to the genus *Saprolegnia* (Neish and Hughes 1980), although a number of species of other genera, such as *Achlya*, *Isoachlya*, *Dictyuchus*, and *Pythium* have been also observed in fish (Wilson 1976; Srivastava 1980; Czeczuga and Woronowicz 1993; Czeczuga 1994; Czeczuga et al. 1995).

Since no studies had been carried out on the fungi growing on the eggs of the *Oncorhynchus* species, except those by Taylor and Bailey (1979), who investigated the effect of NaCl on fungus growth inhabition on the eggs of pink salmon, we decided to determine which fungus species, not only those of the genus *Saprolegnia*, could grow on the eggs of the fish, bred on the American continent (Acara 1977) and the Asian continent (Smirnov 1975), including the Japanese Islands (Hatai et al. 1990).

MATERIAL AND METHODS

The investigations included the eggs (no fertilization) of the following fish species:

1. Oncorhynchus gorbuscha (Walbaum) – pink salmon; 2. Oncorhynchus keta (Walbaum) –

chum salmon; 3. Oncorhynchus keta autumnalis Berg – autumn chum salmon; 4. Oncorhynchus kisutch (Walbaum) – coho salmon; 5. Oncorhynchus masou (Brevoort) – masu salmon; 6. Oncorhynchus mykiss (Walbaum), syn. Salmo gairdneri Richardson – rainbow trout; 7. Oncorhynchus nerka (Walbaum) – sockeye salmon; 8. Oncorhynchus nerka (Walbaum) var. adonis – kokanee salmon, and 9. Oncorhynchus tschawytscha (Walbaum) – chinook salmon which were obtained from Kamchatka (GPO–Dalnyj, Petropavlovsk Kamčatskij; VRPO–Dalryba, Kamčatrybprom) and from Sakhalin (PO–Sachalinrybprom), Russia. The materials were transported in thermos flask (in physiological solution) by air mail. Whereas the eggs Oncorhynchus mykiss were obtained from A. Lityński Stock Centre of PAU in Gawrych Ruda, Suwałki district.

Table 1 Chemical composition (in mg l^{-1}) of the different water (April, 1994)

Specification	Pond	Lake	River
Temperature °C	10.08	9.04	8.12
pH	7.72	7.82	7.68
O_2	8.12	9.86	18.24
Oxidability	14.78	6.72	12.66
CO_2	42.90	15.40	26.40
Alkalinity in CaCO ₃ *	7.6	2.8	5.0
N (NH ₃)	0.220	0.825	0.630
N (NO ₂)	0.006	0.035	0.047
$N (NO_3)$	0.502	0.160	0.200
PO_4	1.306	0.250	0.255
Cl	90.0	36.0	48.0
Total hardness in Ca	97.92	144.74	108.72
Total hardness in Mg	21.07	14.62	20.64
SO ₄	32.09	47.31	48.55
Fe	0.505	0.350	0.545
Dry residue	600.0	256.0	454.0
Dissolved solids	558.0	250.0	417.0
Suspended solids	42.0	6.0	37.0

^{*} in myal 1-1

The water for experiments were collected from three different the water body: river Supraśl, pond in Branicki Park and lake Komosa. Eighteen parameters of these water samples were determined (Tab. 1) according to the generally accepted methods (Golterman and Clymo 1969).

For the determinations of the presence of aquatic fungus species on the eggs, the following procedure was employed: a certain amount of eggs (100–200) of each species of fish were transferred to two for each water a 1.0 litre vessel (together for each species was six vessel) and placed in the laboratory at a temperature approaching that of the given hatchery. The part of the eggs from

each vessel was observed under a microscope and the mycelium (form zoospore, oogonia and conidia) of aquatic fungi growing on the eggs was recorded. The methods were described in detail in paper Smith et al. (1985) and Fuller and Jaworski (1986). The eggs of the various fish species were examined for one to one and a half weeks. The eggs was mostly live but sometimes dead. Length of time of the experiments was three weeks.

For determinations of the fungi the following keys were used: Johnson (1956), Sparrow (1960), Seymour (1970), Kreger van Rij (1984) and Dick (1990).

Table 2

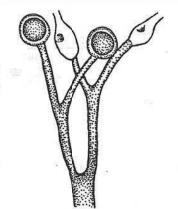
Aquatic fungi were found on the eggs of North Pacific salmon

	Salmon (see
Species of fungi	Materials and
W. S. L. D. L. (2000) III. 1977	Methods)
1 Achlya americana Humphrey	5,6
	4,6
3 Achlva caroliniana Coker	3.9
4 Achlya colorata Pringsheim	2.6
5 Achlya diffusa Harvey et Johnson	2.4
6 Achlya dubia Coker	2,6 2,4 9
7 Achlya flagellata Coker	5
8 Achlya hypogyna Coker et Pemberton	5,6
9 Achlya klebsiana Pieters	1,2,4,7
10 Achlya oligacantha de Bary	1,7
11 Achlya orion Coker et Couch	57
12 Achlya polyandra Hildebrand	2.6
13 Achlya racemosa Hildebrand	2,6 2,4,7
14 Achlya radiosa Maurizio	2,6,9
15 Achlya treleaseana (Humph.) Kauffman	6, 7, 8, 9
16 Allomyces arbuscula Butler	5
17 Aphanomyces laevis de Bary	3
18 Aphanomyces stellatus de Bary	5 3 7
19 Aplanes androgymus (Archer) Humphrey	5
20 Blastocladiopsis parva (Whiffen) Sparrow	1,3,4,5,7
21 Dictyuchus anomalus Nagai	6
22 Dictyuchus monosporus Leitgeb	3, 4, 5, 6, 7, 9
23 Dictyuchus sterilis Coker	3,8,9
24 Isoachlya anisospora (de Bary) Coker	2,4,6,8
25 Isoachlya monilifera (de Bary) Kauffman	6, 8
26 Legendium humanum Karling	3
27 Leptolegnia caudata de Bary	4, 6, 9
28 Leptomitus lacteus (Roth) Agardh	1,2,5,6,7,9
29 Polyphagus euglenae Nowakowski	1
30 Protoachlya paradoxa (Coker) Coker	6
31 Pythium artotrogus de Bary	6
32 Pythium middletonii Sparrow	4
33 Pythium proliferum de Bary	5
34 Pythium ultimum Trow	1,3,5,7
35 Saprolegnia anisospora de Bary	4
36 Saprolegnia asterophora de Bary	6
37 Saprolegnia australis Elliott	2,4,5,8,9 9
38 Saprolegnia delica Coker	9
39 Saprolegnia diclina Humphrey	8
40 Saprolegnia eccentrica Coker	3
41 Saprolegnia ferax (Gruith) Thurnet	1,2,3,4,6,7
42 Saprolegnia hypogyna (Pringsh.) de Bary	8
43 Saprolegnia irregularis Johnson et Seymour	8
44 Saprolegnia litoralis Coker	6,8
45 Saprolegnia mixta de Bery	1, 4, 5, 6, 7
46 Saprolegnia monoica Pringsheim	2,4,5,9
47 Saprolegnia parasitica Coker	1,2,3,4,5,6,
	7, 8, 9
48 Saprolegnia shikotsuensis Hatai et al.	2,4,5
49 Saprolegnia unispora (Coker et Couch) Seymour	4
50 Thraustotheca clavata (de Bary) Humphrey	1
51 Zoopage phanera Drechsler	6,7,9

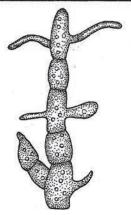
RESULTS

Fifty-one zoosporic fungus species and Candida albicans (Robin) Berk. conidial fungus were observed on the eggs of 9 salmon taxons of the genus Oncorhynchus (Tab. 2). The Achlya and Saprolegnia genera were the most common, each having 15 representatives. Saprolegnia parasitica was found to grow on the eggs of all the Oncorhynchus taxons examined while Blastocladiopsis parva, Dictyuchus monosporus, Leptomitus lacteus, Saprolegnia australis, Saprolegnia ferax and Saprolegnia mixta were observed on the eggs of most taxons. Achlya klebsiana, and Achlya treleaseana were the most common species of the genus Achlya, while Pythium ultimum of the genus Pythium.

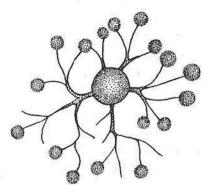
Most aquatic fungus species were found to develop on the eggs of rainbow trout individuals (twenty-one), fewest on the eggs of pink salmon, autumn chum salmon and kokanee salmon (ten on each) (Tab. 3).



a - Blastocladiopsis parva (thallus with spore $-34.5-56.2 \mu m$)



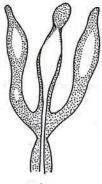
 $b-Lagenidium\ humanum$ (typical thallus)

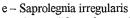


c - Polyphagus euglenae (thallus with rhizoids attached of eggs)



d – Saprolegnia eccentrica (gametangium– oogónium 28-36 µm)





A – proliferated secondary sporangia

B – oogonium (23.6–24.5 μm)

Fig. 1. New aquatic fungi to fishes



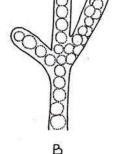


Table 3

Aquatic fungi were found on the eggs of particular North Pacific salmon

Species of salmon	Fungi (see Table 2)	Number of fungi
Oncorhynchus gorbuscha	9,10,20,28,29,34,41,45,47,50	10
Oncorhynchus keta	4,5,9,12,13,14,24,28,37,41,46,47,48	13
Oncorhynchus keta autumnalis	3,17,20,22,23,26,34,40,41,47	10
Oncorhynchus kisutch	2,5,9,13,20,22,24,27,32,35,37,41,45,46,47,48,49	17
Oncorhynchus masou	1,7,8,11,16,19,20,22,28,33,34,37,45,46,47,48	16
Oncorhynchus mykiss	1,2,4,8,12,14,15,21,22,24,25,27,28,30,31,36,41,44,	
	45,47,51	21
Oncorhynchus nerka	9,10,11,13,15,18,20,22,28,34,41,45,47,51	14
Oncorhynchus nerka var. adonis	15,23,24,25,37,39,42,43,44,47	10
Oncorhynchus tschawytscha	3,6,14,15,22,23,27,28,37,38,46,47,51	13

Of 51 species found, Blastocladiopsis parva, Lagenidium humanum, Polyphagus euglenae, Saprolegnia eccentrica and Saprolegnia irregularis are new to fish. However Pythium proliferum, Saprolegnia anisospora and Saprolegnia asterophora are rarely to fish (Fig. 1).

DISCUSSION

The fish species investigated, except rainbow trout, live a two-environmental mode of life: some of them spend one third or even half of their lives in fresh waters (Sano 1959; Tanaka 1965; French et al. 1976; Major et al. 1978; Takagi et al. 1981). The aquatic fungus species found on the eggs of 9 taxons of the genus *Oncorhynchus* have be observed several times in typically freshwater fish species of other genera, except for some fungus species new to fish, whose development we observed on the eggs of certain salmon species of the North Pacific.

The literature data (McKay 1967; Neish and Hughes 1980; Hatai and Hoshiai 1992 a, b, 1993; Hatai et al. 1977 b) reveal the growth of four fungus species of the genus *Saprolegnia – S. australis, S. diclina, S. parasitica*, and *S. shikotsuensis* on individuals of certain fish species of the genus *Oncorhynchus*. The present study confirms the growth of these fungi also on the fish eggs of the genus *Oncorhynchus* examined. The observed development of fungi other than of the genus *Saprolegnia* broadens considerably the list of species that can infect the eggs of the North Pacific salmon. Moreover, this atudy displays fungus species that have never been observed on dead and alive fish or eggs.

Blastocladiopsis parva, a species new to fish, was found in our studies on the eggs of pink salmon, autumn chum salmon, coho salmon, masu salmon and sockeye salmon. It is a common species in the waters of northeastern Poland, found in water bodies of various

types — limnocrenic springs (Czeczuga et al. 1989; Czeczuga and Muszyńska 1993). Marycha-type rivers (Czeczuga et al. 1990), high field peatbogs (Czeczuga 1993) and lakes of different size and trophicity (Czeczuga 1991; Czeczuga and Woronowicz 1991; Czeczuga 1994). Moreover, *Blastocladiopsis parva* grows on the chitin-containing substratum (Czeczuga and Godlewska 1994) and keratin-containing substratum (Czeczuga and Muszyńska 1994).

Another new species – *Lagenidium humanum* developed on the eggs of autumn chum salmon. It is regarded as a saprophyte of human skin and grows on such keratin-containing substrata as human hair and snake-skin (Czeczuga and Muszyńska 1994).

Polyphagus euglenae, an alga parasite, was observed on the eggs of pink salmon. It was also found in the waters of northeastern Poland as a parasite of *Euglena viridis* (Czeczuga and Woronowicz 1994).

Saprolegnia eccentrica, also a new species to fish, grows in soil, seldom in lakes (Seymour 1970). We observed its growth on the eggs of autumn chum salmon only in the water of Lake Komosa. Worth noting is the finding of Saprolegnia irregularis, previously observed only in water in Iceland on the eggs of kokanee salmon (Johnson and Seymour 1975). Rarely fungus to fish, Pythium proliferum, regarded as an aquatic saprophyte, was found to grow on the eggs of masu salmon. This fungus was observed on the eggs of some fish in hatcheries by Florinskaya (1969). Saprolegnia anisospora was observed on the eggs of coho salmon. According to Seymonr (1970), Saprolegnia anisospora is found in fresh waters and in soil; Newby (1948) observed its growth on dead roach, which was, however, not reported in the monographic surveys of fungi found on fish (Wolke 1975; Wilson 1976; Srivastava 1980; Neish and Hughes 1980; Dudka et al. 1989). The same can be said about Saprolegnia asterophora, which is usually found in freshwater and acid soil habitats. In our studies it was observed on the eggs of rainbow trout. Its growth was also noticed on dead fish, particularly on Leuciscus sp. (Petersen 1910) and other species (Hayren 1928).

In the present study, Saprolegnia parasitica was found on the eggs of all the taxons of the genus Oncorhynchus examined. This fungus grows in great mass, causing epizooties of the Atlantic salmon, Salmo salar (Munro 1970). Saprolegnia parasitica also attacks the eggs of other salmonid species (Osipian et al. 1988) and acipenserid species (Czeczuga et al. 1995).

Until 1988, rainbow trout belonged to the genus *Salmo* as *Salmo gairdneri* Richardson (Szlamińska 1991). It is one of the main species bred on a large scale on fish-farms not only in Europe. Thus, since the thirties of our century, myconis causing losses in the populations of this species has been intensively studied. Individuals of this species can be infected by *Aphanomyces laevis*, *Leptomitus lacteus*, *Saprolegnia delica*, *Saprolegnia diclina*, *Saprolegnia monoica* and *Saprolegnia parasitica* (Tiffney 1939; Scott and O'Bier 1962;

Florinskaya 1969; Chien Chiu Yuan 1981; Hatai et al. 1990). Moreover, Czeczuga and Woronowicz (1993) additionally revealed the presence of such fungi as *Achlya polyandra*, *Achlya radiosa* and *Saprolegnia ferax* on the eggs of rainbow trout.

The present study found *Candida albicans* on the eggs of masu salmon in the water of the river Biała. This fungus was previously observed on the eggs of *Coregonus albula* in a hatchery in Węgorzewo (Czeczuga and Woronowicz 1993) and on the fry of eel montee – *Anguilla anguilla* in the waters of Lake Mikołajskie (Czeczuga 1994 b). It should be mentioned that Hatai and Egusa (1975) isolated *Candida sake* cells from the gastrotympanites of amago salmon individuals.

Susceptibility of fish and their parts, eggs inclusive, to mycotic infections depends on a number of factors, both of biotic and abiotic nature. The mucous covering fish acts as a protective barrier and any damage to its layer allows fungi to anchor on the surface of the fish (Richards and Pickering 1978). It is also stress factor-dependent (Wedemeyer 1970; Śnieszko 1974), including excessive density of population, suboptimal water temperature and sublethal concentrations of various toxic chemical substances. Also, chemical composition of water plays a role in the occurrence of different fungus species on the eggs of the same fish species according to the source of water.

The present study has proved that the growth of respective aquatic fungus species on eggs of North Pacific salmon depends on the water body from which water has been collected for the experiment (Tab. 4 and 5). Most species developed on eggs in pond water. The chemical analysis of the water collected from these three water bodies found water differentiation as regards the content of chemical compounds (Tab. 1). Pond water had considerably more biogenic compounds, mainly phosphorus. This would confirm once again our earlier assumptions (Czeczuga and Woronowicz 1993; Czeczuga et al. 1995) that the degree of infection of fish eggs in hatcheries and in laboratory conditions depends largely on the state of cleanness and trophicity of water that supplies a given hatchery or test.

Table 4 Aquatic fungi were found on the eggs of North Pacific salmon in the different water

Water from	Fungi (see Table 2)	Total number
Pond	1,2,34,5,6,7,8,9,13,15,17,19,20,22,23,24,25,27,28,30,31,34,35,36,37, 38,39,41,45,46,47,48,49,50,51	36
Lake	1,2,4,14,15,16,17,19,20,22,23,24,26,27,28,32,33,34,35,36,37,40,41,42, 43,44,45,47,48,51	30
River	3,4,7,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,28,29,34,36,37, 41,43,44,45,47,48,51	31

 ${\bf Table~5}$ Aquatic fungi were found on the eggs of particular North Pacific salmon in the different water

Species	Fungi (see Table 2) in water from		
of salmon	Pond	Lake	Rivers
Oncorhynchus gorbuscha	9,20,34,41,50	20,28,34,45	10,29,41,47
Oncorhynchus keta	5,13,28,46	4,24,28,37,41,48	4,9,12,14,24,28,37,47
Oncorhynchus keta autumnalis	3,17,20,23,34,41	17,20,22,23,26,40,41	17,22,23,41,47
Oncorhynchus kisutch	5,35,37,45,46,47,48,49	2,32,35,37,41,45,48	24,37,41,45
Oncorhynchus masou	1,7,8,19,22,45,46,47	16,19,22,28,33,37,47	11,16,19,20,34,45,47,48
Oncorhynchus myk i ss	2,4,8,24,25,27,30,31,36,47	1,4,22,36,44,47,51	4,14,15,21,22,28,36,45,47
Oncorhynchus nerka	20,22,28,41,51	20,22,45	7,9,10,11,13,15,18,21,34,41,47
Oncorhynchus nerka var. adonis	23,24,27,37,39,47	15,23,24,37,42,43,47	37,43,44,47
Oncorhynchus tschawytscha	6,15,38,46	14,23,27,37,47	3,15,28,37,47,51

Certain fungus species first apper on dead eggs granules and very soon attack alive eggs, which results in great losses, even of the whole population of the incubated eggs on fish-farms (Wolf 1958; Florinskaya 1971; Sati and Khulbe 1981; Dudka et al. 1989; Lartseva and Dudka 1990).

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GRZYBY ZOOSPOROWE ROZWIJAJĄCE SIĘ NA IKRZE PÓŁNOCNOPACYFICZNYCH ŁOSOSI Z RODZAJU *ONCORHYNCHUS* W WARUNKACH LABORATORYJNYCH

STRESZCZENIE

Badano w warunkach laboratoryjnych występowanie grzybów wodnych na ikrze 9 taksonów pólnocnopacyficznych lososi należących do rodzaju Oncorhynchus. Badaniami objęto ikrę Oncorhynchus gorbuscha, Oncorhynchus keta, Oncorhynchus keta autumnalis, Oncorhynchus kisutch, Oncorhynchus masou, Oncorhynchus mykiss (syn. Salmo gairdneri), Oncorhynchus nerka, Oncorhynchus nerka var. adonis oraz Oncorhynchus tschawytscha.

Do doświadczeń używano wody ze stawu, jeziora i rzeki, uwzględniając w niej poszczególne parametry hydrochemiczne.

Ogólnie stwierdzono na ikrze rozwój 51 gatunków grzybów zoosporowych oraz 1 gatunek konidialny (Candida albicans). Najmniej gatunków rozwijało się na ikrze Oncorhynchus gorbuscha, Oncorhynchus keta autumnalis i Oncorhynchus nerka var. adonis (po 10), najwięcej zaś – na ikrze Oncorhynchus mykiss (21). Spośród stwierdzonych grzybów 5 takich gatunków jak Blastocladiopsis parva, Legenidium humanum, Polyphagus euglenae, Saprolegnia eccentrica oraz Saprolegnia irregularis okazały się nowymi dla ryb w ogóle.

Najwięcej gatunków grzybów rozwijało się na ikrze badanych łososi w wodzie ze stawu, która była najzasobniejsza w związki biogenne.

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