Archiv für Hydrobiologie
Vol. 172/2: 161–165, July 2008

A previously undescribed set of *Saprolegnia* spp. in the invasive spiny-cheek crayfish (*Orconectes limosus*, Rafinesque)

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With 1 figure

Abstract: Coinciding with a population decline in the invasive spiny-cheek crayfish *Orconectes limosus* in Lake Constance, SW Germany, we found crayfish specimens with a fungus-like Aufwuchs which after DNA-isolation and sequencing was identified as consisting of a set of previously undescribed *Saprolegnia* species. This finding may have implications for the farming and conservation of native crayfish as well as for the lake's ecosystem. We propose that spiny-cheek crayfish might function as a disease vector for these potential pathogens.

Key words: Oomycetes, invasive crayfish, population dynamics, Lake Constance.

Introduction

Freshwater crayfish are among the most commonly introduced species worldwide. In invaded systems they often constitute the largest part of the invertebrate biomass and they are ecosystem engineers, affecting all trophic levels and potentially causing the complete degradation of a freshwater ecosystem (Lodge et al. 2000, Statzner et al. 2000, Rodriguez et al. 2005). The most prominent impact of invasive freshwater crayfish species in Central Europe is their role as vectors of disease, in particular of the crayfish plague caused by *Aphanomyces astaci* (Schikora) (Unestam 1969, Cerenius et al. 1988). Crayfish native to North America are of special concern since they are resistant to the crayfish plague but can act as vectors for the disease, thus causing infection of native freshwater crayfish that are fully susceptible to the pathogen (Unestam 1972, Diéguez-Uribeondo & Söderhäll 1993, Söderhäll & Cerenius 1999). This is one of the reasons why invasive crayfish are at least in part responsible for an estimated one-third to one-half of the world's crayfish species being at risk of serious decline or even complete extinction (Taylor 2002). Apart from drastic effects on the conservational status of native crayfish, the harvest and aquaculture of native crayfish, both of economic and socio-cultural value in Europe (Taugbøl 2004), were negatively influenced and are still threatened by the spread of invasive crayfish and the diseases they carry. In Germany the establishment of the invasive North American spiny-cheek crayfish *Orconectes limosus* (Rafinesque) is the commonly accepted reason for the decline of native crayfish populations during the last century and the break-down of regional crayfish-farming (Bohl 1999, Dehus et al. 1999). The spiny-cheek crayfish is capable of carrying and transmitting the crayfish plague and was introduced for aquacultural purpose into Germany in 1890 (Schweng 1973, Vey

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DOI: 10.1127/1863-9135/2008/0172-0161 1863-9135/08/0172-0161 $ 1.25

et al. 1983). Today it is widespread in Central Europe and also in Lake Constance, SW Germany, where it appeared in the late 1980s and is of some commercial value to fishermen (Dehus et al. 1999). In this study, a spiny-cheek crayfish population in Lake Constance was identified as carrying oomycetes as Aufwuchs.

Material and methods

Four fyke-nets (see Balik et al. 2005 for fyke-net type) were exposed between the 27th August and the 29th September in 2004 as well as between the 25th August and the 12th September in 2005 in the littoral zone of Lake Constance near the island of Mainau at a riparian-strip of approx. 4 km length in a depth of 0.5 to 1.5 m (Coordinates: 47° 41′ 26″ N / 9° 12′ 10″ E, 47° 41′ 43″ N / 9° 11′ 39″ E). Fyke nets were emptied every four days. The total sampling effort was comparable between the two years. In 2005, white cotton-like patches distributed over the anterior part of the crayfish were frequently observed and investigated in detail. In the laboratory, using a microscope, patches obtained from the conspicuous crayfish were identified as filamentous mycelium. Mycelia from the Aufwuchs as well as from interior parts (abdominal tissues) of several animals were plated out onto selective agar medium as used for the isolation of *Pythium* spp. and other oomycetes (e.g. Nechwatal et al. 2005), and cultures were purified from bacteria. Altogether, seven isolates were obtained and cultivated on V8 and corn meal agar (Nechwatal et al. 2005), as well as on autoclaved hempseed halves (Diéguez-Uribeondo et al. 2007) for morphological studies. After DNA extraction and PCR with primers ITS1 and ITS4 targeting internal transcribed spacer (ITS) regions of the rDNA repeats (White et al. 1990), all isolates were sequenced using the above-named primers. Three representative sequences have been submitted to GenBank (accession ns. EF460349, EF460350 and EF460351). A BLAST search was performed to reveal the most closely related sequences from the GenBank database. Phylogenetic relationships of the taxa involved were analysed using neighbour-joining methods as described by Nechwatal et al. (2005), using the isolates' closest relatives as revealed by BLAST searches as well as sequences used in a recent molecular account of fish pathogenic *Saprolegnia* species (Diéguez-Uribeondo et al. 2007).

Results and discussion

In 2005, 83 crayfish were caught of which almost 25 % (20 out of 83 crayfish) showed white cotton-like patches distributed predominantly over the anterior part of the body. In contrast, in 2004, 547 crayfish were caught none of which showed any sign of filamentous Aufwuchs and therefore no further investigations were made. Sequencing results revealed that all isolates of the 2005 mycelium samples belonged to the genus *Saprolegnia*. The isolates sequenced were not identical in their ITS sequence, and fell into three different phylogenetic clades (a–c). According to BLAST searches in the GenBank database, only one of the clades could be unequivocally assigned to a described species. However, due to the lack of taxonomically unambiguous *Saprolegnia* sequences, species misassignments and species synonymy in GenBank, exhaustive comparative molecular studies in this genus are difficult, and likely to reveal inconsistent results. Hence, some of the isolates studied here gave several contradictory matches. They were most closely related to a) *S. diclina* s. str. (98 % identity), b) *S. australis* (100 %), c) *S. ferax, S. mixta* and *S. anomalies* (100 %), as well as to several unidentified *Saprolegnia* spp. deposited in GenBank. In summary, four of our isolates (b) grouped in molecular clade IV after Diéguez-Uribeondo et al. (2007), and were most likely identical to *S. australis*, two isolates (c) grouped in clade II, and one isolate (a) in clade III. Morphological studies partly confirmed the molecular classification; all isolates remained sterile after several months of storage on various standard agar media (V8 juice agar, oatmeal agar). On hemp seed cultures only the single isolate in clade a) (isolate ID: II-2) abundantly produced spherical, smooth-walled oogonia, measuring ca. 50–105 µm (mean 69.5 ± 13.4 µm SD), and containing ca. 5–30 (mean approx. 15) globose, centric to subcentric oospores relatively uniform in size (mean 23 ± 1.6 µm SD) (Fig. 1).

Saprolegnia spp. belong to the order of *Saprolegniales*, among which some of the most important fish pathogens as well as *A. astaci*, the causative agent of the crayfish plague can be found (Oidtmann et al. 2004). None of our isolates could unambiguously be assigned to a *Saprolegnia* sp. particularly known to be associated with diseased or dying crayfish. However, while isolates from groups a) and c) were affiliated with presumably saprophytic species from plant litter or pond water, most isolates (b) belonged to a phylogenetic clade comprising pathogenic isolates from salmonid fish lesions, originally isolated in Chile and designated as *S. australis* (Diéguez-Uribeondo et al. 2007). *Saprolegnia* spp. as pathogens and egg parasites are of major economic concern since they are responsible for devastating infections on fish and crayfish in aquaculture and farms (Melendre et al. 2006, van West 2006). Moreover, *Saprolegnia* spp. have been assumed to be associated with the decline of populations of the native crayfish species *Austropotamobius pallipes* (Lereboullet) in Spain (Gil-Sánchez & Alba-Tercedor 2006) and have been proven to cause mortality both in European as well as in North American crayfish species (Diéguez-Uribeondo et al. 1994). Therefore, it might well be that the *Saprolegnia* spp. found in this study might have caused disease in the crayfish specimens report-

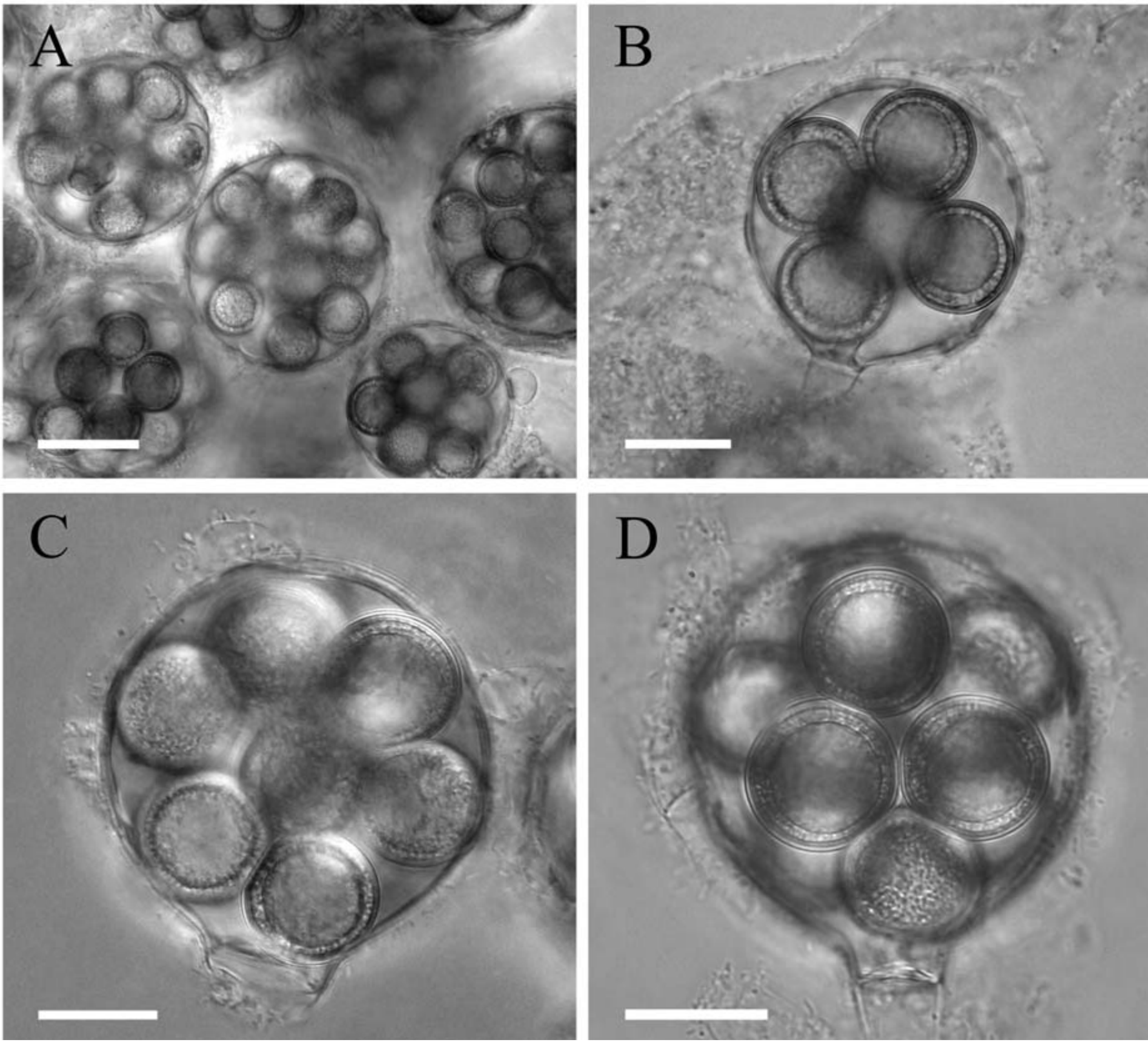

Fig. 1. Oogonia and oospores of *Saprolegnia* sp. isolate II-2 after seven days on autoclaved hemp-seed halves in water culture. **A:** general view (bar = 40 µm); **B–D**: detailed view of small **(B)** and medium-sized **(C–D)** oogonia (bar = 20 µm).

ed here. At least four of the affected crayfish showed signs of paralysis and lack of typical flight reaction in response to handling, which can be interpreted as signs of disease (Oidtmann et al. 1996). The fact that *Saprolegnia* spp. were isolated from Aufwuchs as well as from subcuticular tissues, the latter showing clear signs of local disintegration, further indicates these species might be considerably affecting the animals rather than being merely external colonisers. Various ubiquitous fungi have been assumed to play a role in the density regulation of crayfish populations (Bower & McGladdery 2005) and also Söderhäll et al. (1991) speculate about mortality in crayfish caused by *Saprolegnia*.

In our study, we observed a strong difference in the catch per unit effort between the two years, even though most external factors of the well-monitored drinking water reservoir Lake Constance remained stable during the years 2004 and 2005 (IGKB 2004, 2005). Moreover, also the local fishermen and fishing authorities at Lake Constance reported a drastic decline in crayfish catches between these years, and as was the case in our study the occurrence of white cotton-like patches on caught specimens only in 2005. Therefore, we have to take into account the possibility that the observed almost sevenfold decrease in catch of crayfish in 2005 compared to 2004 may potentially be connected to the observed occurrence of *Saprolegnia,* either acting directly as a pathogen or indirectly by causing stress. Thus, the actual decline might well have been a result of an acute outbreak of the crayfish plague (*A. astaci*) that can occur even in resistant crayfish species of North American origin, particularly if the crayfish's immune system is impeded by stress. If crayfish are chronic carriers of the disease, stressing conditions like sublethal concentrations of pesticides, water stress, infection with other microorganisms (e.g. *Psorospermium heackeli* Hilgendorf) can lead to an acute outbreak (Persson et al. 1987, Cerenius et al. 1988, Cerenius & Söderhäll 1992). To our knowledge, until the present day no test for crayfish plague has been conducted with any crayfish species in Lake Constance. However, in 2005 spiny-cheek crayfish and the fully plague susceptible narrow-clawed crayfish (*Astacus leptodactylus* Eschscholtz) (Balik et al. 2005) were caught within one net, and in 2004 approx. 3 km from our study site a stone crayfish (*Austropotamobius torrentium* Schrank) was caught (Nowotne, F. pers. comm.). Judging from the long-term co-existence with these plague-susceptible species it might be argued that spiny-cheek crayfish in Lake Constance is a not a chronic carrier of the disease (cf. Pöckl & Peckny 2002).

Even though we cannot provide evidence for the pathogenicity of *Saprolenia* spp., the discovery of *Saprolegnia* spp. has implications for the lake ecosystem. In other freshwater systems the introduction of *S. diclina* through healthy carriers has been found to be associated with the decline of native amphibians through frog spawn infestation (Kiesecker et al. 2001) and previous findings of *Saprolegnia* in freshwater crayfish have also raised questions about the potential for crayfish to act as vectors which may transmit the fungi to other susceptible species (Söderhäll et al. 1991, Diéguez-Uribeondo et al. 1994). In general, the negative impact of invasive species as vectors of fungus-like organisms both in aquatic and terrestrial systems is a well recognised problem (Allen & Humble 2002, Beard & O'Neill 2005) and among crayfish the interspecific transmission of such organisms is of particular importance (Vogt 1999). Therefore, the occurrence of *Saprolegnia* spp. in spiny-cheek crayfish in Lake Constance is also of concern, especially since there still are residual populations of the native crayfish species noble crayfish *Astacus astacus* (L.) and stone crayfish existing in the lake (Krämer et al. 1990, Renz & Breithaupt 2000) and in waters in the lake's back country connected to the lake regional crayfish farming with noble crayfish is conducted.

Conclusion

This is the first report of *S. australis* and other *Saprolegnia* spp. on the important freshwater invader spiny-cheek crayfish. Our finding of this new set of *Saprolegnia* spp., with yet unknown origin, distribution and host range suggests negative effects of this freshwater crayfish invader on the native ecosystem, on native crayfish populations and on commercial crayfish harvest through its possible properties as a reservoir and vector for these previously unregarded species. However, further studies on the pathogenic potential of *Saprolegnia* spp. in crayfish are needed to address the role of this genus in aquatic ecosystems.

Acknowledgments

For help in the field we would like to thank M. Hirning, M. Lohr, C. Osseforth, S. Pittlik, A. Sulger and A. Weber. For invaluable information on crayfish in Lake Constance we thank P. Dehus. Comments by J. Diéguez-Uribeondo and one anonymous reviewer greatly improved the manuscript. We appreciated helpful comments on earlier versions of the manuscript by D. Schleuter. This work was supported by the German Research Foundation within the Special Collaborative Project SFB 454 'Littoral of Lake Constance'.

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Submitted: 26 November 2007; accepted: 4 March 2008.