Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 53, No. 1 March 2024
pages (24-30)

sciendo

ISSN 1730-413X
eISSN 1897-3191

First record of *Astathelohania contejeani* (Henneguy, 1892) in the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) from Belarus

by

Karolina Śliwińska*, Elena Skuratovich, Anatoly Alekhnovich

DOI: **https://doi.org/10.26881/oahs-2024.1.04**
Category: **Short communication**
Received: **June 5, 2023**
Accepted: **November 14, 2023**

Scientific and Practical Center for Bioresources, National Academy of Sciences of Belarus, Akademicheskaya 27, Minsk, BELARUS

Abstract

Porcelain disease, caused by the parasite *Astathelohania contejeani*, is a fatal disease for freshwater crayfish. Previously published data provide information on the occurrence of *A. contejeani* in various crayfish species. However, its prevalence in host populations remains largely undetermined. This issue is relevant to the narrow-clawed crayfish *Pontastacus leptodactylus*, a species of high commercial value in Eastern Europe. A single published report addressed a potential microsporidia infection in *P. leptodactylus*, but without specific data on geographical location and prevalence. We present the first detailed information on the prevalence of *A. contejeani* in the *P. leptodactylus* population from Lake Losvido, including an assessment of the infection rate through both visual and molecular assessment. *Astathelohania contejeani* was observed in 1.56% of 128 crayfish examined visually. Of the 37 asymptomatic crayfish samples analyzed, 29.7% were found to be carriers. This finding suggests that parasitism of *A. contejeani* occurs frequently in the *P. leptodactylus* population in Lake Losvido prior to the manifestation of observable disease symptoms.

Key words: Porcelain disease, microsporidiosis, *Astathelohania contejeani*, narrow-clawed crayfish, *Pontastacus leptodactylus*

* Corresponding author: karolina.sliwinska@outlook.com

1. Introduction

Microsporidia (Opisthokonta: Ophisthosporidia) are exclusively parasitic organisms that can only exist in microeukaryotes, animals, and human hosts (Bojko et al. 2022). In aquatic ecosystems, these organisms have the highest diversity within the Crustacea group, including 72 described species from 43 genera (Bojko, Stentiford 2022). A total of 11 known species belonging to the following genera: *Cambaraspora* (Bojko et al. 2020b; Stratton et al. 2023a), *Ovipleistophora* (Bojko et al. 2020a; Stratton et al. 2022a), *Nosema* (Moodie et al. 2003c; Pretto et al. 2018), *Alternosema* (Stratton et al. 2023b), and *Astathelohania* (Moodie et al. 2003a; Moodie et al. 2003b; Stratton et al. 2022b) are known to be crayfish-infecting microsporidia.

Astathelohania contejeani (Henneguy, 1892) (Stratton et al. 2022b) is the causative agent of fatal porcelain disease (Bowler, Brown 1977). Infection with *A. contejeani* microsporidia is chronic, and infected individuals can survive for several months to several years (Brown, Bowler 1977). The gastrointestinal tract of crayfish is the entry point for infection, but it is not known how *A. contejeani* migrates to the sites of proliferation and sporogony. The plasmalemma ruptures in strongly infected crayfish muscle cells, allowing the parasite to spread throughout the body (Diéguez-Uribeondo et al. 1997b). The disease progresses, causing weakness, and in late stages infected crayfish become lethargic and anorexic (Evans, Edgerton 2002). Muscle cells of heavily infected crayfish show milky/whitish color through the carapace. This is a characteristic symptom of infection that distinguishes healthy crayfish from diseased ones (Diéguez-Uribeondo et al. 1997b).

In Europe, symptoms of the porcelain disease were recorded mostly in populations of native species such as the noble crayfish *Astacus astacus* (Sumari, Westman 1969; Voronin 1971; Mažylis 1978; Skurdal et al. 1988; 1990; Lom et al. 2001), the white-clawed crayfish *Austropotamobius pallipes* (Brown, Bowler 1977; Diéguez-Uribeondo et al. 1997a; Mori and Salvidio 2000; Imhoff et al. 2011; Longshaw et al. 2012; Pretto et al. 2018), and the narrow-clawed crayfish *Pontastacus leptodactylus* (Mažylis 1978). Symptoms of microsporidiosis infection, characterized by whitish discoloration of crayfish muscle, have also been found in invasive species: the spiny-cheek crayfish *Faxonius limosus* (Krucińska, Simon 1968) and the signal crayfish *Pacifastacus leniusculus* (Dunn et al. 2009; Imhoff et al. 2010; 2011). There are inaccuracies in the published literature regarding the occurrence of the porcelain disease pathogen in the narrow-clawed crayfish. Many authors have misquoted the results obtained by Krucińska and Simon (1968) regarding the observation of porcelain disease in a narrow-clawed crayfish population (Voronin 1971; Quilter 1976; Edgerton et al. 2002; McGriff, Modin 1983; Quaglio et al. 2011; Longshaw 2011), while the work of Krucińska and Simon focuses solely on *Faxonius limosus* (published as *Cambarus affinis*) as the host species of *A. contejeani*. To the best of our knowledge, only one report of porcelain disease in *P. leptodactylus* has been published to date (Mažylis 1978). However, the study mentions that the narrow-clawed crayfish could be infected with microsporidia, probably *A. contejeani*, although neither the causative agent nor the precise location of the observation was identified.

There is a widespread perception that research on diseases that affect European crayfish is insufficient (Longshaw 2011; Dragičević 2021). Research on the distribution and prevalence of infections is essential to identify factors contributing to mortality rates among individuals and populations (Mažylis 1978). The availability of such information is a crucial aspect to consider in management plans for crayfish populations (Edgerton et al. 2004).

The narrow-clawed crayfish is represented by numerous populations in Belarus, and its resources are widely used for commercial purposes (Alekhnovich 2016). The exploitation of crayfish is strictly regulated by local and national law. Operating business entities are required by the norms to introduce exploited crayfish populations to new habitats in order to increase the overall crayfish production in the region. These actions are regulated and documented. However, it is noteworthy that veterinary control measures are not incorporated into these activities. Relocation of infected populations may deviate from the intended goal of increasing the size of productive crayfish stocks. On the other hand, the spread of infected populations may also threaten other populations of wild crayfish in the region. Therefore, research into the distribution of *A. contejeani*, the pathogen that affects crayfish populations, is crucial for both managing crayfish resources and preserving rare, endangered species.

When evaluating the commercial stocks of narrow-clawed crayfish in Lake Losvido, we observed individuals exhibiting pronounced microsporidiosis symptoms. Therefore, in order to provide scientific evidence for proper management of this population, we endeavored to identify the causative agent and assess the infection rate.

2. Materials and methods

Lake Losvido is a naturally formed mesotrophic water body of glacial origin, located in the Gorodok district, Vitebsk region, northeastern Belarus (55°23'27.2"N; 30°02'07.4"E). The eastern outflow of Lake Losvido forms a connection with Lake Tsyganovo. Lake Tsyganovo, in turn, is connected to the Khrapovlyanka River, which belongs to the Baltic Sea drainage basin. Therefore, Lake Losvido is not an isolated aquatic habitat.

Crayfish were collected in May 2022 using unbaited fyke nets (N = 39) to avoid competition between crayfish of different size classes. Traps were set at a depth of 2–3 m at different sampling sites along the shoreline of the lake, in accordance with the official permission from the Ministry of Natural Resources and Environmental Protection of the Republic of Belarus. A total of 128 crayfish were caught, which corresponds to the catch per unit effort CPUE = 1.62. Each individual was inspected for expressed symptoms of infection manifested by whitish coloration of the muscles (Fig. 1). Diseased individuals (N = 2) were subsequently euthanized. From all visually healthy crayfish (without visible muscle coloration), we randomly selected a sample of 37 individuals for PCR diagnostics. Muscle tissue samples were collected from the fifth pair of pereiopods (Imhoff et al. 2010) and stored in 96% ethanol for further analysis. After sampling, visually healthy individuals were released back into Lake Losvido.

Figure 1

General comparative ventral view of the narrow-clawed crayfish *Pontastacus leptodactylus* from Lake Losvido – left: an individual showing symptoms of porcelain disease in the abdominal muscles; right: a visually healthy individual.

Total DNA from crayfish muscle tissue was extracted using the Genomic DNA Animal and Fungi DNA Preparation Kit (Jena Bioscience, Germany). DNA concentrations were determined using a NanoPhotometer P-class, P-360 (Implen GmbH, Germany).

The causative agent of porcelain disease has been identified using nested PCR (El-Matbouli, Soliman 2006). For the first PCR reaction, microsporidia-specific primers V1f and 1492r were used to amplify the coding DNA of the small ribosomal subunit (SSU rRNA; Weiss et al. 1994). The product of the first PCR reaction (outer nest) was used as a template for the second PCR reaction (inner nest), which was performed using *A. contejeani*-specific primers MIC 5-1 and MIC3-4 (Imhoff et al. 2009).

PCR for the outer nest was carried out using 0.4 µM of each primer, 1 U of Taq DNA polymerase, 1X PCR buffer (65 mM Tris-HCl, 16.6 mM $(NH_4)_2SO_4$, 0.02% Tween 20, pH 8.8), 0.2 mM of dNTPs, 3 mM $MgCl_2$ (Primetech, Belarus), and from 20 to 50 ng of template DNA. The reaction volume was filled with PCR-grade water to a total volume of 25 µl. PCR amplification was performed in a C1000 Touch Thermal Cycler (Bio-Rad, Germany). The protocol for amplifying the outer nest involved an initial denaturation at 95°C for 5 min, followed by 45 cycles at 95°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR mix for the inner nest specific to *A. contejeani* was the same as for the outer nest, except for 1 µl of the PCR product from the previous reaction used as a template. The amplification protocol used in this case was the one suggested by Imhoff et al. (2009). All PCR runs included positive and negative controls. A tissue sample obtained from a specimen showing visible symptoms of *A. contejeani* infection was used as a positive control. Amplicons of the second PCR were visualized in a 1.5% agarose gel stained with 0.5 µM of ethidium bromide. To determine the size of the amplicons, we used molecular weight markers in the range of 50–500 base pairs (50+ bp DNA Ladder, Evrogen, Russia). A positive result of the second PCR reaction was considered to indicate the presence of the pathogen in the diagnosed individual.

Successfully amplified inner nest PCR products were purified using PCR Clean-Up & Gel Extraction (Thermo Fisher Scientific, Lithuania) and sequenced on a commercial basis by the Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus

(ABI 3500 Genetic Analyzer, Applied Biosystems, USA) to confirm species identification. The obtained sequences were manually edited using the BioEdit 7.2 software (Hall 1999), followed by multiple alignments using the CrustalW algorithm in MEGA X (Kumar et al. 2018), and compared with records in GenBank (Benson et al. 2013). Available sequences from the GenBank database were downloaded to construct a neighbor-joining phylogenetic tree. The orthologous sequence of *Astathelohania parastaci* (Moodie et al. 2003b) was used as an outgroup. The obtained sequences were then published in GenBank with assigned accession numbers: OQ780520–OQ780522.

3. Results

Visual examination of the captured individuals revealed that of the 128 specimens, two showed gross signs of infection, which corresponds to 1.56% of the analyzed sample. Crayfish muscles with a white coloration indicated the presence of infection (Fig. 1).

The outcomes of the nested-PCR diagnostics indicated that the *A. contejeani* gene was amplified in 11 out of the 37 samples analyzed, providing confirmation of the infection in the individuals under study with *A. contejeani*. The prevalence of the etiological agent responsible for porcelain disease in the narrow-clawed crayfish population inhabiting Lake Losvido is 29.7%.

Three isolates of *A. contejeani* from different crayfish individuals were successfully sequenced. One sequence was obtained from crayfish with visual symptoms of the disease (Genbank accession No. OQ780520), while the other two sequences were obtained from animals without visible symptoms of infection. BLAST analysis of our samples showed 100% identity with 100% sequence coverage with an *E* value of 1e-79 compared to the *A. contejeani* sequence with GenBank accession No. MF344632. All obtained sequences (aligned to 167 bp) represented one haplotype, which is also known from populations of *A. astacus* from France (Lom et al. 2001) and *A. pallipes* from Italy (Fig. 2; Pretto et al. 2018).

4. Discussion

Visual examination of infected individuals revealed that the prevalence of infection in Lake Losvido was 1.56%, which is comparable to results from other European crayfish populations. For various populations of *A. astacus* from Lithuania, between 0.7 and 3.7% of crayfish showed symptoms of porcelain disease (Mažylis 1978). Similarly, Skurdal et al. (1988; 1990) identified infection rates ranging from 0.05% to 2.11% in individuals from Norway. Gross symptoms of *A. contejeani* infection in *A. pallipes* were present in 0–0.7% of Spanish crayfish populations (Diéguez-Uribeondo et al. 1997a) and in 0.17–4.3% of

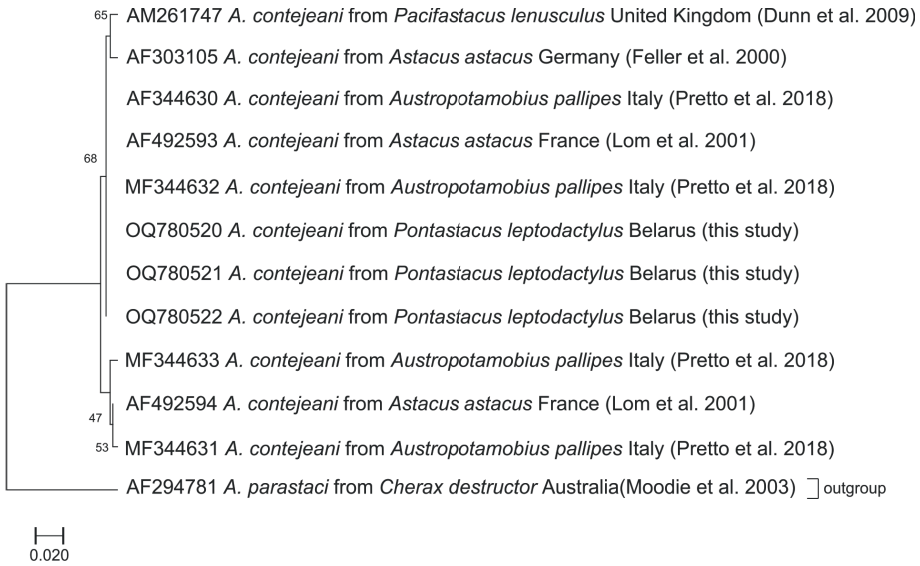

Figure 2

Neighbor-joining tree for *Astatelohania contejeani* from Lake Losvido and GenBank data based on partial small ribosomal subunit sequences (Kimura two-parameter distance model, 167 bp). Numbers on nodes represent bootstrap support values (1000 replicates).

crayfish from Italy (Mori, Salvidlo 2000; Quaglio et al. 2011). In most cases, the prevalence of *A. contejeani* with pronounced symptoms of infection is low and rarely exceeds 10% (Vey & Vago 1973; Brown & Bowler 1977; Imhoff et al. 2009; Longshaw et al. 2012).

According to published data, studies based on the PCR diagnostics of *A. contejeani* in wild crayfish populations are scarce and have so far been applied in only a few research projects (Dunn et al. 2009; Imhoff et al. 2011). Imhoff et al. (2011) found that *A. pallipes* and *P. leniusculus* specimens exhibited infection rates of 14% and 12.3%, respectively, despite the absence of observable symptoms of the disease. Other results based on DNA analysis revealed a high prevalence of the pathogen in *P. leniusculus,* showing a 38% infection rate in a population from Great Britain (Dunn et al. 2009). According to Dunn et al. (2009), the nested PCR method is 10 times more effective for detecting microsporidian infection in crayfish populations than the conventional visual inspection method, which is consistent with our findings in Lake Losvido.

The infection rate of *A. contejeani* in crayfish populations can vary depending on the population density and size, as well as the type of its habitat and the presence of interdependent factors (Cossins 1973; Mažylis 1978; France, Graham 1985; Imhoff et al. 2009). Infection with the causative agent of porcelain disease is thought to be more prevalent in dense populations (Cossins 1973). In Lake Losvido, *P. leptodactylus* is characterized by a high relative abundance (CPUE = 1.62) compared to other populations in the region (Alekhnovich 2016), which may be related to the high prevalence of *A. contejeani*, in line with the prevailing opinion on the widespread distribution of *A. contejeani* in dense crayfish populations.

The effects of *A. contejeani* parasitism on crayfish have not been extensively researched. Until now, it has been known that infected crayfish are able to grow and molt, but the growth rate has been shown to be slower than that of the control group (Bowler, Brown 1977). Food intake and digestion in infected individuals are 30% lower than in the control group, which researchers attribute to a disease-related restriction in mobility (Haddaway et al. 2012). The results of studies on the effects of *A. contejeani* on the reproductive process of crayfish are inconclusive. According to some studies, microsporidian parasitism has no effect on the reproduction of *A. pallipes* (Cossins, Bowler 1974; Imhoff et al. 2009), whereas others claim that infected *A. astacus* females are incapable of laying eggs because of the absence of eggs in the ovaries (Mažylis 1978). There are also anecdotal reports that microsporidiosis can cause massive mortality in *A. pallipes* populations (Duffield 1933; Goodrich 1956). According to published data, the presence of this pathogen in crayfish populations has a negative impact on their well-being. The reintroduction of crayfish infected with *A. contejeani* through restocking is considered inappropriate (Mažylis 1978; Diéguez-Uribeondo et al. 1997a). Therefore, we would like to stress the importance of preventing the spread of this pathogen in the context of narrow-clawed crayfish restocking initiatives in Belarus.

Acknowledgements

The work was partially supported by the Belarusian Republican Foundation for Fundamental Research, grant No. B21M-079.

References

Alekhnovich, A. (2016). Crayfish of Belarus in modern conditions: Distribution, population dynamics, production and commercial potential. [In Russian]. Belaruskaya navuka, Minsk, Belarus.

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2013). GenBank. *Nucleic Acids Research, 41*(D1), D36–D42. https://doi.org/10.1093/nar/gks1195 PMID:23193287

Bojko, J., & Stentiford, G. D. (2022). Microsporidian Pathogens of Aquatic Animals. W L. M. Weiss & A. W. Reinke (Red.), *Microsporidia* (T. 114, pp. 247–283). Springer International Publishing. https://doi.org/10.1007/978-3-030-93306-7_10

Bojko, J., Behringer, D. C., Moler, P., & Reisinger, L. (2020a). *Ovipleistophora diplostomuri*, a parasite of fish and their trematodes, also infects the crayfish *Procambarus bivittatus*. *Journal of Invertebrate Pathology, 169*, 107306. https://doi.org/10.1016/j.jip.2019.107306 PMID:31836486

Bojko, J., Behringer, D. C., Moler, P., Stratton, C. E. L., & Reisinger, L. (2020b). A new lineage of crayfish-infecting Microsporidia: The *Cambaraspora floridanus* n. gen. n. sp. (Glugeida: Glugeidae) complex from Floridian freshwaters (USA). *Journal of Invertebrate Pathology, 171*, 107345. https://doi.org/10.1016/j.jip.2020.107345 PMID:32067978

Bojko, J., Reinke, A. W., Stentiford, G. D., Williams, B., Rogers, M. S. J., & Bass, D. (2022). Microsporidia: A new taxonomic, evolutionary, and ecological synthesis. *Trends in Parasitology, 38*(8), 642–659. https://doi.org/10.1016/j.pt.2022.05.007 PMID:35667993

Brown, D., & Bowler, K. (1977). A population study of the British freshwater crayfish *Austropotamobius pallipes (Lereboullet)*. *Freshwater Crayfish, 3*(1), 33–49.

Cossins, A. R. (1973). *Thelohania contejeani* Henneguy, microsporidian parasite of *Austropotamobius pallipes*

Lereboullet - an histological and ultrastructural study. *Freshwater Crayfish, 1*(1), 151–164.

Cossins, A. R., & Bowler, K. (1974). An histological and ultrastructural study of *Thelohania contejeani* Henneguy, 1892 (Nosematidae), Microsporidian parasite of the crayfish *Austropotamobius pallipes* Lereboullet. *Parasitology, 68*(1), 81–91. https://doi.org/10.1017/S003118200004539X

Diéguez-Uribeondo, J., Pinedo-Ruiz, J., & Mùzquiz, J. L. (1997a). *Thelohania contejeani* in the province of Alava, Spain. *Bulletin Francais de la Peche et de la Pisciculture, 347*, 749–752. https://doi.org/10.1051/kmae/1997057

Diéguez-Uribeondo, J., Cerenius, L., Dyková, I., Gelder, S., Henntonen, P., Jiravanichpaisal, P., Lom, J., & Söderhäll, K. (1997b). Pathogens, parasites and ectocommensals. In C. Souty-Grosset, D.M. Holdich, P.Y. Noël, J.D. Reynolds, P. Haffner (Eds.), *Atlas of Crayfish in Europe* (pp. 135–155). Muséum national d'Histoire naturelle, Paris: Collection Patrimoines Naturels.

Dragičević, P., Bielen, A., Petrić, I., & Hudina, S. (2021). Microbial pathogens of freshwater crayfish: A critical review and systematization of the existing data with directions for future research. *Journal of Fish Diseases, 44*(3), 221–247. https://doi.org/10.1111/jfd.13314 PMID:33345337

Duffield, J. E. (1933). Fluctuations in Numbers among Freshwater Crayfish, *Potamobius pallipes* Lereboullet. *Journal of Animal Ecology, 2*(2), 184–196. https://doi.org/10.2307/956

Dunn, J. C., McClymont, H. E., Christmas, M., & Dunn, A. M. (2009). Competition and parasitism in the native White Clawed Crayfish *Austropotamobius pallipes* and the invasive Signal Crayfish *Pacifastacus leniusculus* in the UK. *Biological Invasions, 11*(2), 315–324. https://doi.org/10.1007/s10530-008-9249-7

Edgerton, B. F., Evans, L. H., Stephens, F. J., & Overstreet, R. M. (2002). Synopsis of freshwater crayfish diseases and commensal organisms. *Aquaculture (Amsterdam, Netherlands), 206*(1–2), 57–135. https://doi.org/10.1016/S0044-8486(01)00865-1

Edgerton, B. F., & Jussila, J. (2004). Keynote presentation and roundtable session 4. Crayfish pathology in Europe: Past, present and a programme for the future. *Bulletin Francais de la Peche et de la Pisciculture, 372–373*, 473–482. https://doi.org/10.1051/kmae:2004021

El-Matbouli, M., & Soliman, H. (2006). Molecular diagnostic methods for detection of *Thelohania contejeani* (Microsporidia), the causative agent of porcelain disease in crayfish. *Diseases of Aquatic Organisms, 69*, 205–211. https://doi.org/10.3354/dao069205 PMID:16724564

Evans, L., & Edgerton, B. (2002). Pathogens, parasites and commensals. In D. M. Holdich (Ed.), Biology of Freshwater Crayfish (pp. 377–438). Blackwell Science.

Feller, C., Rinder, H., El-Matbouli, M., & Hoffmann, R. W. (2000). A PCR method for the detection of *Thelohania contejeani*. Retrieved accessed March 13, 2023, from https://www.ncbi.nlm.nih.gov/nuccore/AF303105

France, R. L., & Graham, L. (1985). Increased microsporidian parasitism of the crayfish *Orconectes virilis* in an experimentally acidified lake. *Water, Air, and Soil Pollution, 26*(2), 129–136. https://doi.org/10.1007/BF00292063

Goodrich, H. P. (1956). Crayfish epidemics. *Parasitology, 46*(3-4), 480–483. https://doi.org/10.1017/S0031182000026615 PMID:13378890

Haddaway, N. R., Wilcox, R. H., Heptonstall, R. E. A., Griffiths, H. M., Mortimer, R. J. G., Christmas, M., & Dunn, A. M. (2012). Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. *PLoS One, 7*(2), e32229. https://doi.org/10.1371/journal.pone.0032229 PMID:22359673

Hall, T. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Imhoff, E., Mortimer, R., Christmas, M., & Dunn, A. (2009). Porcelain disease in white-clawed and signal crayfish in the UK. In Crayfish Conservation in the British Isles, Proceedings of a conference held on 25th March 2009 in Leeds (pp. 49–56). Leeds, Great Britan.

Imhoff, E., Mortimer, R., Christmas, M., & Dunn, A. (2010). Non-lethal tissue sampling allows molecular screening for microsporidian parasites in signal, *Pacifasticus leniusculus* (Dana), and vulnerable white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet). *Freshwater Crayfish, 17*(1), 145–150.

Imhoff, E., Mortimer, R., Christmas, M., & Dunn, A. (2011). Invasion progress of the signal crayfish (*Pacifastacus leniusculus* (Dana)) and displacement of the native white-clawed crayfish (*Austropotamobius pallipes* (Lereboullet)) in the River Wharfe, UK. *Freshwater Crayfish, 18*(1), 45–53. https://doi.org/10.5869/fc.2011.v18.45

Krucińska, J., & Simon, E. (1968). On the parasites and epibionts of the branchial cavity in crayfish at Wrocław and vicinity. [In Polish]. *Przegląd Zoologiczny, 7*(3), 288–290.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution, 35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096 PMID:29722887

Lom, J., Nilsen, F., & Dyková, I. (2001). *Thelohania contejeani* Henneguy, 1892: Dimorphic life cycle and taxonomic affinities, as indicated by ultrastructural and molecular study. *Parasitology Research, 87*(10), 860–872. https://doi.org/10.1007/s004360100436 PMID:11688894

Longshaw, M. (2011). Diseases of crayfish: A review. *Journal of Invertebrate Pathology, 106*(1), 54–70. https://doi.org/10.1016/j.jip.2010.09.013 PMID:21215355

Longshaw, M., Stebbing, P. D., Bateman, K. S., & Hockley, F. A. (2012). Histopathological survey of pathogens and commensals of white-clawed crayfish (*Austropotamobius*

pallipes) in England and Wales. *Journal of Invertebrate Pathology, 110*(1), 54–59. https://doi.org/10.1016/j.jip.2012.02.005 PMID:22366663

Mažylis, A. (1978). On *Astacus astacus* L. infected with *Thelohania contejeani* Henneguy. *Freshwater Crayfish, 4*(1), 471–473.

McGriff, D., & Modin, J. (1983). *Thelohania contejeani* parasitism of the crayfish, *Pacifastacus leniusculus*, in California. *California Fish and Game, 69*(3), 178–183.

Moodie, E. G., Le Jambre, L. F., & Katz, M. E. (2003a). *Thelohania montirivulorum* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae): fine ultrastructure, molecular characteristics and phylogenetic relationships. *Parasitology Research, 91*(3), 215–228. https://doi.org/10.1007/s00436-003-0948-9 PMID:12923630

Moodie, E. G., Le Jambre, L. F., & Katz, M. E. (2003b). *Thelohania parastaci* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae). *Parasitology Research, 91*(2), 151–165. https://doi.org/10.1007/s00436-003-0941-3 PMID:12923627

Moodie, E. G., Le Jambre, L. F., & Katz, M. E. (2003c). Ultrastructural characteristics and small subunit ribosomal DNA sequence of *Vairimorpha cheracis* sp. nov., (Microspora: Burenellidae), a parasite of the Australian yabby, *Cherax destructor* (Decapoda: Parastacidae). *Journal of Invertebrate Pathology, 84*(3), 198–213. https://doi.org/10.1016/j.jip.2003.11.004 PMID:14726242

Mori, M., & Salvidio, S. (2000). The occurrence of *Thelohania contejeani* Henneguy, a microsporidian parasite of the crayfish *Austropotamobius pallipes* (Lereboullet), in Liguria Region (NW Italy). *Journal of Limnology, 59*(2), 167–169. https://doi.org/10.4081/jlimnol.2000.167

Pretto, T., Montesi, F., Ghia, D., Berton, V., Abbadi, M., Gastaldelli, M., Manfrin, A., & Fea, G. (2018). Ultrastructural and molecular characterization of *Vairimorpha austropotamobii* sp. nov. (Microsporidia: Burenellidae) and Thelohania contejeani (Microsporidia: Thelohaniidae), two parasites of the white-clawed crayfish, *Austropotamobius pallipes* complex (Decapoda: Astacidae). *Journal of Invertebrate Pathology, 151*, 59–75. https://doi.org/10.1016/j.jip.2017.11.002 PMID:29122615

Quaglio, F., Capovilla, P., Fioravanti, M. L., Marino, F., Gaglio, G., Florio, D., Fioretto, B., & Gustinelli, A. (2011). Histological analysis of thelohaniasis in white-clawed crayfish *Austropotamobius pallipes* complex. *Knowledge and Management of Aquatic Ecosystems, 401*, 27. https://doi.org/10.1051/kmae/2011045

Quilter, C. G. (1976). Microsporidan parasite *Thelohania contejeani* Henneguy from New Zealand freshwater crayfish. *New Zealand Journal of Marine and Freshwater Research, 10*(1), 225–231. https://doi.org/10.1080/00288330.1976.9515609

Skurdal, J., Qvenild, T., Taugbol, T., & Fjeld, E. (1990). A 6-year study of *Thelohania contejeani* parasitism of the noble crayfish, *Astacus astacus* L., in Lake Steinsfjorden, S. E. Norway. *Journal of Fish Diseases, 13*(5), 411–415. https://doi.org/10.1111/j.1365-2761.1990.tb00800.x

Skurdal, J., Taugbol, T., Fjeld, E., Hessen, D. O., & Hastein, T. (1988). *Thelohania contejeani* Henneguy parasitizing the noble crayfish, *Astacus astacus* L., in Norway. *Journal of Fish Diseases, 11*(5), 433–435. https://doi.org/10.1111/j.1365-2761.1988.tb00739.x

Sumari, O., & Westman, K. (1969). The crayfish parasite *Thelohania contejeani* Henneguy (Sporozoa, Microsporidia) found in Finland. *Annales Zoologici Fennici, 7*, 193–194.

Stratton, C. E., Kabalan, B. A., Bolds, S. A., Reisinger, L. S., Behringer, D. C., & Bojko, J. (2023a). *Cambaraspora faxoni* n. sp. (Microsporidia: Glugeida) from native and invasive crayfish in the USA and a novel host of *Cambaraspora floridanus. Journal of Invertebrate Pathology, 199*, 107949. https://doi.org/10.1016/j.jip.2023.107949 PMID:37276936

Stratton, C. E., Moler, P., Allain, T. W., Reisinger, L. S., Behringer, D. C., & Bojko, J. (2022a). The plot thickens: *Ovipleistophora diplostomuri* infects two additional species of Florida crayfish. *Journal of Invertebrate Pathology, 191*, 107766. https://doi.org/10.1016/j.jip.2022.107766 PMID:35472375

Stratton, C. E., Reisinger, L. S., Behringer, D. C., Reinke, A. W., & Bojko, J. (2023b). *Alternosema astaquatica* n. sp. (Microsporidia: Enterocytozoonida), a systemic parasite of the crayfish *Faxonius virilis. Journal of Invertebrate Pathology, 199*, 107948. https://doi.org/10.1016/j.jip.2023.107948 PMID:37276935

Stratton, C. E., Reisinger, L. S., Behringer, D. C., & Bojko, J. (2022b). Revising the Freshwater Thelohania to Astathelohania gen. Et comb. Nov., and description of two new species. *Microorganisms, 10*(3), 636. https://doi.org/10.3390/microorganisms10030636 PMID:35336214

Vey, A., & Vago, C. (1973). Protozoal and fungal diseases of *Austropotamobius pallipes*, Lereboullet in France. *Freshwater Crayfish, 1*(1), 165–179.

Voronin, V. (1971). New data on microsporidiosis of the crayfish, *Astacus astacus* (L. 1758). *Parazitologiia, 5*(2), 186–191.

Weiss, L. M., Zhu, X., Cali, A., Tanowitz, H. B., & Wittner, M. (1994). Utility of microsporidian rRNA in diagnosis and phylogeny: A review. *Folia Parasitologica, 41*(2), 81–90. PMID:7927064