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NOTE

First report of microsporidians in the non-native shrimp *Neocaridina davidi* from a temperate European stream

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ABSTRACT: The release of ornamental pets outside their native range can directly or indirectly impact the recipient community, e.g. via the co-introduction of associated pathogens. However, studies on parasites associated with non-native species, in particular freshwater decapods, have focused mainly on a limited set of pathogens. Here we provide data for the first time on microsporidian parasites of the non-native ornamental shrimp *Neocaridina davidi*, collected in a stream in Germany. Furthermore, we confirm an ongoing range expansion of the warm-adapted *N. davidi* from thermally polluted colder water. In the investigated shrimps, the microsporidian parasite *Enterocytozoon hepatopenaei* and an unknown microsporidian isolate were detected, raising concerns about their transmission potential and pathogenicity on native crustacean species.

KEY WORDS: *Enterocytozoon hepatopenaei* · Atyidae · Invasive species · DNA barcoding · Shrimp diseases

1. INTRODUCTION

The ornamental pet trade has flourished in recent years, which has increased the risk of non-native species introductions (Patoka et al. 2018). Freshwater habitats, especially those experiencing degradation, are prone to biological invasions (Casatti et al. 2006). Thermal pollution of temperate streams or rivers, for example, may facilitate the establishment of exotic species (Klotz et al. 2013, Weiperth et al. 2019). The introduction of non-native species can have negative impacts on the recipient community by directly or indirectly affecting ecosystem functioning and biodiversity, even without establishment, e.g. via pathogen transmission (Simberloff et al. 2013).

Biological invasions are often linked to commercial success in the global pet trade (Gippet & Bertelsmeier 2021), and decapods are no exception. Warm-adapted decapods have a low probability of becoming established in temperate waters, except for in thermally polluted waterbodies (Weiperth et al. 2019). Established populations of the atyid shrimp *Neocaridina davidi*, a popular aquarium pet originating from eastern and central China, have been reported in the USA, Japan, Israel, and Europe (Klotz et al. 2013, Jabłońska et al. 2018, Levitt-Barmats et al. 2019, Weiperth et al. 2019). Until now, *N. davidi* has primarily been investigated for epibionts such as *Holtodrilus truncatus*, *Scutariella* sp., *Vorticella* sp., and *Cladogonium* sp. (Ohtaka et al. 2012, Liao et al. 2018, Bauer et al. 2021), which have been found in native and non-native populations of *N. davidi* (Ohtaka et al. 2012, Patoka et al. 2016, Liao et al. 2018, Maci-

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aszek et al. 2021). However, other potential parasites such as microsporidians, of which the genus *Triwangia* has been described from atyid shrimps (Weng et al. 2022), have received little or no attention.

Microsporidians are eukaryotic obligate intracellular parasites with simple or complex life cycles (Stentiford et al. 2013). They infect all major animal groups, have zoonotic potential, and can cause important diseases in aquatic organisms (Stentiford et al. 2013). The spread of microsporidians as pathogens may occur via invasive species that introduce these parasites into new areas. For instance, the microsporidian *Cucumispora dikerogammari*, which was initially described in the invasive amphipod *Dikerogammarus villosus*, has spread to native amphipod species, leading to high mortality levels (Cormier et al. 2021).

We sampled *N. davidi* from the Finkelbach, a cold-water tributary of the thermally polluted River Erft, North Rhine-Westphalia, Germany, for the presence of microsporidian parasites. The present study provides new insights into parasites of this non-native shrimp. We also provide further evidence of an ongoing range expansion of *N. davidi* in European temperate streams.

2. MATERIALS AND METHODS

Neocaridina davidi took were sampled on 14 December 2021 at the Finkelbach (50.9808° N, 6.5781° E), a tributary of the River Erft located 25 km upstream of the nearest reported shrimp population (Klotz et al. 2013, Schoolmann & Arndt 2018; Fig. 1). *N. davidi* was first reported from the Gillbach in 2013, another tributary of the River Erft, and later from the lower Erft and the Rhine (Klotz et al. 2013, Schoolmann & Arndt 2018). The Finkelbach receives water from a communal wastewater treatment plant via the Elsdorfer Fließ. Upstream of the estuary of the Elsdorfer Fließ, the Finkelbach is dry and serves only as a drainage during heavy rain. For several decades, the Finkelbach, like most streams in this region, has not had contact with groundwater due to coal mining activities. Until 2009, the stream also directly received water from 3 wastewater treatment plants. Additionally, a sugar factory released warm water into the Elsdorfer Fließ until the

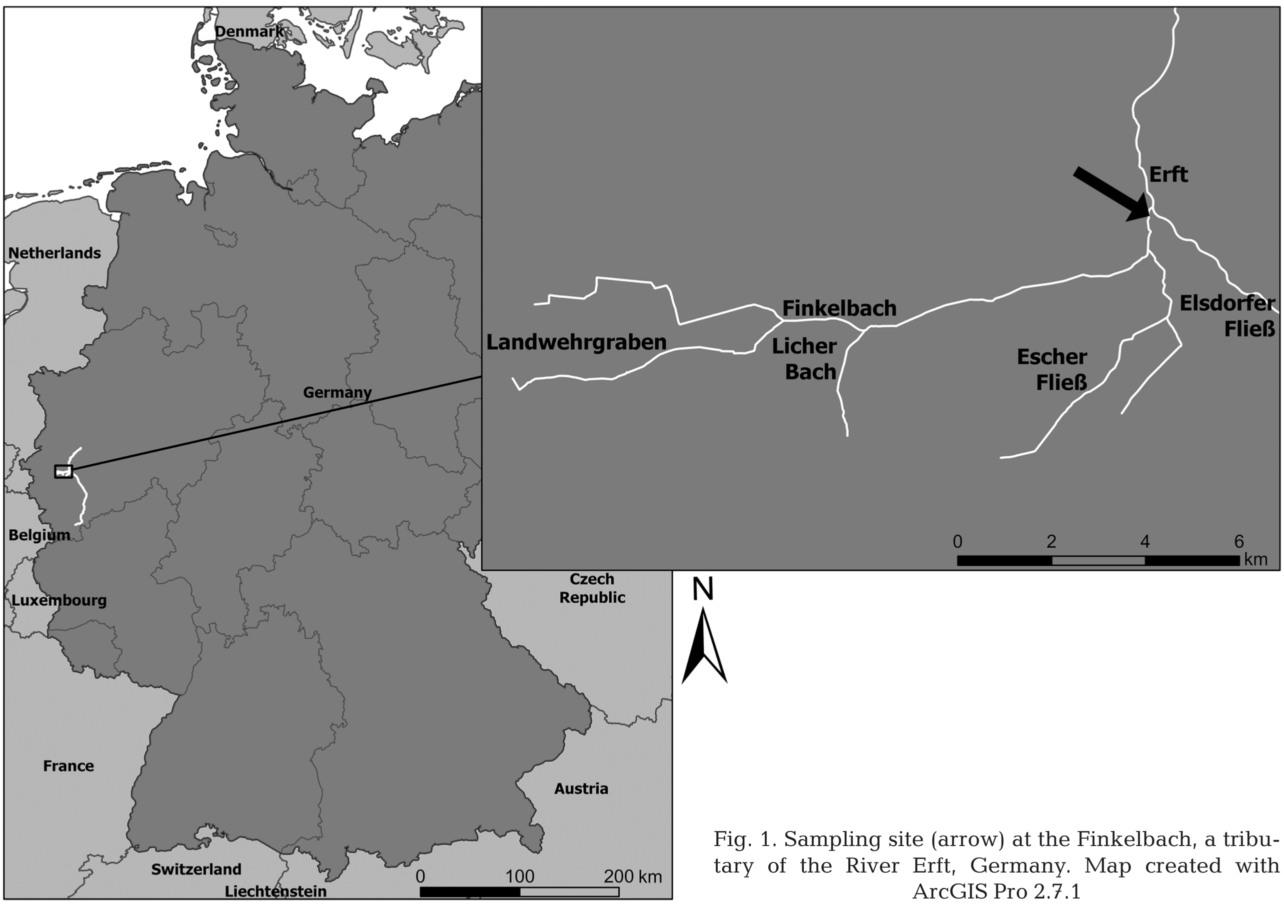

Fig. 1. Sampling site (arrow) at the Finkelbach, a tributary of the River Erft, Germany. Map created with ArcGIS Pro 2.7.1

early 2000s. Currently, there are irregular rinse-water discharges from the sugar factory and the local water company into the Elsdorfer Fließ (U. Rose pers. comm.). At the time of sampling, water temperature was 9°C, conductivity 620 ms cm^{-1}, dissolved oxygen 9.8 mg l^{-1}, and pH 7.65. Individuals of *N. davidi* were collected using dip nets and preserved in 96% ethanol. Morphological identification, carapace length measurements, and parasitological analyses were performed in the laboratory. Additionally, a small sample of muscular tissue was retrieved for molecular identification of both host and microsporidian parasites.

DNA was isolated from muscular tissue using a modified salt precipitation protocol described by Grabner et al. (2015). Molecular identification of 3 randomly selected *N. davidi* individuals was achieved with the universal eukaryotic primers LCO1490 and HCO2198 (Folmer et al. 1994) and that of microsporidians with the universal microsporidian primers V1 and Micuni3R (Weigand et al. 2016). PCR products of infected amphipods were sent to Microsynth Seqlab (Göttingen, Germany) for sequencing using LCO1490 and V1 primers, respectively. Obtained sequences were compared against GenBank records using BLAST (blast.ncbi.nlm.nih.gov). Sequences were edited in Geneious version 2022.0.1 (Biomatters) and aligned using the MUSCLE algorithm, and maximum likelihood phylogenetic trees were produced in MEGA 11 version 11.0.8 (Tamura et al. 2021) using the K2+G substitution model for microsporidians and the T92+G substitution model for *N. davidi*.

3. RESULTS

A total of 75 adult shrimps (31 females and 44 males) were collected, including 1 ovigerous female. Morphological identification of *Neocaridina davidi* was confirmed by molecular identification. All 3 sequenced shrimp individuals belonged to the same haplotype (GenBank accession nos. OM468121, OM-468122, OM468123) and showed 99.68% similarity to *N. davidi* collected in Taiwan (MG734300). Altogether, PCR revealed that 4 shrimps (5.3% prevalence) were infected with microsporidians. Three of those individuals (4% prevalence), were infected with a microsporidian that was 99.56% similar to *Enterocytozoon hepatopenaei* (EHP) isolates found in India, China, and Vietnam (MH260592, KX981865, and KP759285, respectively). However, only 2 (OM-467903, OM467904) out of the 3 EHP sequences were of suitable quality to be used in our phylogenetic analyses and were consequently uploaded to GenBank. One of the 4 microsporidian-positive individuals (1.3% prevalence) was infected with an unknown microsporidian isolate, referred to here as Microsporidium sp. EFB01 (OM467902) showing 85.24% similarity to Microsporidium sp. BIVIC3 from lake Baikal (FJ756174) and 84.54% similarity to the *Vittaforma*-like Microsporidium sp. Q2-DKR-D (MF-374895) found in Taiwanese drinking water (Fig. 2). The most closely related and taxonomically characterized species is *V. corneae*, with 84.06% similarity (MH351756). Reference sequences of the genera *En-*

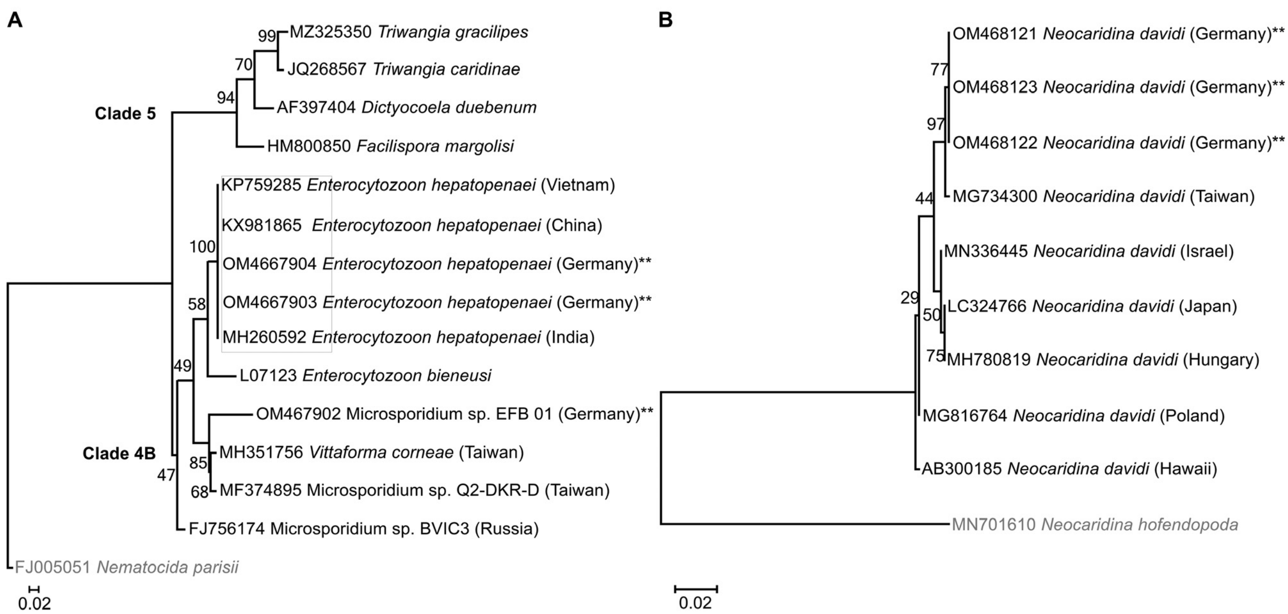

Fig. 2. Maximum likelihood phylogenetic tree with bootstrap values (999 replicates) of (A) microsporidian isolates (K2+G substitution model) and (B) *Neocaridina davidi* (T92+G substitution model). Outgroup sequences are indicated in grey font. **Specimens from this study

terocytozoon and *Vittaforma* all belong to the microsporidian Clade 4B (Park & Poulin 2021) (Fig. 2).

With an average carapace length of 4.04 ± 0.19 mm (SD), *N. davidi* individuals infected with EHP were smaller than uninfected individuals (5.47 ± 0.86 mm, Table 1). No epibionts or other parasites were found in or on *N. davidi*.

4. DISCUSSION

The release of ornamental pets and their associated parasites into novel environments can seriously threaten native biodiversity (Svoboda et al. 2017, Patoka et al. 2018). Despite that, there is a lack of knowledge on 'hitchhiking' pathogens, in particular concealed ones like microsporidians. The present study provides the first report on the occurrence of microsporidian parasites in non-native *Neocaridina davidi* from a German stream flowing into a thermally polluted river. To our knowledge, this is the first record of microsporidians in *N. davidi* to date.

The discovery of EHP in *N. davidi* is surprising, as this microsporidian is mainly known from brackish and marine water, where it has become a critical threat to the shrimp farming industry, particularly in Southeast Asia (Kim et al. 2022). However, EHP has been reported from waters with low to high salinity (2–40 ppt) (Aranguren Caro et al. 2021). Moreover, EHP is not limited to warm tropical and subtropical waters, as evidenced by its recent discovery in grow-out ponds located in the Korean province of Chungcheongnam-do, an area with climatic conditions similar to central Europe (Kim et al. 2022). Therefore, the survival of this parasite under German climatic conditions is plausible.

The occurrence of EHP and *Mirosporidium* sp. EFB01 in the Finkelbach is likely the result of co-introduction of *N. davidi* by aquarium owners in the Erft catchment. *N. davidi* is farmed intensively in ponds, mainly in southern Taiwan, in close proximity to the coast, an area known for intensive farming of penaeid shrimps such as *Litopenaeus vannamei* and *Penaeus monodon*. Penaeid shrimps such as *L. vannamei*, a euryhaline shrimp species, are common hosts for EHP (Aranguren Caro et al. 2021, Kim et al. 2022). Thus, it cannot be excluded that EHP might have inadvertently entered *N. davidi* ponds and infected shrimps destinated for aquarium trade export. Although EHP sequences from penaeid shrimp farmed in the same area are not available, the similarity with sequences found across Asia seems to support this transmission route. On the other hand, the origin of Microsporidium sp. EFB01 remains uncertain due to a low similarity to the best matching isolates currently hosted on GenBank.

The exclusive occurrence of EHP in small-sized *N. davidi* might be an indication of reduced growth of infected shrimps. Observations from brackish and marine shrimp farms indicate reduced feeding and retarded growth in infected shrimps (Kim et al. 2022). However, due to the small number of infected *N. davidi* individuals, this observation should be treated with caution. The prevalence of EHP in cultured shrimps such as *L. vannamei* typically ranges between 11 and 93% (Shen et al. 2019). Therefore, it is likely that the low infection level observed in *N. davidi* is related to reduced water salinity, which might hinder EHP infectivity, as observed by Aranguren Caro et al. (2021). Alternatively, *N. davidi* might not constitute an ideal host for this parasite, constraining its reproduction and transmission rate. An analogy could be the case of *Cucumispora dikerogammari*, a microsporidian that typically infects the amphipod *Dikerogammarus villosus* but is also able to infect other crustacean species in which it can be found at low prevalences (Bacela-Spychalska et al. 2012).

Ectoparasites and epibionts are commonly reported from *N. davidi*, including non-native populations (Ohtaka et al. 2012, Patoka et al. 2016, Liao et al. 2018, Maciaszek et al. 2021). For instance, the prevalence of *Scutariella japonica* (Platyhelminthes: Temnocephalida) in native *N. davidi* populations was between 25.2 and 100% (Ohtaka et al. 2012), while that of *Vorticella* sp., a peritrich ciliate, in non-native populations reached up to 82.5% (Maciaszek et al. 2021). Their absence in our sample might be due to a very low prevalence in the study area or their inability to adapt to the local water parameters. Similarly, a previous study in the same catchment also did not detect epibionts (Klotz et al. 2013).

Table 1. Infection, sex, and carapace length of *Neocaridina davidi*

	n (prevalence [%])	Sex	Carapace length (mm) Mean ± SD	Range
Enterocytozoon hepatopenaei	3 (4.00)	1F/2M	4.04 ± 0.19	3.88–4.30
Microsporidium sp. EFB01	1 (1.33)	1F	4.29	4.29
Uninfected	71	29F/42M	5.47 ± 0.86	3.67–7.99
Total	75	31F/44M	5.40 ± 0.89	3.67–7.99

The presence of *N. davidi* in the cold water of the Finkelbach suggests a range expansion towards colder water. Despite the relatively cold temperature of 9°C, an ovigerous female was present among the collected shrimps, indicating that local reproduction even at low temperature seems possible. In a previous study, non-native *N. davidi* individuals were observed sporadically during winter at a water temperature of 6°C in a thermally polluted stream (Weiperth et al. 2019). However, no ovigerous females were found in that study. As *N. davidi* has been present in the Erft catchment for over a decade, it is likely that, over time, the gradual insurgence of cold-adapted individuals among the shrimp population allowed for an expansion towards temperate water, as predicted by Klotz et al. (2013). Thus, a further spread into nearby watercourses can be expected.

The range expansion of *N. davidi*, coupled with the presence of microsporidian parasites, is cause for concern, as the transmission potential of EHP and its pathogenicity for the native crustacean fauna are unknown. Given the scarcity of information, further investigations, including screening of the native fauna to assess the transmission potential of these pathogens, are recommended.

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