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First record and DNA barcodes of the aquarium shrimp, *Neocaridina davidi*, in Central Europe from thermally polluted River Oder canal, Poland

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Abstract – *Neocaridina davidi* (Bouvier, 1904) is an exotic freshwater shrimp originating from Asia and often kept as a pet in amateur aquarium cultures. Herewith, we report on the second finding of *N. davidi* in fresh waters of Europe and the first discovery of that species both in Poland and in Central Europe. The species was found in samples collected in 2003, 2013 and 2017 in the thermally polluted canal connected to the River Oder, south of Gryfino, in the vicinity of the Dolna Odra Power Plant. The taxonomic identity of the collected shrimp was confirmed by the standard DNA barcoding procedure, using a 610 bp-long fragment of cytochrome oxidase I (COI). The findings spanning more than a decade suggest that *N. davidi* may have established a self-reproducing population at this site. Following the finding of *Atyaephyra desmarestii* (Millet, 1831) in 2000, *Neocaridina davidi* is the second freshwater shrimp species found in the River Oder and in Poland.

Keywords: Atyidae / freshwater shrimp / alien species / DNA barcoding / thermal pollution

Résumé – Premier signalement et code-barres ADN de la crevette d'aquarium, Neocaridina davidi, en Europe centrale dans le canal de l'Oder pollué thermiquement, Pologne. *Néocaridina davidi* (Bouvier, 1904) est une crevette exotique d'eau douce originaire d'Asie et souvent conservée dans des aquariums amateurs. Ici, nous présentons le deuxième signalement de *N. davidi* dans les eaux douces d'Europe et la première découverte de cette espèce en Pologne et en Europe centrale. L'espèce a été trouvée dans des échantillons prélevés en 2003, 2013 et 2017 dans le canal thermiquement pollué relié à l'Oder, au sud de Gryfino, à proximité de la centrale électrique de Dolna Odra. L'identité taxonomique des crevettes collectées a été confirmée par la procédure standard de code-barres ADN, utilisant un fragment de cytochrome oxydase I (COI) de 610 pb de longueur. Les résultats sur plus d'une décennie suggèrent que *N. davidi* a peut-être établi une population autoreproductrice sur ce site. Après la découverte d'*Atyaephyra desmarestii* (Millet, 1831) en 2000, *Neocaridina davidi* est la deuxième espèce de crevettes d'eau douce trouvée dans l'Oder et en Pologne.

Mots clés : Atyidae / crevette d'eau douce / espèce exotique / code-barres ADN / pollution thermique

Human-mediated introductions of alien species into fresh waters is a well-known phenomenon world-wide. Tropical and subtropical organisms are able to inhabit thermally polluted water bodies of the temperate zone (Maczulak, 2010). In Europe, classic examples of alien invertebrates thriving in water bodies with elevated water temperatures include *Branchiura sowerbyi* (Oligochaeta) and *Sinanodonta woodiana* (Bivalvia), both originating from tropical and subtropical Asia (Gruszka, 1999, Kraszewski and Zdanowski, 2011, Jabłońska *et al.*, 2015).

The shrimp *Neocaridina davidi* (Bouvier, 1904) occurs naturally in fresh waters of South-East Asia (Cai 1996). It has already been introduced to Hawaii (Englund and Cai, 1999)

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Table 1. Sequences used in analysis.

Accession number	Species	Locality	Reference
AB300183	*Neocaridina davidi*	Taiwan (Taichung County)	Shih & Cai 2007
AB300184	*Neocaridina davidi*	Taiwan (Yunlin County)	Shih & Cai 2007
AB300185	*Neocaridina davidi*	Hawaii (Oahu)	Shih & Cai 2007
AB300186	*Neocaridina davidi*	Hawaii (Oahu)	Shih & Cai 2007
AB300187	*Neocaridina davidi*	Taiwan (Kinmen County)	Shih & Cai 2007
AB300190	*Caridina cantonensis*	China (Guangdong Province)	Shih & Cai 2007
MG816764	*Neocaridina davidi*	Poland (Oder River)	This study
MG816766	*Neocaridina davidi*	Poland (Oder River)	This study
MG816769	*Neocaridina davidi*	Poland (Oder River)	This study
MG816771	*Neocaridina davidi*	Poland (Oder River)	This study
MG816772	*Neocaridina davidi*	Poland (Oder River)	This study
MG816774	*Neocaridina davidi*	Poland (Oder River)	This study
MG816775	*Neocaridina davidi*	Poland (Oder River)	This study
MG816765	*Neocaridina davidi*	Poland (aquarium collection)	This study
MG816767	*Neocaridina davidi*	Poland (aquarium collection)	This study
MG816768	*Neocaridina davidi*	Poland (aquarium collection)	This study
MG816770	*Neocaridina davidi*	Poland (aquarium collection)	This study
MG816773	*Neocaridina davidi*	Poland (aquarium collection)	This study
MG816776	*Neocaridina davidi*	Poland (aquarium collection)	This study

and Japan (Nishino and Niwa, 2004). In Europe, this species is an extremely popular aquarium pet (Nur and Christianus, 2013, Horwath, 2015, Pantaleão *et al.*, 2017) but was only recently found in nature, in thermally polluted tributaries of the River Rhine, Western Europe (Klotz *et al.*, 2013).

In this study, we provide a first report on the occurrence of *N. davidi* in Poland, Central Europe. The shrimp were collected in a ca. 4.5 km-long artificial canal connected to the lower section of the Oder River, south of Gryfino (N 53°12′42.36″, E 14°28′2.70″). The canal receives heated waters from the large Dolna Odra Power Station cooling system. Bottom sediments at the sampling site were composed of fine-grained sands and mud. The banks were partially covered by concrete. The water temperature at the sampling site in June – July was 27–29 °C, while in November, it dropped to 15 °C. A total of 15 animals were found in qualitative benthic samples, collected using a hydrobiological hand-net from submerged vegetation in July 2003 (3 individuals), November 2013 (3 ind.) and June 2017 (9 ind.). The sample from June 2017, was taken from a 1 m^2 surface in about 15 min sampling time. For comparison and taxonomic verification, in 2017, we acquired six specimens of *N. davidi* var. "Red Cherry" from an amateur aquarium culture in Łódź, Poland, ca. 500 km south-east from the sampling site. All individuals, except the sample from 2003 (which was preserved in 4% formaldehyde), were instantly preserved in 96% ethanol.

Morphological examination of shrimp was carried out using descriptions in papers by Englund and Cai (1999) and Klotz *et al.* (2013). Individuals were then DNA-barcoded using the cytochrome *c* oxidase subunit I (COI) marker. Total DNA was extracted from the abdominal tissue of 13 specimens, collected both from the field and from aquarium using the Chelex procedure (Casquet *et al.*, 2012). The PCR reaction followed the protocol provided by Hou *et al.* (2007), with the primer pair HCOJJ/LCOJJ (Astrin and Stüben, 2008). PCR products were purified with Exonuclease I and FastAP alkaline phosphatase (Werle *et al.*, 1994), and then sequenced by Macrogen Inc., Korea. The obtained sequences are deposited in GenBank (Benson *et al.*, 2005). The GenBank accession numbers are provided in Table 1. In addition, we acquired five COI sequences of *Neocaridina davidi* from Taiwan and Hawaii, published and deposited in GenBank, under the name *Neocaridina denticulata sinensis* (Kemp, 1918), by Shih and Cai (2007) (Tab. 1). The sequences were aligned and trimmed to the length of 610 bp in Geneious 6.1.8 (Kearse *et al.*, 2012). Haplotypes were identified using the DnaSP software (Librado and Rozas, 2009). The Neighbour-Joining method was applied using Mega 7.0 (Kumar *et al.*, 2016). *Caridina cantonensis* COI sequence was chosen as an outgroup (Tab. 1).

The material examined morphologically, contained five males and 16 females (one ovigerous, collected in November 2013). All individuals were identified morphologically as *Neocaridina davidi* using the following characteristics (based on all the individuals found at the sampling site). Body length, 13 to 24.5 mm. Average body length of males: 15.5 mm, average body length of females: 19.3 mm. Rostrum, with 13–18 dorsal teeth (2–4 postorbital) and 4–8 ventral teeth, reaching the distal edge of the third antennular segment. The tip of the rostrum slender, slightly curved, without teeth. Eyes, normally developed. Carapace smooth. Supraorbital spine absent. Antennal spine well-developed. Pterygostomian angle acute and sharply pointed. Pereiopods normally developed, characteristic of the family Atyidae. Spines on dactylus of the 3rd pair of pereiopods in males stronger and thicker than in females. Endopod of the 1st male pleopod, pear-shaped, broad and rounded in its distal part. Telson, shorter than uropods, with 4–5 pairs of dorsal spines, margin rounded, armed with 8–10 long spines.

DNA COI barcodes also confirmed that all the studied shrimps belong to *Neocaridina davidi*. The haplotype assignment showed that all the individuals, collected either

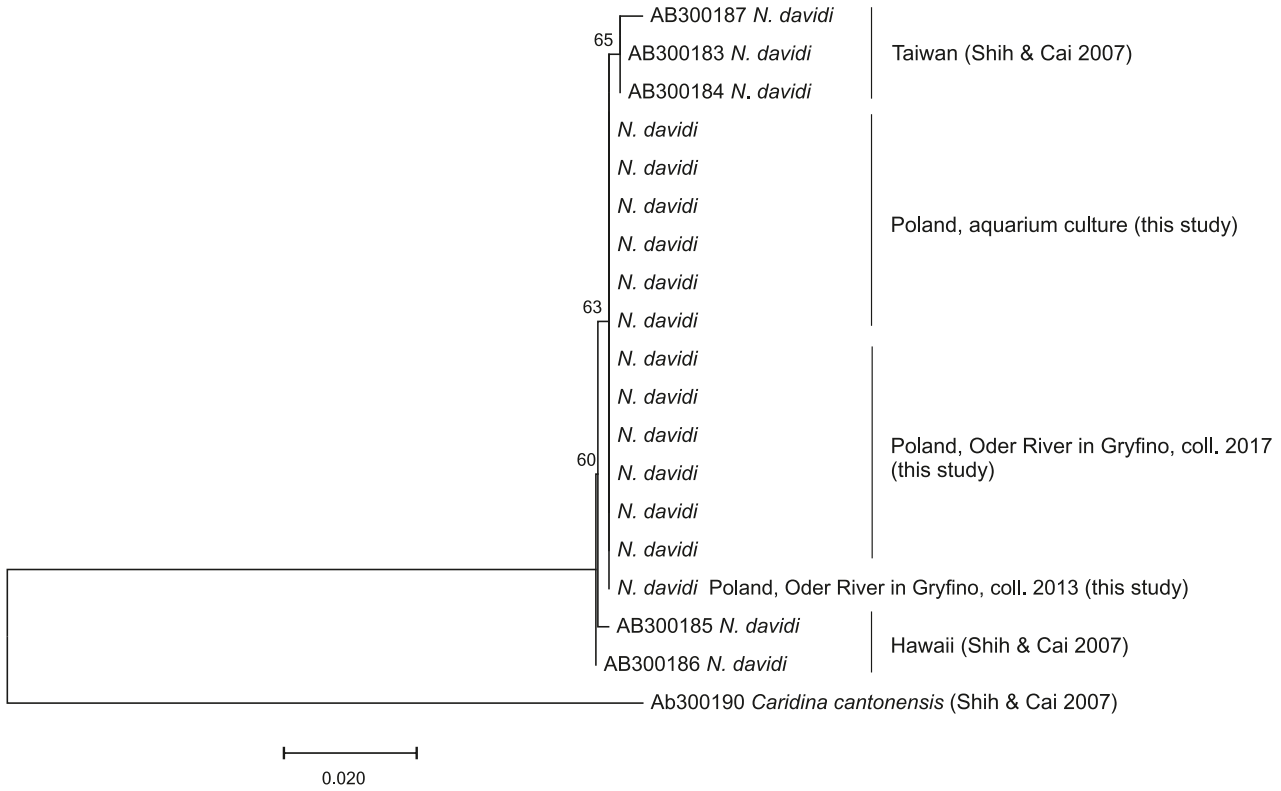

Fig. 1. Neighbour-Joining tree constructed for newly obtained and GenBank-stored sequences of *Neocaridina davidi*.

in the wild or from the aquarium culture, belonged to a single haplotype (Fig. 1). Moreover, four other COI haplotypes of individuals from Hawaii and Taiwan, acquired from GenBank, differed from the sequences we obtained, by only one to three mutations.

The results of our morphological and genetic analyses confirms the presence of *N. davidi* in the thermally-polluted canal connected to River Oder near Gryfino, Poland. Low numbers of shrimp found in the samples may indicate low abundance and density. The population in the sampling site is distributed pointwise and it is probable that numerous suitable habitats are inaccessible for sampling, enclosed in the Power Plant's grounds. This is the second record of this species, from European surface waters and the first from Central Europe. *Neocaridina davidi* is the second freshwater shrimp species reported from Poland after *Atyaephyra desmarestii* recorded previously from the western, natural, branch of the Oder River near Gryfino (Gruszka, 2001). Although both shrimps, *N. davidi* and *A. desmarestii*, belong to family Atyidae, the differences in their morphology are clear (Klotz *et al.*, 2013).

Neocaridina davidi, particularly the variety "Red Cherry", is a very popular aquarium pet world-wide, including Europe (Nur and Christianus, 2013, Horwath, 2015, Pantaleão *et al.*, 2017). We propose that, as in the Rhine tributaries in western Germany, the population occurring in the canal near Gryfino derives from an aquarium culture, especially since the wild individuals share the same haplotype as the cultured ones. Moreover we cannot exclude the possibility that the population has been settled in this place for more than a decade. The individuals (including one ovigerous female) were collected in 2003, 2013 and 2017, the temperature in the canal reaches 27–28 °C, which are the most favorable values for breeding of *N. davidi* (Nur and Christianus, 2013, Tropea *et al.*, 2015). Another possibility, although less probable over such a long period, would be a series of yearly introductions supplying the population regularly with captive-bred individuals. Thus, the population should be investigated in the coming years to check its stability at the study site. It would be also interesting to study a wider area to ascertain if the species is spreading.

Although releasing aquarium pets into open waters can be harmful for natural ecosystems, this phenomenon is quite common (Gherardi *et al.*, 2007, Lipták and Vitázková, 2015). Aquarists often believe this is a more humanitarian solution than killing unwanted pets (Duggan, 2011). In some cases, stable populations of exotic species pose a threat to native species and entire ecosystems including increased predatory pressure on indigenous fauna or introduction of new diseases or pathogens (Gherardi *et al.*, 2007, Klotz *et al.*, 2013, Weber and Traunspurger, 2016). So far, no such threats associated with *N. davidi* have been found in nature. On the other hand, most tropical organisms introduced to natural waters in temperate climates are not able to survive the winter. Some, however, do find the new conditions advantageous. Klotz *et al.* (2013) noted that the native habitat of *N. davidi* is not tropical, since the water temperatures range from 6 °C in winter to 30 °C in summer. Such winter temperatures are still 2–4 °C higher than those in Central Europe (Wetzel, 2001). Nevertheless, the global warming associated with prolonged periods without cold spells in winters and heat waves in summers (Anders *et al.*, 2014), makes the temperate zones more prone for acclimatization of thermophilic species. Such is the case of the predominantly Mediterranean scarlet dragonfly, *Crocothemis*

erythraea (Brullé, 1832), recently expanding its breeding range in Central Europe (Ott, 2010). We suppose that thermally polluted water bodies may serve as gateway ecosystems in which such species adapt to local conditions and, in the future, colonize natural waters. After acclimatization, the further spread of alien freshwater species is usually promoted by other human activities, such as building artificial channels connecting previously isolated river basins, inland waterway transportation, tourist movement as well as by ecosystems degradation (Gherardi, 2007). Thus, we suspect that, with increasing propagule pressure from amateur aquarists, further climate warming and connection of inland waterways, *N. davidi* will likely spread in Central Europe and become a stable element of local fauna, as the thermophilic *Procambarus clarki* (Girard, 1852) has in many other regions of Europe (Souty-Grosset *et al.*, 2016). It would be beneficial to promote the education and awareness of the problems of releasing non-native aquarium species into water systems. This activity is an adverse procedure for natural environments, particularly because controlling such activities is very difficult.

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