Further steps of Cryptorchestia garbinii invasion in Polish inland waters with insights into its molecular diversity in Central and Western Europe

Tomasz Rewicz1,2,* , Jaroslaw Brodecki1, Karolina Bącła-Spychalska1, Alicja Konopacka1 and Michał Grabowski1
1 University of Lodz, Department of Invertebrate Zoology and Hydrobiology, 12/16 Banacha str., 90-237 Lodz, Poland
2 University of Guelph, Centre for Biodiversity Genomics, 50 Stone Road East Guelph, ON N1G2W1, Canada

Received: 18 November 2019 / Accepted: 27 February 2020

Abstract – Cryptorchestia garbinii Ruffo, Tarocco and Latella, 2014 (Amphipoda: Talitridae), a semiterrestrial amphipod, reported (as Orchestia cavimana Heller, 1865) from the Polish inland waters, e.g., the lowermost Vistula River, for the first time in 2009. Now, we have reported this species from the upper Oder River, which is c.a. 350 km SE from the closest known localities near Berlin, Germany. We confirmed its identity by the DNA barcodes and showed that all individuals found so far in other countries in Central and Western Europe represent the same haplotype, excluding a singleton from the locus typicus of this species in Lake Garda, Italy.

Keywords: Amphipoda / Talitridae / beach hoppers / non-indigenous species / DNA barcoding

1 Introduction

The Oder is the second biggest river in Poland and thirtieth in Europe with a length of 854 km and the drainage area of 1,18,861 km². The river flows into the Baltic Sea and is the most significant waterway in Poland, navigable on most of its length. The Oder is fed, among others, by such rivers as Warta, Nysa Łużycka, Bóbr and connected through artificial channels to Havel, Spree, Vistula and Kłodnica rivers, which supports the migration of non-indigenous species through the so-called Central Invasion Corridor (CIC) sensu Bij de Vaate et al. (2002). Additionally, the Szczecin Lagoon, being a part of the Oder deltaic system (Osaduczuk, 2004), with a large traffic port playing an important role in marine and inland shipping, is considered to be an essential gateway for the introduction of invasive species (Gryszka, 1999; Galil et al., 2008; Gryszka et al., 2013; Messner and Zettler, 2018). The number of alien macroinvertebrate species in the Oder River is still growing. Altogether, at least 33 non-indigenous species have been so far identified in the Oder and in the Szczecin Lagoon (Gryszka, 1999; Szlauer-Łukaszewska and Grabowski, 2012; Jabłońska et al., 2015; Pabis et al., 2017; Szlauer-Łukaszewska et al., 2017; Jabłońska et al., 2018; Cebulska and Krodkiewska, 2019). Among them, nine amphipod species were identified: Chelicorophium curvispinum (Sars G.O., 1894), Dikerogammarus villosus (Sowinsky, 1894), D. haemobaphes (Eichwald, 1841), Echinogammarus marinus (Martynov, 1932),...
Melita nitida Smith, 1873, Obesogammarus crassus (Sars G.O., 1894), Pontogammarus robustoides (Sars, 1894), Gammarus tigrinus Sexton, 1939 and Cryptorchestia garbinii (Orchestia cavimana) Ruffo, Tarocco and Latella, 2014 (Konopacka et al., 2009; Rachalewski et al., 2013; Zettler and Zettler, 2017).

Lowry and Fanini (2013) erected the genus Cryptorchestia to accommodate semi-terrestrial and terrestrial species, associated with fresh and brackish waters, set apart from the typically marine, supralittoral beach hoppers of the genus Orchestia Leach, 1814 (see Davolos et al., 2018a and literature therein). Currently, Cryptorchestia cavimana (Heller, 1865) is considered as endemic to Cyprus (Ruffo et al., 2014; Davolos et al., 2017). In contrast, Cryptorchestia garbinii (encompassing records of O. cavimana outside Cyprus), has a wide European distribution including sea coasts, deltas and estuaries of the Baltic, Black, Marmara, Mediterranean, and North seas (Herkül et al., 2006; Konopacka et al., 2009; Davolos et al., 2018a, Tab. S1)(Fig. 1). Cryptorchestia garbinii is continually spreading in the European inland freshwater ecosystems, such as large rivers (e.g., Dnieper, Danube, Thames, Rhine) (Rehage, 1987; Martens et al., 1999; Ruffo et al., 2014) and lakes (e.g., Albano, Balaton, Doirani, Garda, Ohrid, Prespa) (Carasu et al., 1955; Karaman, 1993; Juhász et al., 2006; Ruffo et al., 2014; Davolos et al., 2018a).

In Poland, the first observations come from the brackish waters of the Szczecin Lagoon, the islands of Wolin and Uznam, the Vistula Lagoon, the Martwa Wisha (Jaźdżewski and Konopacka, 1995) and the Puck Bay (Spicer and Janas, 2006). The first record from inland waters (as an O. cavimana) was provided by Konopacka et al. (2009) from the lowest part of the Vistula River, 3 km upstream from its mouth (Fig. 1). No further localities from the Polish inland waters have been registered since then. Even Tykarska et al. (2019), in their work about the distribution of talitrids at the Polish Baltic coast, regarded this species as restricted only to the Vistula mouth.

Our study aims to document the southward spread of C. garbinii to the upper Oder river as well as to provide first molecular confirmation for the species identity in Poland. Taxonomic consistency is crucial for the studied species as its assignments to O. cavimana are still ongoing. Even four years after the taxonomic revision of Orchestia and redescription of C. garbinii and C. cavimana (Lowry and Fanini, 2013, Ruffo et al., 2014), C. garbinii is still commonly reported as O. cavimana (Davolos et al., 2018b), generating more confusions
Molecular identification based on the portion of cytochrome oxidase (COI) gene used as a DNA barcode as well as comparison with other European sequences of *C. garbinii* (including *locus typicus*) can be sufficient to reveal species assignment and potential cryptic diversity. Such a method has already been successfully implemented in studies upon various invasive invertebrates, including amphipods (*e.g.*, Rewicz et al., 2015, 2016; Hupalo and Grabowski, 2018; Mauvisseau et al., 2019).

### 2 Materials and methods

The individuals of *Cryptorchestia garbinii*, were collected on the groin near Krapkowice (11th of October, 2018) and Zdzieszowice (17th of October, 2019) (Fig. 2). The samples were collected by hand and with a benthic hand net from plant debris and grassroots along the water’s edge and from among the stones and instantly preserved in 96% ethanol. Additional specimens that originated from previous sampling campaigns: Poland 2009, 2016 as well as Italy 2011, 2016 were used (Tab. 1, Fig. 1). All the material was identified to the species level under the Nikon SMZ-800 stereomicroscope, based on available literature (*i.e.*, Spicer and Janas, 2006; Ruffo et al., 2014) and has been stored in the permanent collection of the Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Poland.

The total DNA was extracted from the fifth pereiopod or pleopods of the dissected individuals. DNA extraction, PCR reaction, enzymatic purification, and sequencing was proceeded following Rewicz et al. (2019). To verify the identification of obtained sequences, we used the BLAST tool (Altschul et al., 1990). Then, the sequences were edited and aligned using Geneious 10.2.6 software package (Kearse et al., 2012), and deposited in GenBank under accession numbers MK721831–MK721847; MN701467 – MN701476. The COI haplotypes were identified using the DnaSP v5 software (Librado and Rozas, 2009). All 22 sequences were deposited in the online database of the Barcode of Life Data Systems (BOLD) Systems (Ratnasingham and Hebert, 2007). We obtained the Barcode Index Numbers (BIN) which are indexing DNA sequences based on the genetic distance approach (Ratnasingham and Hebert, 2013). Additional COI sequences of *C. garbinii* (eight individuals), as well as the three outgroup sequences, were acquired from GenBank and BOLD (see Tab. 1). Sequences were finally trimmed to 500 bp. Phylogenetic tree was constructed in MEGA 6 (Tamura et al., 2013) using the neighbor-joining method (Saitou and Nei, 1987) based on the K2p distance (Kimura, 1980) with a bootstrap test performed on 1000 replicates (Fig. 3). Relevant voucher information is accessible through the public dataset “DS-CGARPOL” (DOI: http://dx.doi.org/10.5883/DS-CGARPOL) in the Barcode of Life Data Systems (BOLD; http://v4.boldsystems.org).

### 3 Results

DNA barcoding confirmed the identity of all the 22 individuals identified as *C. garbinii* (Fig. 3). We have found only two haplotypes in the analyzed dataset. Almost all individuals from Great Britain, Italy, Germany, and Poland represented one haplotype. Only in the locality from Lake Garda (*locus typicus*), we found another unique haplotype, differing by a single mutation from the common one (Fig. 3). All the sequences grouped under one BIN (BOLD: AAD87110) which is up to now the only BIN known from the BOLD publically available data and is widespread in Western and Central Europe.

### 4 Discussion

The known distribution of *C. garbinii* stretches to the Balkans and the Ponto-Caspian region on the south-eastern part of Europe and Great Britain, France and Italy on the western part (Ruffo et al., 2014, own unpublished data). We can observe an ongoing spatial expansion of *C. garbinii* in Europe, as new records have been reported in recent years from northern Germany, Netherlands, Belgium, Poland, Baltic States, Ukraine, Balkan Peninsula and from Turkey (Herkül et al., 2006; Ramm and Scholtz, 2017; Jelassi et al., 2018).
Konopacka et al., 2009; Rewicz et al., 2016; GBIF, 2019; Davolos et al., 2018a; Fig. 1, Tab. S1). Revealing the presence of this talitrid in the upper Oder confirms its ongoing expansion. The question arises of what the source for this new population is. The obtained DNA barcode sequences unequivocally support the identification of *C. garbinii* in Poland (Fig 3). The molecular diversity of this species in Central and Western Europe is quite interesting. Only two haplotypes have been identified in an area of more than 750,000 km², stretching N-S about 1360 km and W-E about 1300 km (Fig. 1). One of them is present all over the studied range, while the second haplotype occurs only in Lake Garda, which is a *locus typicus* of this species. Such low haplotype diversity may be, on the one hand, a result of fast colonization process, on another, a result of under-sampling. Davolos et al. (2017, 2018a) conducted studies upon phylogeny of the genus *Cryptorchestia* and found different haplotypes occurring in Lake Ohrid, Northern Macedonia, and in Turkey. However, since they used nuclear Histone 3, and the mitochondrial COI 3P markers, we cannot compare our data. Interestingly, *C. garbinii* and two other *Cryptorchestia* species, *C. cavimana*, and *C. ruffoi*, which are endemic to the east Mediterranean islands of Cyprus and Rhodes, respectively, form a monophyletic clade (Davolos et al., 2018a). That may suggest a potentially different place of origin and diversification of this species, than Western Europe (*locus typicus*). Studies upon another amphipod, *Gammarus roeseli* Gervais, 1835, pointed out the Balkan Peninsula as the most probable origin of the species (Grabowski et al., 2017). This region is regarded as an essential faunal biodiversity hotspots in Europe (e.g., Wysocka et al., 2013, 2014; Sworobowicz et al., 2015; Mamos et al., 2016; Jabłońska et al., 2020). The Pontic region was, on the other hand, the place of origin of *Gammarus varsoviensis* Jázdżewski, 1975 (Grabowski et al., 2012). Both amphipod species were described hundreds of kilometers westward (vicinity of Paris, France, and Warsaw, Poland, respectively) from their factual place of origin. In the case of both species, we can observe much higher molecular diversity in the real place of origin than in the recently colonized area. We can expect a similar situation in the case of *C. garbinii*, and further phylogeographic studies including individuals from the Ponto-Caspian and Balkan regions, are undeniably needed to solve this problem.

In conclusion, we provide the first molecular evidence of the presence of *Cryptorchestia garbinii* in Poland and insight into the molecular diversity of the species in Central and Western Europe. This species is in the spatial expansion stage in Europe, and its population structure, as well as wide-scale phylogeography (especially in the Balkans and in the Ponto-Caspian region), are still insufficiently known and require thorough molecular research.
Acknowledgments. We would like to thank Berenika Georgievová, Kamil Hupało, Ewa Janowska, Piotr Janowski, Tomasz Mamos, and Remi Wattier for their support in-field sampling as well as to Kamil Hupało and Halyna Morhun for their assistance in laboratory work. We would like to thank Andrii Khomenko, Halyna Morhun, Vadim Panov, Alexandr Prokin and Mikhail Son for information about occurrence data of Cryptorchestia garbinii in Ukraine and in Russia. The study was supported partially from the National Science Centre (Poland), projects 2018/31/D/NZ8/03061, 2014/15/B/NZ8/00266; Polish Ministry of Sciences and Higher Education grant N N304 350139 and partially from the internal funds of the University of Lodz. Tomasz Rewicz was supported by the Scholarship of the Polish National Agency for Academic Exchange (NAWA) at Bekker Programme nb. PPN/BEK/2018/1/00162/U/00001. Jarosław Brodecki participates in the University of Lodz programme for higher school students “Zdolny uczeń - świetny student”.

References


Fig. 3. Neighbor-joining tree based on COI K-2p distance for sequences of Cryptorchestia garbinii, C. cavimana, and two other talitrid outgroup species. Sequences from the Oder river are in bold. Each sequence name consists of two parts separated by a lower dash: (1) BOLD Process ID, (2) species name.

Supplementary Material

Supplementary table.

The Supplementary Material is available at https://www.kmaejournal.org/10.1051/kmae/2020009/olm.


GBIF.org (06 November 2019) GBIF Occurrence Download https://doi.org/10.15468/dl.giszu


Pabis K, Krodkiewska M, Cebulska K. 2017. Alien freshwater polychaetes Hypania invalida (Grube 1860) and Laonome valida,
Capa 2007 in the Upper Odra River (Baltic Sea catchment area).

Knowl Manag Aquat Ecosyst 418: 46.


