

DNA barcoding of alien Ponto-Caspian amphipods from the Belarusian part of the Central European invasion corridor

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Abstract

Here we focus on alien Ponto-Caspian amphipods in the Belarusian part of the Central European Invasion Corridor and their identification through DNA barcoding. A new alien Ponto-Caspian amphipod species, *Echinogammarus trichiatus*, was identified in the Belarusian part of the Dnieper River using DNA barcoding. This species expands the national checklist of Ponto-Caspian amphipods to nine species. In addition, DNA barcoding confirmed the misidentification of *Dikerogammarus bispinosus* in Belarus.

Background

Invasive alien species (IAS) are considered an important cause in the global biodiversity loss and their role was recognized by the Convention on Biological Diversity which included IAS among the Aichi Biodiversity Targets to be reached by 2020. For fast and accurate identification of IAS, DNA barcoding arises as an optimal method. Eight species of Ponto-Caspian amphipods are known to have invaded the water bodies of Belarus (Figure 1): *Chelicorophium curvispinum*, *Ch. robustum*, *Dikerogammarus haemobaphes*,

D. villosus, *Echinogammarus ischnus*, *Obesogammarus crassus*, *O. obesus*, and *Pontogammarus robustoides* [2, 3]. Despite a well-developed checklist of alien amphipods for Belarus, the presence of additional IAS (such as *Echinogammarus trichiatus*, *Dikerogammarus bispinosus*, *Chelicorophium mucronatum*, or *Chaetogammarus warpachowskyi*) might go undetected due to misidentification (especially in early developmental stages) or cryptic speciation.

Methods

DNA was extracted from whole legs of amphipods using a silica-based protocol with glass fiber filtration plates [4]. The 658-bp barcode region of the mitochondrial COI gene [5] was amplified with two primer sets: CrustDF1 / CrustDR1 [6] or C_LepFolF / C_LepFolR [7]. Bidirectional sequencing was performed at the Canadian Center for DNA Barcoding (University of Guelph, Canada) according to standard protocols (<http://ccdb.ca/resources/>). Sequence editing was performed with CodonCode Aligner v. 5.1.5. All details regarding taxonomy, collection, vouchers, images, traces and sequences have been uploaded to BOLD [8] and will be released upon publication.

Figure 1: Map of the Belarusian part of the Central European Invasion Corridor (adapted from [1])

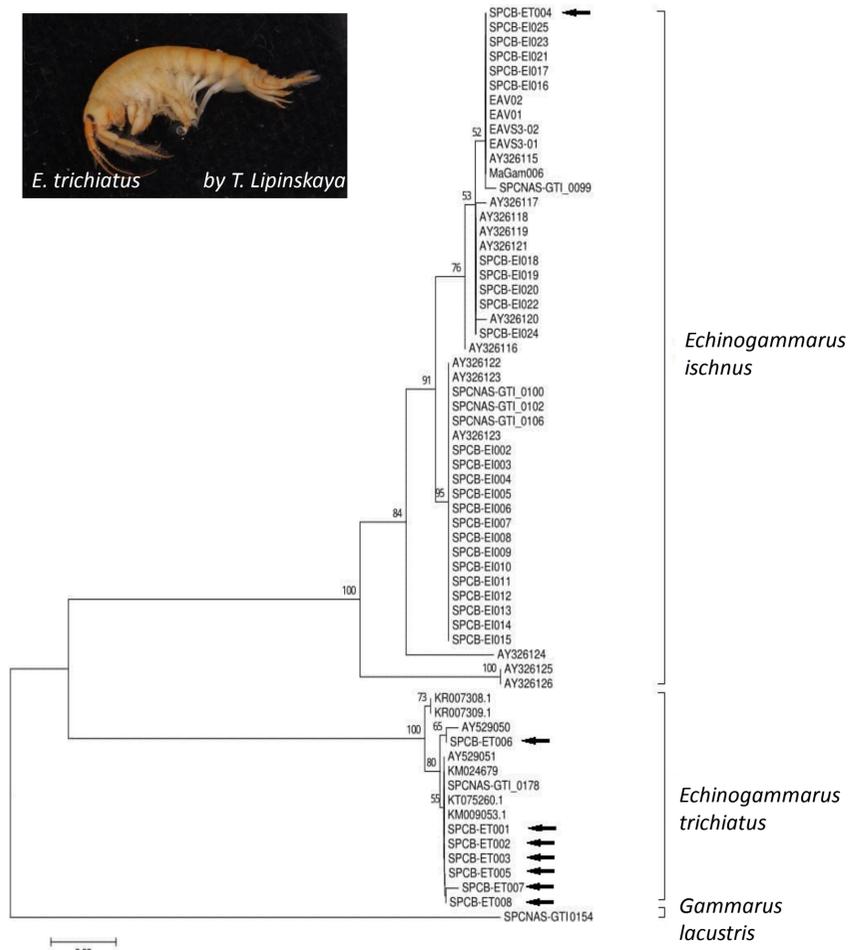
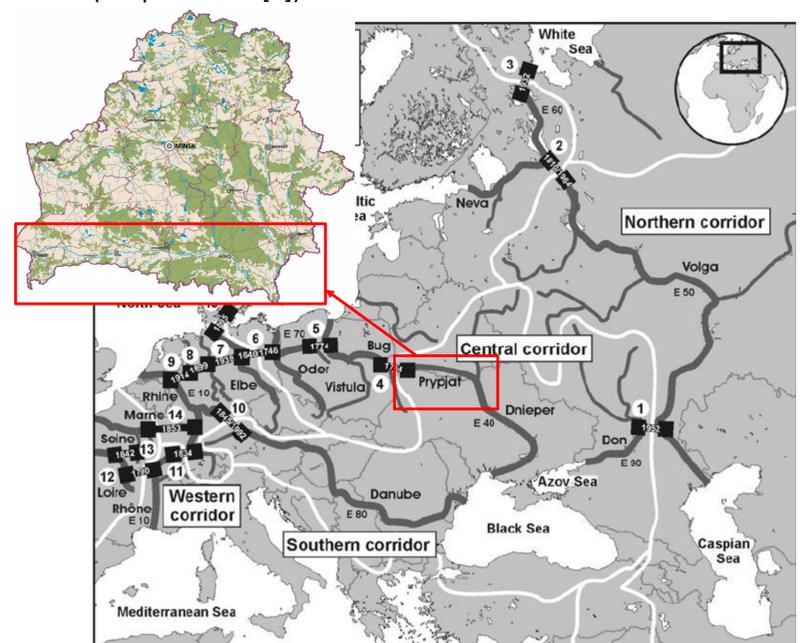


Figure 2: Neighbor-joining tree based on COI p-distances for sequences of *Echinogammarus ischnus* and *Echinogammarus trichiatus*. *Gammarus lacustris* was used as an outgroup. Arrows indicate samples from the Dnieper River (this study). Bootstrap values are indicated for each node. Samples with SPC* code were generated in this study.

References

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Results and discussion

DNA barcodes were obtained from 395 specimens, representing all nine species of alien amphipods of Belarus. BIN assignments [9] corresponded perfectly with current species boundaries in 89 % of cases. Two species showed patterns of intraspecific divergence: *E. ischnus* was split into two BINs (BOLD:AAB7817 and BOLD:ADF4395 separated by 1.25% distance; November 8, 2017), while *Ch. curvispinum* displayed intraspecific distances of 5%, although all sequences were grouped into one BIN (BOLD:ACW3521; November 8, 2017).

DNA barcoding confirmed the misidentification of *D. bispinosus* in Belarus.

Eight specimens identified morphologically as *E. trichiatus* were analyzed and seven sequences were generated (SPCB* codes in Figure 2). These sequences matched *E. trichiatus* sequences from Ukraine, Romania, Belgium, Netherlands, Germany, and Turkey, available in BOLD. Together, they form a single cluster with 100% bootstrap support (Figure 2). The mean K2P between 15 compared sequences of *E. trichiatus* was 0.0041 (± 0.0021 SE). In conclusion, DNA barcoding confirmed the presence of a new alien amphipod species in Belarus.

This is the first effort to provide a molecular inventory of alien Ponto-Caspian amphipod species known from the Belarusian part of the Central European Invasion Corridor. The results of this study will be used to update the national checklist of alien amphipods of Belarus.

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