

Discovery of *Dreissena rostriformis bugensis* (Andrusov 1897) in Western Europe

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Abstract Live individuals of *Dreissena rostriformis bugensis* were found in a trawl net sample taken in April 2006 near Willemstad in the Hollands Diep, part of the main distributary in the Rhine delta in The Netherlands. This represented the first record of this species in Western Europe and a major westward range extension for this Ponto-Caspian species. Density was very low, with the ratio of *Dreissena polymorpha* to *D. rostriformis bugensis* in the order of 100:1. Mean length ($n = 5$) was 22.5 mm (range 20.4–24.6 mm), suggesting that they were at least two years old and that the original introduction into the Hollands Diep had occurred in 2004 or earlier. Species identity was confirmed by mor-

phological and molecular methods. We speculate that the invasion occurred through the Main-Danube Canal – a pathway that is becoming increasingly recognized as a southern corridor for the potential movement of non-indigenous aquatic species between Eastern and Western Europe.

Keywords Introduced species · zebra mussels · *Dreissena* · Main-Danube Canal · Hollands Diep

Introduction

The Ponto-Caspian freshwater bivalve *Dreissena polymorpha* (Pallas 1771) quickly spread from Eastern to Western Europe during the 19th century through a corridor of river basins interconnected by man-made canals (Kinzelbach 1992). Over the last two centuries, this dreissenid species has continued its expansion into inland waters throughout much of Western Europe.

In contrast, another Ponto-Caspian species, *Dreissena rostriformis bugensis* (Andrusov 1897), has been far slower in its range expansion. Native to the Dnieper and Bug rivers and limans of the northern Black Sea, its distribution was limited to the Ukraine until about the middle of the 20th century (Therriault et al. 2005). Since then, an eastward expansion has occurred into Russia (Orlova et al. 2004; Zhulidov et al. 2004, 2005). Recently there have also been two reports

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of *D. rostriformis bugensis* in the Danube River within Romania, i.e., west of its native range. Micu and Telembici (2004) indicated finding *D. rostriformis bugensis* at Cernavoda (44°21' N, 28°02' E) in 2004 (#1 in Fig. 1), and Popa and Popa (2006) observed this species the next year further westward (>500 km upriver) at Drobeta Turnu Severin (44°37' 13.68" N, 22°40' 49.40" E) (#2 in Fig. 1). Noting these two recent Danube records, Mienis (2006) predicted that *D. rostriformis bugensis* would soon arrive in the Rhine River since these two rivers were now reconnected by the Main-Danube Canal. Since its reopening in 1992, this canal has been recognized as a potential new southern corridor for movement of non-indigenous aquatic species between Western and Eastern Europe (Bij de Vaate et al. 2002, Müller et al. 2002). Herein we provide evidence from The Netherlands that *D. rostriformis bugensis* is present in Western Europe and that its introduction likely occurred in 2004 or earlier.

Discovery and Species Identification

As part of a field project intended to document zebra mussel endosymbionts (Molloy and Giamberini, unpublished data), several thousand *Dreissena* were collected on April 19, 2006 with a trawlnet at a depth of 5–7 m in the Hollands Diep (51°42' 11" N, 04°28' 34" E), part of the main distributary in the Rhine delta in The Netherlands (#3 in Fig. 1). The zebra mussels collected were mainly attached to unionid shells (empty shells and live specimens). Since *D. polymorpha* was the only *Dreissena* sp. previously recorded from Western Europe, it was surprising to see that about 1% of the *Dreissena* completely lacked the distinctive ventro-lateral carina typical of *D. polymorpha* (Pathy and Mackie 1993). These latter specimens were subsequently identified as *D. rostriformis bugensis* since they morphologically possessed the key external and internal shell characteristics of this species (see description of the “quagga mussel” in Pathy and

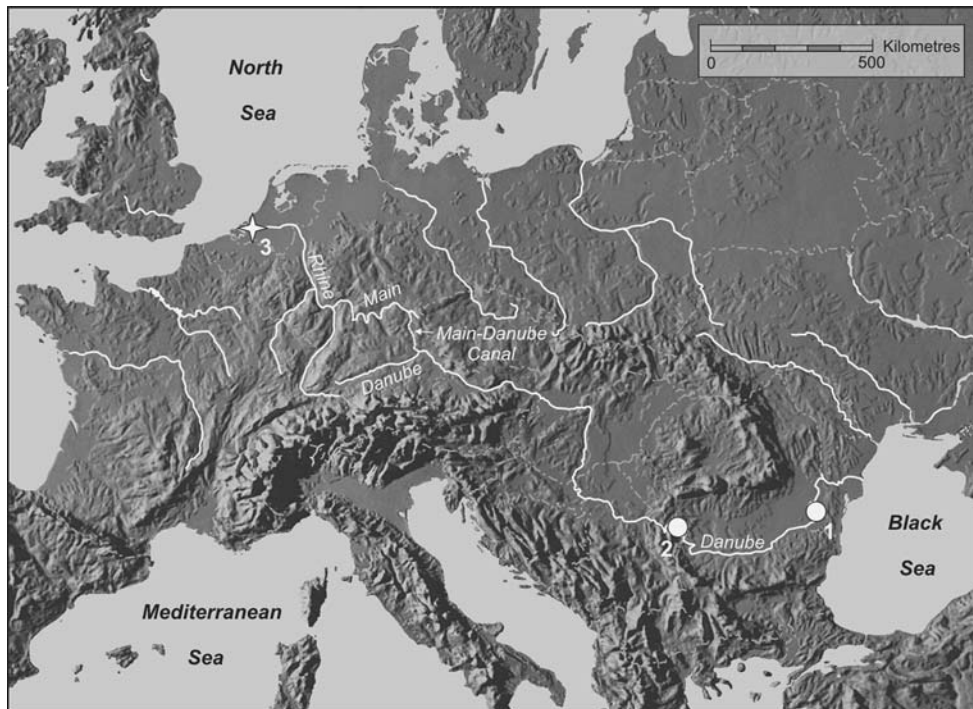


Fig. 1 Locations west of its native range where *D. rostriformis bugensis* has been field collected. (1) Danube River, Cernavoda, Romania in 2004 (Micu and Telembici 2004). (2) Danube River, Drobeta Turnu Severin, Roma-

nia in 2005 (Popa and Popa 2006). (3) Hollands Diep, Willemstad, The Netherlands (present study). Map adapted from Mountain High Maps® Copyright© 1993 Digital Wisdom®, Inc.

Mackie 1993). Five of these *D. rostriformis bugensis* were randomly chosen and deposited as voucher specimens (#159740) at the malacological department of the Zoological Museum Amsterdam. Their mean length (range) was 22.5 (20.4–24.6) mm, suggesting that they were at least two years old and that the original introduction into the Hollands Diep had occurred in 2004 or earlier (Jantz and Neumann 1992, Smit et al. 1992).

To confirm the species identification using genetic markers, tissue samples from two of the above voucher specimens were fixed in 96% ethanol. DNA from each mussel was isolated using the CTAB protocol of Wilke et al. (2006). For amplification, we utilized the variable mitochondrial gene for cytochrome oxidase *c* subunit I (COI), which is the most widely used marker for testing species level relationships in molluscs. The primers for amplifying a fragment of the COI gene were LCO1490 and HCO2198 as described by Folmer et al. (1994). Sequences (forward and reverse) of the two specimens were determined using the LI-COR (Lincoln, NE) DNA sequencer Long ReadIR 4200 and the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ). The DNA work yielded two identical, 638 base pair long fragments (GenBank accession numbers EF080861 and EF080862), one of which was used for BLAST searches in NCBI GenBank. The search returned, among others, three completely identical sequences of the COI gene, all belonging to *D. rostriformis bugensis*, i.e., GenBank accession number AF510504 of Therriault et al. (2004), DQ840132 of Gelembiuk et al. (2006), and DBU47651 of Baldwin et al. (1996).

There is an extreme degree of homogeneity in the mitochondrial COI genes of *D. rostriformis bugensis*. Although we suspect that the source of the introduction was the Main-Danube Canal, it was beyond the scope of this investigation to conduct additional molecular research involving highly variable markers needed to test this invasion route hypothesis. Additional sampling is now required in the Danube-Main-Rhine waterway between Romania and The Netherlands to ascertain if *D. rostriformis bugensis* has invaded the entire corridor or simply been vectored

long-distance from the Romanian section of the Danube to The Netherlands' Rhine delta.

Dreissena polymorpha was first recorded in The Netherlands in 1827 (Van Benthem Jutting 1954). Thus, it has taken almost two centuries for *D. rostriformis bugensis* to catch up to the Western European populations of its Ponto-Caspian congeneric, *D. polymorpha*. Now that it has, will there be ecological consequences? Displacement of *D. polymorpha* populations by *D. rostriformis bugensis* has been commonly observed both in North American (Ricciardi and Whoriskey 2004) and in Eastern European (Orlova et al. 2004) waterbodies. The very low percentage of the *Dreissena* population represented by *D. rostriformis bugensis* at Hollands Diep suggests that if a similar pattern of displacement is occurring, it is only in its early stage.

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