

Rapid Communication***Gammarus tigrinus* Sexton, 1939 continues its invasion in the Baltic Sea: first record from Bornholm (Denmark)**

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Received: 6 April 2019**Accepted:** 11 July 2019**Published:** 25 October 2019**Handling editor:** Calum MacNeil**Thematic editor:** Amy Fowler**Copyright:** © Rewicz et al.This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).**OPEN ACCESS****Abstract**

The paper provides a new record of the North-American invasive amphipod *Gammarus tigrinus* from the Bornholm island (Denmark), accompanied by DNA barcodes. We found this species in two locations 9 km apart: 1. Baltic Sea in Svaneke, 2. Østersøen stream, Nexø. Our finding shows further range expansion of this species in the Baltic Sea. Earlier findings of this invader from Germany (1985), Poland (1988), Lithuania (2004), Latvia (2011), Estonia (2003), Finland (2003) and Russia (2005). However, taking into account, the current locality, spreading directions and speed it is only a matter of time, before the species colonizes coastal waters all around the Baltic Sea and its islands.

Key words: Amphipoda, distribution, North America, alien species, DNA barcoding**Introduction**

Amphipods are macroinvertebrates with a high invasive potential in fresh- and brackishwater ecosystems (Holdich and Pöckl 2007). The invasive amphipods are frequently characterized by a unique set of physiological and behavioral traits which allows their successful colonization and overwhelm local species (e.g. Grabowski et al. 2007; Rewicz et al. 2014). The Baltic Sea is the world's largest brackish-water basin (382,000 km² or 415,000 km² with the Kattegat included) and a unique ecosystem (Leppäkoski et al. 2002). Most of the local animals and plants are postglacial immigrants, many of them living close to their salinity tolerance limits (Leppäkoski and Olenin 2001). The Baltic Sea has often been regarded as very exposed and sensitive to biological invasions due to the low number of native species, its environmental instability, and increasing intensity of freight transportation as well as of anthropogenic pressure (Leppäkoski et al. 2002; Reissalu et al. 2016). Currently, more than 120 non-indigenous species (NIS) have been recorded in the Baltic Sea (Lehtiniemi et al. 2015). Among them there are at least 11 species of invasive amphipods, with seven species of Ponto-Caspian origin: *Chaetogammarus warpachowskyi* Sars, 1897, *Chelicorophium*

curvispinum (G.O. Sars, 1895), *Dikerogammarus villosus* (Sowinsky, 1894), *D. haemobaphes* (Eichwald, 1841), *Echinogammarus ischnus* (Stebbing, 1899), *E. trichiatus* (Martynov, 1932), *Obesogammarus crassus* (Sars G.O., 1894), *Pontogammarus robustoides* (Sars, 1894) (e.g. Herkül and Kotta 2007; Messner and Zettler 2018); one, *Gmelinoides fasciatus* (Stebbing, 1899), from Lake Baikal (Berezina and Panov 2003) and two, *Melita nitida* Smith, 1873 and *Gammarus tigrinus*, originating from the Atlantic coast of North America (Bulnheim 1976; Messner and Zettler 2018).

Gammarus tigrinus is widely distributed from the Labrador Peninsula in Canada to the eastern coast of Florida in the United States. It occurs most frequently in tidal estuaries with salinities up to 25 PSU (Bousfield 1973). This amphipod has an extensive invasion history. It was first recorded in 1931 in England (Sexton and Cooper 1939) and then in 1950s in Lough Neagh, Northern Ireland (Hynes 1955) (Supplementary material Table S1). Then it was intentionally introduced to the Werra River in Germany in 1957 (Schmitz 1960), to enrich its fauna after the populations of native gammarids declined in result of salt-pollution. In 1960 some dozens of individuals were introduced to IJsselmeer in the Netherlands (Nijssen and Stock 1966). Both populations successfully established and started to spread in river systems of Western Europe, interconnected by artificial channels. *Gammarus tigrinus* reached the Baltic Sea in 1975 in Germany (Bulnheim 1976), and continued its eastward expansion. Notes about first records were obtained from: the Szczecin Lagoon in Poland in 1988 (Gruszka 1999), the Vistula Lagoon in 1998 (Jażdżewski and Konopacka 2000), the coasts of Lithuania (Daunys and Zettler 2006) in 2004, Estonia in 2003 (Herkül and Kotta 2007), Finland in 2003 (Pienimäki et al. 2004), the Gulf of Riga in 2003 (Kotta 2005), and Russia in 2005 (Berezina 2007) (Figure 1A). The species was also reported in 1990 from the Baltic Sea in Denmark (Stæhr et al. 2016).

Here, we present the first record of *G. tigrinus* from the isle of Bornholm, Denmark, documenting further range expansion of this species and provide genetic identification of the local population based on the portion of cytochrome oxidase (COI) gene used as a DNA barcode, to show which of the already known lineages it belongs to (see Kelly et al. 2006a, b for comparison), and to reveal the most probable source of the Bornholm populations. This marker has sufficient variability to be used commonly in studies upon molecular population structure and cryptic diversity of various invasive invertebrates, including amphipods (e.g. Rewicz et al. 2015, 2016; Hupało and Grabowski 2018; Lipinskaya et al. 2018; Mauvisseau et al. 2019). The data presented here shows also an updated European distribution of *G. tigrinus* (Figure 1A, Table S1).

Materials and methods

The amphipods were collected from two localities: 1. Baltic Sea, Svaneke, 23.07.2018, N55.13293; E15.15201 (Figure 1B, C); 2. Østersøen stream, Nexø,

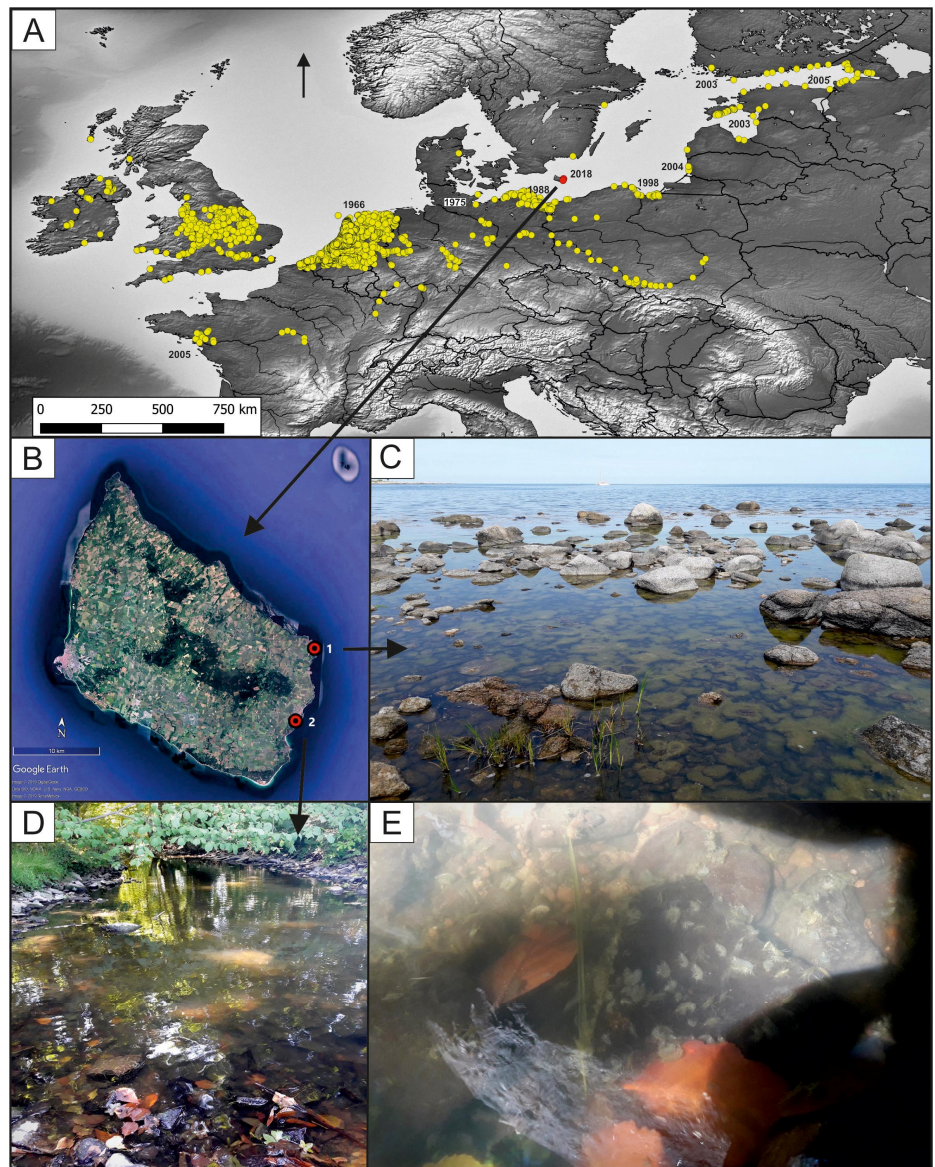


Figure 1. A) Distribution of *Gammarus tigrinus* in Europe. Yellow circles – previously known records of the species (combined literature and own data – see details in Table S1). Red circles – localities of *G. tigrinus* in Bornholm revealed in 2018. B) Map of Bornholm with big red dots, numbers on data points correspond to the locality 1 and 2. C) Habitat of *G. tigrinus* in Svaneke (locality 1). D) Østersøen stream, Nexø (locality 2). E) Numerous individuals of *G. tigrinus* in Østersøen stream.

25.07.2018, N55.05695; E15.12698 (Figure 1D, E). The samples were gathered with a benthic hand-net, from all the available habitats and preserved in 96% ethanol directly in the field. In the laboratory, the material was identified to the species level under a Nikon SMZ-800 stereomicroscope, based on available literature (i.e. Zettler and Zettler 2017) and stored in the permanent collection of the Department of Invertebrate Zoology & Hydrobiology, University of Lodz.

Genetic analysis

The total DNA of four individuals (one from Svaneke and three from Nexø) identified as *G. tigrinus* was extracted from their fifth pereopod,

with the Chelex procedure (Casquet et al. 2012). The COI fragments were amplified using the LCO1490 JJ/HCO2198 JJ primer pair (Astrin and Stüben 2008). We used reaction conditions following Hou et al. (2007). PCR products (5 µl) were cleaned up by Exonuclease I (2 U, Thermo Scientific) and alkaline phosphatase FastAP (1 U, Thermo Scientific) treatment, according to the manufacturer's guidelines and sent for sequencing to the MacroGen Inc., Korea. The identity of the obtained sequences was verified with BLAST (Altschul et al. 1990). The obtained sequences were edited, aligned and trimmed to 540 bp using BIOEDIT[®] 7.2.5 (Hall 1999) and deposited in GenBank under accession numbers (MK403733–MK403736). Simultaneously, the DNA sequences were deposited in the online database of the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007), in order to obtain the Barcode Index Numbers (BIN) that group DNA sequences based on the genetic distance, as tentative equivalents of species (Ratnasingham and Hebert 2013). Obtaining BINs for sequences deposited in BOLD provides an additional verification of species identification. Haplotypes were identified using the DnaSP 5.10.01 software (Librado and Rozas 2009). The phylogenetic tree was constructed using the haplotype data in MEGA 6 (Tamura et al. 2013) with the neighbor-joining method (NJ) (Saitou and Nei 1987) based on the p-distance (Nei and Kumar 2000) with a bootstrap test performed on 10,000 replicates (Figure S1). The K2p pairwise genetic distances were calculated in MEGA6 software (Tamura et al. 2013). Additional COI sequences of *Gammarus tigrinus* (117 individuals) as well as the outgroup sequence (four individuals) of the *Gammarus daiberi* Bousfield, 1969 were acquired from GenBank and BOLD (Table S2) and included in the dataset to build the NJ tree. Relevant voucher information is accessible through the public data set "GTIGBOR" (<http://dx.doi.org/10.5883/DS-GTIGBOR>) in the Barcode of Life Data Systems (BOLD; <http://v4.boldsystems.org>).

Results

The results of the morphological identification revealed the presence of 18 individuals (ind.) of *Gammarus tigrinus* (11♀, 7♂) accompanied by six ind. of *Gammarus locusta* (Linnaeus, 1758) and 175 ind. of *Gammarus duebeni* Lilljeborg, 1852 at site 1, and 124 ind. (47♀, 77♂) of *G. tigrinus* at site 2 (Figure 2).

DNA barcoding

The DNA barcoding confirmed that the obtained sequences (1 ind. – Svaneke, 3 ind. – Nexø) belonged to *Gammarus tigrinus*. We have identified three haplotypes. The pairwise K2p distance between individuals from Bornholm only, did not exceed 3.6% and 11.6% for all 121 sequences of *G. tigrinus* gathered in our dataset. The sequences from Bornholm grouped under two



Figure 2. Male specimen of *Gammarus tigrinus* collected in Bornholm.

BINs (BOLD: AAA4302; ADP7774) which are widespread in Europe. Two haplotypes identified in this study occurring in Bornholm were identical with those already recorded from Poland, Germany, Estonia and Finland. The third haplotype was new, however it fits inside the lineage known from all over Europe (Figure S1).

Discussion

In its native range, *G. tigrinus* is represented by six genetically distinct lineages grouped in two main clades defined as the “northern” and “southern” species (Kelly et al. 2006a). The “southern species” includes two lineages occurring along the south-eastern Atlantic coast from North Carolina to Florida. The “northern species” including four lineages spreads from Virginia towards St. Lawrence river. Our updated molecular dataset including new records from Bornholm and other European localities shows that *G. tigrinus* in Europe is represented by two lineages (N1 and N4) of the “northern species” *sensu* Kelly et al. (2006b), equal to two BIN’s (respectively AAA4302 and ADP7774) and occurring in sympatry.

The rapid expansion of this North-American invasive gammarid is a serious threat to the local ecosystems of the Baltic Sea. Established populations of the species often achieve very high abundances, high fecundity and predation pressure, which significantly dominate or even displace populations of native gammarids (e.g. Szaniawska et al. 2003; Jażdżewski et al. 2004; Kotta et al. 2013; Jänes et al. 2015; Reialu et al. 2016). Our results showing its low abundance in Svaneke, Bornholm (locality 1) but high abundance and domination at the locality 2 may suggest its preferences to lower salinity in the stream compared to the sea which is congruent with findings of Boets et al. (2011a) from the polder waters in Flanders (Belgium). Lack of other gammarid species in Østersøen stream (locality 2) could facilitate

colonization, or on the other hand may be a result of successful outcompeting of native amphipods.

The species is known to exhibit wide tolerance for salinity conditions (Lewin et al. 2018), probably allowing to survive in the ballast waters of ships during the initial crossing of the Atlantic Ocean (Savage 1982), but also enhancing the secondary spread all over Europe.

There can be at least two plausible scenarios of the species' introduction to Bornholm. One is the spread from Kołobrzeg, Ustka and Darłowo in Poland to Nexø, as there have been direct ferry connections between those towns and *G. tigrinus* was previously noted in each of those Polish ports (Jazdzewski et al. 2005, Table S1). On the other hand it is possible, that the initial place of introduction was an area around the capital of the Bornholm – Ronne, which has ferry connections with many cities in Germany, Denmark and with Świnoujście in Poland (Stettin Lagoon). However, to verify this hypothesis, further studies of gammarid communities near Ronne area would be necessary. Dispersal with ballast water or attaching to biofilm fouling the hulls is often observed in the case of other invasive amphipods, such as *Dikerogammarus villosus* (Baćela-Spychalska et al. 2013; Minchin et al. 2019), *Crangonyx pseudogracilis* Bousfield, 1958 (Slothouber Galbreath et al. 2010), *C. floridanus* Bousfield, 1963 (Mauvisseau et al. 2019). Thus, such pathway of introduction for *G. tigrinus* seems to be very probable.

Independently to invasion along the Baltic Sea coast, *G. tigrinus* has been entering the rivers of central and western Europe, extending its range very quickly (Zettler and Zettler 2017). After establishing stable populations all along the course of the Oder river and in the Vistula delta, the species appeared unexpectedly in the upper Vistula tributaries (Lewin et al. 2018). According to our unpublished data, *G. tigrinus* was present in the upper Vistula River already in 2011. Just two years later, in 2013, we found it c.a. 300 km downstream from the place where it was reported by Lewin et al. (2018) (see Figure 1A, Table S1) and it looks that the invasion is still ongoing.

In conclusion, we provide the first morphological and molecular evidence of the presence of *Gammarus tigrinus* in Bornholm, Denmark. The ongoing invasion is undeniable. This species has almost encircled the Baltic Sea during the last 40 years. Its wide salinity tolerance, high intraspecific molecular diversity and observed outcompeting of native and other invasive amphipods indicates it is a highly invasive component of invertebrate communities.

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Author contributions

T.R., M.G., K.B.S, designed the study and its methodology; T.R., performed fieldwork and genetic analyses; G.T., performed microscopic photography; A.K., performed species identification. The manuscript was written by T.R., M.G. and K.B.S. and edited by all authors.

Competing interest

The authors declare no competing interests.

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410, [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Astrin JJ, Stüben PE (2008) Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). *Invertebrate Systematics* 22: 503–522, <https://doi.org/10.1071/IS07057>
- Bączela-Spychalska K, Grabowski M, Rewicz T, Konopacka A, Wattier R (2013) The ‘killer shrimp’ *Dikerogammarus villosus* (Crustacea, Amphipoda) invading Alpine lakes: overland transport by recreational boats and scuba-diving gear as potential entry vectors? *Aquatic Conservation - Marine and Freshwater Ecosystems* 23: 606–618, <https://doi.org/10.1002/aqc.2329>
- Berezina NA (2007) Expansion of the North American amphipod *Gammarus tigrinus* Sexton, 1939 to the Neva Estuary (easternmost Baltic Sea). *Oceanologia* 49: 129–135
- Berezina NA, Panov V (2003) Establishment of new gammarid species in the eastern Gulf of Finland (Baltic Sea) and their effects on littoral communities. *Proceedings of the Estonian Academy of Sciences: Biology, Ecology* 52(3): 284–304
- Boets P, Lock K, Goethals PLM (2011a) Shifts in the gammarid (Amphipoda) fauna of brackish polder waters in Flanders (Belgium). *Journal of Crustacean Biology* 31: 270–277, <https://doi.org/10.1651/10-3357.1>
- Bousfield EL (1973) Shallow-water Gammaridean Amphipoda of New England. Cornell University Press, Ithaca, pp 1–312
- Bulnheim HP (1976) *Gammarus tigrinus*, ein neues Faunenelement der Ostseeförde Schlei. *Schriften des Naturwissenschaftlichen Vereins für Schleswig-Holstein* 46: 79–84
- Casquet J, Thebaud C, Gillespie RG (2012) Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Molecular Ecology Resources* 12: 136–141, <https://doi.org/10.1111/j.1755-0998.2011.03073.x>
- Daunys D, Zettler MD (2006) Invasion of the North American amphipod (*Gammarus tigrinus* Sexton, 1939) into the Curonian Lagoon, South-eastern Baltic Sea. *Acta Zoologica Lituanica* 16: 20–26, <https://doi.org/10.1080/13921657.2006.10512705>
- Grabowski M, Jazdzewski K, Konopacka A (2007) Alien Crustacea in Polish waters - Amphipoda. *Aquatic Invasions* 2: 25–38, <https://doi.org/10.3391/ai.2007.2.1.3>
- Gruszka P (1999) The river Odra estuary as a gateway for alien species immigration to the Baltic Sea basin. *Acta hydrochimica et hydrobiologica* 27: 374–382, [https://doi.org/10.1002/\(SICI\)1521-401X\(199911\)27:5<374::AID-AHEH374>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1521-401X(199911)27:5<374::AID-AHEH374>3.0.CO;2-V)
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98
- Herkül K, Kotta J (2007) New records of the amphipods *Chelicorophium curvispinum*, *Gammarus tigrinus*, *G. duebeni*, and *G. lacustris* in the Estonian Coastal Sea. *Proceedings of the Estonian Academy of Sciences: Biology, Ecology* 56: 290–296
- Holdich DM, Pöckl M (2007) Invasive crustaceans in European inland waters. In: Gherardi F (ed), *Biological invaders in inland waters: profiles, distribution and threats*. Springer, Berlin, pp 29–75, https://doi.org/10.1007/978-1-4020-6029-8_2
- Hou ZG, Fu JH, Li SQ (2007) A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution* 45: 596–611, <https://doi.org/10.1016/j.ympev.2007.06.006>
- Hupała K, Grabowski M (2018) A first insight into the transatlantic population genetic structure of the beach flea, *Platorchestia platensis* (Krøyer, 1845). *BioInvasions Records* 7: 165–170, <https://doi.org/10.3391/bir.2018.7.2.08>
- Hynes HBN (1955) Distribution of some freshwater Amphipoda in Britain. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 12: 620–628, <https://doi.org/10.1080/03680770.1950.11895341>
- Jänes H, Kotta J, Herkül K (2015) High fecundity and predation pressure of the invasive *Gammarus tigrinus* cause decline of indigenous gammarids. *Estuarine, Coastal and Shelf Science* 165: 185–189, <https://doi.org/10.1016/j.ecss.2015.05.014>
- Jazdzewski K, Konopacka A (2000) Immigration history and present distribution of alien crustaceans in Polish waters. In: von Vaupel Klein JC, Schram FR (eds), *The biodiversity*

- crisis and Crustacea, Proceedings of 4th International Crustacean Congress, Brill, Leiden, Crustacean Issues 12: 55–64
- Jazdzewski K, Konopacka A, Grabowski M (2004) Recent drastic changes in the gammarid fauna (Crustacea, Amphipoda) of the Vistula River deltaic system in Poland caused by alien invaders. *Diversity and Distributions* 10: 81–87, <https://doi.org/10.1111/j.1366-9516.2004.00062.x>
- Jazdzewski K, Konopacka A, Grabowski M (2005) Native and alien malacostracan Crustacea along the Polish Baltic Sea coast in the 20th century. *Oceanological and Hydrobiological Studies* 34(Suppl. 1): 175–193
- Kelly DW, MacIsaac HJ, Heath DD (2006a) Vicariance and dispersal effects on phylogeographic structure and speciation in a widespread estuarine invertebrate. *Evolution* 60: 257–267, <https://doi.org/10.1111/j.0014-3820.2006.tb01104.x>
- Kelly DW, Muirhead JR, Heath DD, MacIsaac HJ (2006b) Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters. *Molecular Ecology* 15: 3641–3653, <https://doi.org/10.1111/j.1365-294X.2006.03012.x>
- Kotta J (2005) *Gammarus tigrinus* arrived in the Gulf of Riga in July 2003. Report of the Benthos Ecology Working Group (BEWG), 19-22 April 2005, Copenhagen, ICES BEWG, Rep. No 14, 93 pp
- Kotta J, Pärnoja M, Katajisto T, Lehtiniemi M, Malavin SA, Reisalu G, Panov VE (2013) Is a rapid expansion of the invasive amphipod *Gammarus tigrinus* Sexton, 1939 associated with its niche selection: a case study in the Gulf of Finland, the Baltic Sea. *Aquatic Invasions* 8: 319–332, <https://doi.org/10.3391/ai.2013.8.3.08>
- Lehtiniemi M, Johannes D, Buschbaum C, Florin AB, Näslund J, Wikström S, Strake S, Orlova M, Olenin S, Zaiko A (2015) Trends in arrival of new non-indigenous species. HELCOM core indicator report, 17 pp
- Leppäkoski E, Olenin S (2001) The meltdown of biogeographical peculiarities of the Baltic Sea: The interaction of natural and man-made processes. *Ambio* 30: 202–209, <https://doi.org/10.1579/0044-7447-30.4.202>
- Leppäkoski E, Olenin S, Gollasch S (2002) The Baltic Sea - a field laboratory for invasion biology. In: Leppäkoski E, Olenin S, Gollasch S (eds), *Invasive Aquatic Species of Europe: Distribution, Impacts and Management*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 253–259, https://doi.org/10.1007/978-94-015-9956-6_27
- Lewin I, Halabowski D, Rymarski Z (2018) The first records of the occurrence of a North American invader *Gammarus tigrinus* Sexton, 1939 in the tributaries of the upper Vistula River. *Knowledge and Management of Aquatic Ecosystems* 419: 31, <https://doi.org/10.1051/kmae/2018021>
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452, <https://doi.org/10.1093/bioinformatics/btp187>
- Lipinskaya T, Radulovici A, Makaranka A (2018) First DNA barcoding based record of *Echinogammarus trichiatus* (Martynov, 1932) (Crustacea, Gammaridae) in Belarus. *BioInvasions Records* 7: 55–60, <https://doi.org/10.3391/bir.2018.7.1.08>
- Mauvisseau Q, Davy-Bowker J, Bryson D, Souch GR, Burian A, Sweet M (2019) First detection of a highly invasive freshwater amphipod *Crangonyx floridanus* (Bousfield, 1963) in the United Kingdom. *BioInvasions Records* 8: 1–7, <https://doi.org/10.3391/bir.2019.8.1.01>
- Messner U, Zettler ML (2018) The conquest (and avoidance?) of the brackish environment by Ponto-Caspian amphipods: A case study of the German Baltic Sea. *BioInvasions Records* 7: 269–278, <https://doi.org/10.3391/bir.2018.7.3.07>
- Minchin D, Arbačiauskas K, Daunys D, Ezhova E, Grudule N, Kotta J, Molchanova N, Olenin S, Višinskienė G, Strake S (2019) Rapid expansion and facilitating factors of the Ponto-Caspian invader *Dikerogammarus villosus* within the eastern Baltic Sea. *Aquatic Invasions* 14: 165–181, <https://doi.org/10.3391/ai.2019.14.2.02>
- Nei M, Kumar S (2000) *Molecular Evolution and Phylogenetics*. New York, NY, USA: Oxford University Press, 333 pp
- Nijssen H, Stock JH (1966) The amphipod, *Gammarus tigrinus* Sexton, 1939, introduced in the Netherlands (Crustacea). *Series of Miscellaneous Publications* 13: 197–206
- Paiva F, Barco A, Chen Y, Mirzajani A, Chan FT, Luringson V, Baltazar-Soares M, Zhan A, Bailey SA, Javidpour J, Briski E (2018) Is salinity an obstacle for biological invasions? *Global Change Biology* 24: 2708–2720, <https://doi.org/10.1111/gcb.14049>
- Pienimäki M, Helavuori M, Leppäkoski E (2004) First findings of the North American amphipod *Gammarus tigrinus* Sexton, 1939 along the Finnish coast. *Memoranda Societatis pro Fauna et Flora Fennica* 80: 17–19
- Ratnasingham S, Hebert PD (2007) BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Resources* 7: 355–364, <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Ratnasingham S, Hebert, PD (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS ONE* 8: e66213, <https://doi.org/10.1371/journal.pone.0066213>
- Reisalu G, Kotta J, Herkül K, Kotta I (2016) The invasive amphipod *Gammarus tigrinus* Sexton, 1939 displaces native gammarid amphipods from sheltered macrophyte habitats of the Gulf of Riga. *Aquatic Invasions* 11: 45–54, <https://doi.org/10.3391/ai.2016.11.1.05>

- Rewicz T, Grabowski M, Macneil C, Bączela-Spychalska K (2014) The profile of a ‘perfect’ invader - the case of killer shrimp, *Dikerogammarus villosus*. *Aquatic Invasions* 9: 267–288, <https://doi.org/10.3391/ai.2014.9.3.04>
- Rewicz T, Wattier R, Grabowski M, Rigaud T, Bączela-Spychalska K (2015) Out of the Black Sea: Phylogeography of the invasive killer shrimp *Dikerogammarus villosus* across Europe. *PLoS ONE* 10: e0118121, <https://doi.org/10.1371/journal.pone.0118121>
- Rewicz T, Konopacka A, Bączela-Spychalska K, Özbek M, Grabowski M (2016) First records of two formerly overlooked Ponto-Caspian amphipods from Turkey: *Echinogammarus trichiatus* (Martynov, 1932) and *Dikerogammarus villosus* (Sovinsky, 1894). *Turkish Journal of Zoology* 40: 328–335, <https://doi.org/10.3906/zoo-1505-31>
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425
- Savage AA (1982) The survival and growth of *Gammarus tigrinus* Sexton (Crustacea: Amphipoda) in relation to salinity and temperature. *Hydrobiologia* 94: 201–212, <https://doi.org/10.1007/BF00016400>
- Schmitz W (1960) Die Einbürgerung von *Gammarus tigrinus* Sexton auf dem europäischen Kontinent. *Archives of Hydrobiology* 57(1–2): 223–225
- Sexton EW, Cooper LHN (1939) On a new species of *Gammarus* (*G. tigrinus*) from Droitwich district. *Journal of the Marine Biological Association of the United Kingdom* 23: 543–551, <https://doi.org/10.1017/S0025315400014065>
- Slothouber Galbreath JGM, Smith JE, Becnel JJ, Butlin RK, Dunn AM (2010) Reduction in post-invasion genetic diversity in *Crangonyx pseudogracilis* (Amphipoda: Crustacea): a genetic bottleneck or the work of hitchhiking vertically transmitted microparasites? *Biological Invasions* 12: 191–209, <https://doi.org/10.1007/s10530-009-9442-3>
- Stæhr PA, Jakobsen HH, Hansen JLS, Andersen P, Storr-Paulsen M, Christensen J, Lundsteen S, Göke C, Carausu MC (2016) Trends in records and contribution of non-indigenous species (NIS) to biotic communities in Danish marine waters. Aarhus University, DCE - Danish Centre for Environment and Energy. Scientific Report from DCE - Danish Centre for Environment and Energy No. 179, 44 pp, <http://dce2.au.dk/pub/SR179.pdf>
- Szaniawska A, Łapucki T, Normant M (2003) The invasive amphipod *Gammarus tigrinus* Sexton, 1939, in Puck Bay. *Oceanologia* 45(3): 507–510
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729, <https://doi.org/10.1093/molbev/mst197>
- Zettler ML, Zettler A (2017) Marine and freshwater Amphipoda from the Baltic Sea and adjacent territories. Conchbooks, Harxheim, 845 pp

Supplementary material

The following supplementary material is available for this article:

Figure S1. Neighbor-joining tree based on COI p-distances for sequences of *Gammarus tigrinus* COI from populations across the northwest Atlantic coast and Europe. Black arrows indicates sequences coming from Bornholm, obtained in this study. Particular shadings in the tree indicates respectively: blue – outgroup; orange – “southern” clades of North America Atlantic coast after Kelly et al. (2006a); green – sequences obtained from European invasive populations. Each sequence name consist of three parts separated by lower dash: 1) BOLD Process ID, 2) GenBank accession number, 3) locality. On right side of the tree BINs and codes of clades proposed by Kelly et al. (2006a) are presented. *Gammarus daiberi* was used as an outgroup. Only bootstrap supports above 50% were reported.

Table S1. Records of *Gammarus tigrinus* in Europe. Samples from Bornholm are in bold.

Table S2. Cytochrome oxidase I (COI) sequences of *Gammarus tigrinus* and *Gammarus daiberi* used in the study. Samples from Bornholm are in bold. NA - not available

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