BioInvasions Records (2021) Volume 10, Issue 1: 119–135

Research Article

Additional records of the bivalves *Mytilopsis leucophaeata* (Conrad, 1831) (Dreissenidae) and *Arcuatula senhousia* (Benson, 1842) (Mytilidae) in the Ponto-Caspian region

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Citation: Zhulidov AV, Kozhara AV, Son MO, Morhun H, van der Velde G, Leuven RSEW, Gurtovaya TY, Zhulidov DA, Kalko EA, Kuklina YA, Kosmenko LS, Santiago-Fandino VJR, Nalepa TF (2021) Additional records of the bivalves *Mytilopsis leucophaeata* (Conrad, 1831) (Dreissenidae) and *Arcuatula senhousia* (Benson, 1842) (Mytilidae) in the Ponto-Caspian region. *BioInvasions Records* 10(1): 119–135, https://doi.org/10.3391/bir.2021.10.1.14

Received: 9 April 2020
Accepted: 1 September 2020
Published: 9 December 2020

Handling editor: Fred Wells
Thematic editor: Andrew David

Abstract

Conrad's false mussel *Mytilopsis leucophaeata* (Conrad, 1831) and the Asian date mussel *Arcuatula senhousia* (Benson, 1842) are highly invasive bivalves actively spreading in the Ponto-Caspian region, which includes the basins of the Black, Azov, and Caspian seas. This study provides new records of both species from this region and gives a synopsis of known information on their ecology, substrate preferences, tolerance and adaptive potential to environmental factors such as salinity. *Mytilopsis leucophaeata* and *A. senhousia* are at different stages of colonization in the Ponto-Caspian region. While *M. leucophaeata* has become established in the region, approaching circumferential distribution in each sea basin, *A. senhousia* is in the initial phase of its dispersal. Although both species are distributed in estuarine and coastal areas, because of different salinity tolerances they are confined to zones of differing degrees of hydrological fluctuation: *M. leucophaeata* occurs in more estuarine conditions with fluctuating river discharges. *Arcuatula senhousia* avoids low salinity and is mainly absent near large rivers. In spite of this, *A. senhousia* generally prefers substrates of riverine origin (so called mixed sediments with prevalence of the soft fraction) and therefore its distribution reflects a compromise pattern with settlement occurring at the outer periphery of estuaries, whereas *M. leucophaeata* occupies areas with substantial freshwater influence. Unlike *A. senhousia*, *M. leucophaeata* usually occurs on hard substrates, however, our findings indicate that it is able to occupy other substrates in the absence of typical biotopes. Compared to *A. senhousia*, *M. leucophaeata* colonizes more diverse localities with a broader range of fluctuating conditions such as salinity and of substrate types. Both *M. leucophaeata* and *A. senhousia* in the Black Sea tend to settle on substrates that are not fully occupied by native byssus-attached bivalves.

Key words: Asian date mussel, Conrad's false mussel, dreissenids, mytilids, non-indigenous species, invasion ecology, Black Sea, Azov Sea, Caspian Sea

Introduction

Biological invasions can have profound effects on native biotic communities. Invasive non-indigenous species may affect local communities through competition, predation, parasitism, alterations of trophic webs, as well as through habitat modification (Vitousek 1990; Williamson 1996; Robinson et al. 2007; Simberloff et al. 2013). Over the last century, invasion rates have greatly accelerated due to the expansion of international commerce. This expansion has led to the breakdown of biogeographic barriers through the massive trade in live organisms and to the inadvertent transport of many organisms in cargo ships as well as in a variety of other kinds of transportation and by the construction of canals connecting formerly separate biogeographic regions (Williams et al. 2013; Seebens et al. 2018; Zhulidov et al. 2018). Dreissenid and mytilid bivalve species such as Conrad's false mussel or dark false mussel *Mytilopsis leucophaeata* (Conrad, 1831) (Dreissenidae) and the Asian date mussel *Arcuatula senhousia* (Benson, 1842) (Mytilidae) are among the most successful invaders (Bachelet et al. 2009; Zhulidov et al. 2015). Both species can reach very high population densities of more than 100,000 ind. m^{-2} (Munari 2008; Van der Gaag et al. 2017). *Mytilopsis leucophaeata* is an invasive, brackish water bivalve species that originates from the North American Atlantic coast and the northern part of the Gulf of Mexico. It is mainly found in subtropical and warm-temperate regions, both in its native and invaded range (Marelli and Gray 1983; Van der Velde et al. 2010a). Presently, this species is expanding its distribution in European waters (Zhulidov et al. 2015, 2018; Forsström et al. 2016) and becoming a real concern since it causes severe biofouling problems in brackish water systems (Mackie and Claudi 2010; Rajagopal and Van der Velde 2012). These problems include fouling of ship hulls, growth on underwater constructions, and clogging of cooling water systems and pump houses of power plants and industries (Van der Velde et al. 2010b).

The history of earlier findings of *M. leucophaeata* in the Ponto-Caspian basin is described in Zhulidov et al. (2018). The new records reported by these authors confirmed establishment of the species in the north-western part of the Black Sea and indicated its further spread to the eastern part of the sea (Tuapse River bay, Russian Federation, 2014). Moreover, the species was recorded in the Damchik area of the Astrakhan Biosphere Reserve in the Volga Delta at the Caspian Sea (Russian Federation, 2014). The easternmost known record of an established population of this species in the Black Sea basin was in Patara Paliastomi Lake, Georgia in 2010 (Mumladze et al. 2019).

Arcuatula senhousia, a species from the Western Pacific, was accidentally introduced into coastal areas of North America, Oceania, the European Atlantic coast (Bachelet et al. 2009; Barfield et al. 2018; Faasse 2018), Western Australia from where it has been extirpated due to a freshwater

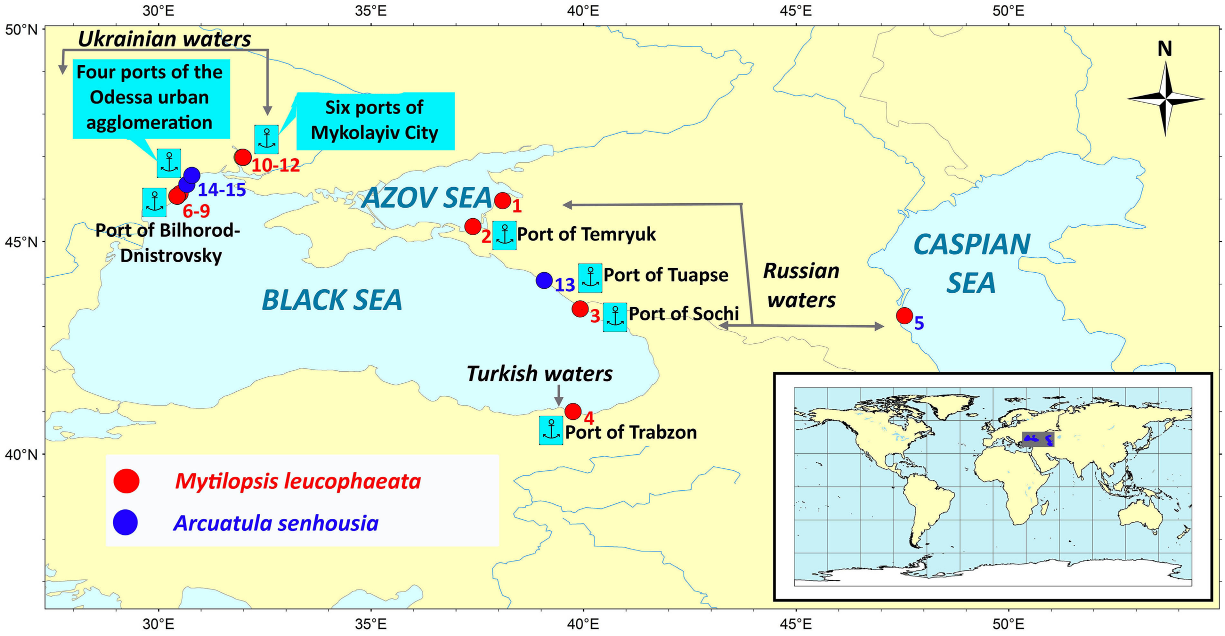

Figure 1. Map of the study area (Black, Azov and Caspian Seas) with new sampling locations of the species and nearby ports: red circles refer to *Mytilopsis leucophaeata*; blue circles refer to *Arcuatula senhousia*; location enumeration as in Table 1.

event (McDonald and Wells 2010), and the Mediterranean region (Hoenselaar and Hoenselaar 1989), with significant impact on local benthic communities (Crooks 1996; Mistri 2002; Magni et al. 2006). *Arcuatula senhousia* was recorded for the first time in the Black Sea in 2002, in the Constanța port area, Romania (Micu 2004). This was, however, just a single finding of a live animal and there has been no further confirmation of the occurrence of this species in Romania. A second finding of two live specimens was reported in 2015 in the Kerch strait, the Russian Azov-Black Sea (Kovalev et al. 2017). In Bulgaria, a single juvenile specimen was found in Burgas Bay (the Bulgarian Black Sea) in 2017 (Chartosia et al. 2018). The appearance of *A. senhousia* in Bulgaria was expected based on its environmental requirements and its presence in adjacent areas (Karachle et al. 2017). This species was earlier recorded at two locations of the neighbouring Marmara Sea in 2012 (Öztürk et al. 2017). Further monitoring of this species' spread is especially important at early stage of its invasion in the Black Sea basin as it can provide valuable information on its establishment in this region.

In this paper, we present additional records of both species in the water bodies of the Ponto-Caspian region and discuss the significance of these findings in relation to the ecology, invasion pathways and impact of these species.

Materials and methods

Sample collection

Specimens were collected in the years 2017–2019 at various locations in the Azov Sea, the Black Sea and the Caspian Sea (Figure 1, Table 1). At all locations, snorkel surveys at depths of 0–3 m were performed and specimens

Table 1. New recent localities, date, salinity, substrate and number of individuals of *Mytilopsis leucophaeata* and *Arcuatula senhousia* observed in the Azov Sea (1, 2), Black Sea (3, 4, 6–15), and Caspian Sea (5). * localities from which *cox1* sequences were obtained. "—" = no data.

№	Locality	Coordinates (Latitude N, Longitude E)	Date	Salinity (PSU)	Substrate	Number of individuals
Mytilopsis leucophaeata						
1	Azov Sea close to the Kirpili River mouth, Primorsko-Akhtarsk surroundings, Russian Federation	—	19 September 2018	1.1–2.4	Mixed sediments (muddy sand and shell gravel)	7 live specimens and 3 empty shells
2	Azov Sea close to the Kuban River mouth, Temryuk surroundings, Russian Federation	45.352908, 37.397940	25 September 2018	1.1–2.6	Mixed sediments (muddy sand and shell gravel)	10 live specimens and 2 empty shells
3	Black Sea close to the Mzymta River mouth, Adler surroundings, Russian Federation	43.413988, 39.924069	7 September 2018	2.4–4.9	Stones, sand and underwater litter	5 live specimens and 4 empty shells
4	Black Sea close to the Muchka River mouth, Trabzon surroundings, Turkey	41.002641, 39.755611	1 September 2018	13–14.5	—	2 separate valves of different empty shells
5	Caspian Sea close to the Sulak River mouth, Sulak surroundings, Russian Federation	43.254152, 47.551842	20 August 2017	4.2–7.3	Shell gravel	11 live specimens and 3 empty shells
6	Dniester Liman (=estuary), Ukraine	46.130111, 30.508639	18 May 2019	0.6	—	2 ind. m^{-2}
7	Fish ladder between the Dniester and Budak Limans, Ukraine	46.064806, 30.445556	02–08 June 2018	13 (in 2018)	Reed	Single live specimens
8	Fish ladder, place of entering to the Dniester Liman, Ukraine	46.069556, 30.433667	02–08 June 2018, 18 May 2019	6 (in 2018) 1.2-1.7 (in 2019)	Stones	Up to 1600 ind. m^{-2} (in 2018) 50 ind. m^{-2} (in 2019)
9	Fish ladder, place of entering to the Dniester Liman, Ukraine	46.070522, 30.434228	18 May 2019	1.1	Stones	1 live specimen
10*	Ingul River, Mykolayiv (Nikolaev) City, Ukraine	46.980056, 31.995167	12 May 2019	2.7	Stones and underwater litter	8 ind. m^{-2}
11	Southern Bug River mouth, Mykolayiv City, Ukraine	46.982441, 31.969698.	10 August 2018	—	Stones	2 live specimens
12	Southern Bug River, Mykolayiv City, Ukraine	46.982667, 31.990472	12 May 2019	2.2	Muddy sand, attached to druses of *Amphibalanus*	500 ind. m^{-2}
Arcuatula senhousia						
13	Tuapse River bay, Russian Federation	44.086389, 39.078611	30 August 2016	16.4	Mixed sediments (sand, mud, and pebbles)	2 live specimens
14*	Sukhoy Liman, Odessa surroundings, Ukraine	46.344167, 30.669444	10 June 2018, 22 June 2018	15	Mixed sediments (mud, shell gravel, pebbles, and marine litter)	7 live specimens
15	Open coast of the Black Sea, north of Odessa, Ukraine	—	Summer 2018	—	Shell wracks	1 dead specimen with remnants of the soft body

were collected by hand or with a frame sampler that scraped organisms from hard substrate. The population density was estimated using a benthic frame sampler with an area of 25 × 25 cm. The description of substrates is according to the terminology used in the EUNIS habitat classification (European Environment Agency 2012).

The specimens were preserved in 96% ethanol and then examined using both morphological and molecular methods. The specimens are stored in the collection of invertebrates of the Institute of Marine Biology NAS of Ukraine and Department of Invertebrate Zoology and Hydrobiology (University of Lodz, Poland).

Salinity measurements

Salinity was determined from measurements of conductivity using a YSI Pro Plus handheld multiparameter meter. The instrument was calibrated

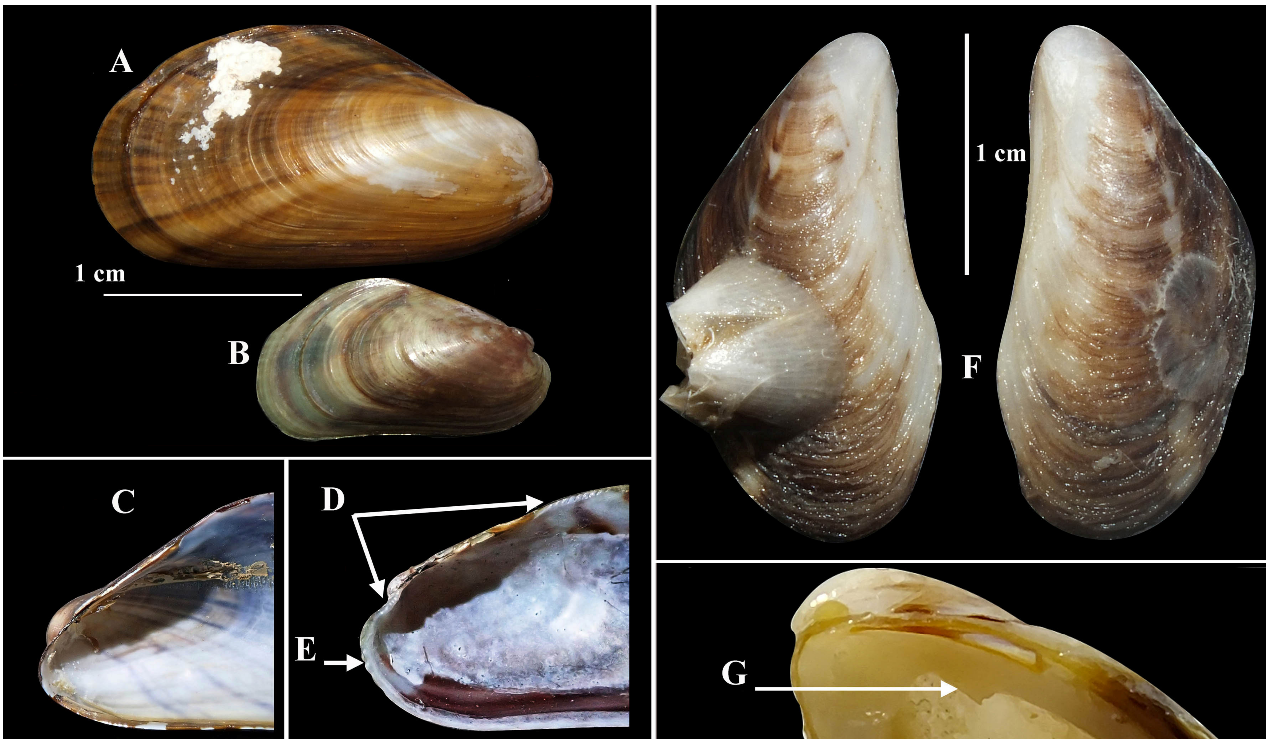

Figure 2. General and hinge views of the studied species: native *Modiolus adriaticus* from Dzharylhach Bay (A, C), *Arcuatula senhousia* from Sukhoy Liman (B, D, E) and *Mytilopsis leucophaeata* from the Southern Bug River (F, G); arrows indicate the diagnostic features of the species: hinge teeth (D) and crenulate anterior margin near the umbo (E) in *A. senhousia* (*vs.* smooth anterior margin in *M. adriaticus*); the apophysis of *M. leucophaeata* (G). Photos by M. Son.

using a KCl standard solution having a conductivity of 10 mS/cm, which is recommended by the manufacturer for brackish to estuarine waters. Salinity values were determined from measured conductivity values according to the Practical Salinity Scale of 1978 (PSS78; Unesco 1981).

A portable refractometer (RHS-4ATC) was used to measure salinity in estuarine waters where salinity values between 6 and 15 were encountered. In areas where salinity was very low (< 3) due to seasonal flooding, salinity was measured using a handheld TDS meter (TDS-3) that was calibrated manually for a range of 0–9990 ppm.

Species identifications

Mytilopsis leucophaeata (Figure 2) can be recognized by the presence of an apophysis, a tubercular structure under the anterior interior of the shell, where the foot muscles are attached (Figure 2G). This structure distinguishes this species from all local species of Dreissenidae and Mytilidae. Other genera that possess such apophyses comprise a number of newly described very small *Rheodreissena* species, which occur in fast flowing rivers of South America, and cave-dwelling Balkan endemic *Congeria kusceri* Bole, 1962 (Cristina et al. 2019). Both are limited in their distribution to specific freshwater habitats and their co-existence with *Mytilopsis* spp. is unlikely.

However, one should be alert to the possible presence of *Mytilopsis sallei* (Récluz, 1849) in the region as its native range is adjacent to that of *M. leucophaeata* (Fernandes et al. 2018). A characteristic feature that distinguishes *M. leucophaeata* from its congener *M. sallei* (as stated in the description of the latter species) is a straightened dorsal margin of the shell *vs*. curved dorsal margin in *M. sallei* (Marelli and Gray 1983). However, this sign may be relatively vague, especially given the different interpretations of *M. sallei* as a taxon. A more reliable diagnostic feature may be the relatively small, often rounded occasionally almost pointed apophysis of *M. leucophaeata*, in contrast to a large, posteriorly pointed or hook shaped apophysis in *M. sallei* (Marelli and Gray 1983). In most of our *M. leucophaeata* specimens the apophysis is not visible at all in standard projection, but it is clearly discernible when the shell is held at an angle (Figure 2G). The height to width ratio of the shell is always smaller than 1.21:1 in *M. leucophaeata*, but greater than 1.3:1 in *M. sallei*. The beak of the shell is rounded to bluntly pointed and directed antero-ventrally at an angle of much less than 45 degrees in *M. leucophaeata* while in *M. sallei* the beak is bluntly to sharply pointed, directed antero-ventrally at an angle of more than 45 degrees. In general the shell of *M. leucophaeata* is longer, lower and wider and that of *M. sallei* shorter, higher and narrower. The septum of the *M. leucophaeata* valve is more cup shaped and laterally deeper. In *M. sallei* it is laterally shallow. The apophysis of *M. leucophaeata* lies more laterally to the hinge margin than does that of *M. sallei* (Marelli and Gray 1983, 1985).

Arcuatula senhousia (Figure 2) differs from the native *Modiolus adriaticus* Lamarck, 1819 by its shell and hinge sculpture (Figure 2D, E). There are anteriorly several mild ridges causing a crenulate anterior margin of the shell of *A. senhousia* (Zenetos et al. 2004), which is more clearly visible on the inside of the valves. The hinge of *A. senhousia* bears distinct rows of teeth, while in *M. adriaticus* these are reduced. Distinguishing *A. senhousia* from juveniles of *M. adriaticus* is hardly possible without an extensive comparative study. By its shell shape, *A. senhousia* cannot be confused with all other Black Sea bivalve species.

Because in European taxonomic keys other Asian mytilid species may be absent, these features are only relevant when compared with native species or other well-known invaders. Therefore, there was a strong need for molecular confirmation of *A. senhousia.*

DNA extraction, amplification and sequencing

Two specimens of *M. leucophaeata* from the River Ingul (Mykolayiv, Ukraine) and one specimen of *A. senhousia* from location Sukhoy Liman (Odessa, Ukraine) were transferred to the laboratory of the Department of Invertebrate Zoology and Hydrobiology (University of Lodz, Poland) for molecular analysis. A small piece of muscle tissue was taken for the DNA extraction. Genomic DNA was isolated using a GeneMATRIX Tissue DNA Purification Kit (EURx, Poland).

The mitochondrial cytochrome oxidase subunit one (*cox1*) fragment was chosen as a standard animal DNA barcode gene region (Herbert et al.

2003). It was amplified using the following primers (Folmer et al. 1994): HCO2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ and LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′. The amplification was conducted under the following PCR conditions: 94 °C for 3 min; 5 cycles of 30 sec at 94 °C, 1:30 min at 45 °C, and 1 min at 72 °C; 35 cycles of 30 sec at 94 °C, 1:30 min at 51 °C, and 1 min at 72 °C; 5 min of denaturation step at 72 °C (Hou et al. 2007). PCR products (5 μl) were cleaned up by Exonuclease I (20 U/μl; EURx, Poland) and alkaline phosphatase Fast Polar-BAP (1 U/μl, EURx, Poland) treatment, according to the manufacturer's guidelines and then sequenced by Macrogen Inc. (Korea) using the same primers as at the amplification stage. Contigs were aligned and manually assembled in FinchTV v. 1.5.0 (Geospiza Inc.). The length of the aligned *cox1* sequences were 622 bp for *M. leucophaeata* and 596 bp for *A. senhousia*.

The correctness of sequences was verified at the amino acid level using MEGA X (Kumar et al. 2018) and then compared with the GenBank database (https://blast.ncbi.nlm.nih.gov) by using the blastn and discontiguous megablast programs to find the matching species (Zhang et al. 2000).

Results

Studies carried out in the years 2017–2019 showed the presence of *M. leucophaeata* and *A. senhousia* in the Azov Sea, the Black Sea, and in the Caspian Sea (Table 1, Figure 1). In addition to our own samplings, one dead specimen of *A. senhousia* was found at a site in the open coast of the Ukrainian Black Sea in summer 2018 (Table 1, Figure 1) (information kindly provided to us by Dr. Ekaterina Kalashnik from the Institute of Marine Biology, Odessa).

Mytilopsis leucophaeata

No stop codons were observed after conversion of nucleotides into amino acids, and no nucleotide variation among specimens was found in the *cox1* sequences. For the appropriate taxonomic identifications based on molecular data, two sequences of *M. leucophaeata* (Accession: MT878229–MT878230) were blasted in a GenBank database. The result revealed 100% nucleotide identity with specimens of *M. leucophaeata* from the following localities: Chesapeake Bay, USA (Accession: KU905904, Aguilar et al. *unpublished*), Antwerp harbour, Belgium (Accession: HM100251, Heiler et al. 2010), the bay of Morlaix, Brittany, France (Accession: MN064628, Couton et al. 2019), Kiel Canal, near Baltic Sea and North Sea, Germany (HM100253-54, Heiler et al. 2010), Caspian Sea, Iran (Accession: HM100257, Heiler et al. 2010).

In the Southern Bug River connected to the Black Sea, individual specimens of *M. leucophaeata* were earlier found on bridge piers in 2012 (Zhulidov et al. 2018). Subsequently throughout 2018–2019, this species was found in several nearby neighbouring locations, both in the Southern

Bug River and its tributary Ingul River on various natural (stones, sand) and artificial (wood, tires, bricks) substrates. Shells of the species have become common in alluvial deposits and bottom sediments. In 2012, *M. leucophaeata* occurred together with *Dreissena polymorpha* (Pallas, 1771), which was also a common species at these locations. However, in 2018–2019, *M. leucophaeata* was the only species occupying the niche of *D. polymorpha,* being found on solid substrates at densities up to 8 ind. m^{-2}. *Mytilopsis leucophaeata* was also found on sand substrates with densities of up to 500 ind. m^{-2}, where it overgrew *Amphibalanus* clusters (also called druses).

In the Dniester Liman on the shores of the Black Sea, the distribution of *M. leucophaeata* can change dramatically due to differences in salinity from year-to-year. In 2009–2010, the distribution of *M. leucophaeata* covered a significant part of the estuary adjacent to its mouth, as well as within a system of fish ladders (structures enabling fish to pass around artificial or natural barriers) between the Dniester and Budak Limans with a salinity range of 1.0–2.4. These fish ladders form a network of intersecting channels, individual sections of which can be closed or opened, thus creating the conditions of salinity and flow in the whole system in different years and seasons. This is used to control the processes of migration, wintering, and fishing of mullet.

The species was originally absent in the semi-isolated part of the fish ladders, which at that time (2009–2010) was stagnant and reached the salinity of the Black Sea (about 18). In spring 2018, after a strong surge of sea water the salinity in the Dniester Liman also increased up to 7–15. Consequently, the population of *M. leucophaeata* declined sharply in the estuary and only one specimen was found in a canal close to the estuary. Further, the benthic community changed dramatically; many Ponto-Caspian relics and other species that previously lived here disappeared and were replaced by marine species, including species previously not recorded in the estuary.

However, similar population changes did not occur within the fish ladder system between the Dniester and Budak Limans. In 2009–2010 these systems were semi-isolated, but were then connected to other canals and the estuary where salinity increased to 4–6. Despite this change, stable populations of *M. leucophaeata* persisted on cane stems and stones, with a density of 1600–1800 ind. m^{-2}.

In spring 2019, there was a sharp desalination of the estuary and in the investigated area the water became almost fresh. At the same time, *M. leucophaeata* recolonized most locations within a salinity range of 0.6–1.1 in low densities (2–4 ind. m^{-2}). In the inner part of fish ladders system, where the salinity was 1.2–1.7, *M. leucophaeata* was observed with a moderate density of 50 ind. m^{-2}.

In contrast to the north-western part of the Black Sea where the species has formed sustainable populations with higher abundance, in the remaining part of the research area only rare, individual specimens have so far been recorded for which no density data could be provided. Accordingly, no massive druses were observed.

The shell lengths ranged 9.7–12.3 mm in the Black Sea near Adler (Table 1, Locality #3), 9.0–14.5 mm in the Azov Sea near Primorsko-Akhtarsk (Table 1, Locality #1), 10.0–15.2 mm in the Azov Sea near Temryuk (Table 1, Locality #2), and 12.1–19.3 mm in the Caspian Sea near the settlement of Sulak (Table 1, Locality #5). At most sampling locations listed above, live specimens of *M. leucophaeata* were present. Near Trabzon (Table 1, Locality #4) only two separate shell valves, belonging to different individuals with shell lengths of 9.0 and 10.3 mm, were collected, respectively.

Arcuatula senhousia

Live specimens of *A. senhousia* were found at two new localities in the Black Sea (Table 1). The sequences of *A. senhousia* from Sukhoy Liman (Odessa) were submitted to GenBank (Accession: MT878230). No stop codons were observed after conversion of nucleotides into amino acids. The blast search of sequence showed 100% query cover and 97% of nucleotide identity to the *A. senhousia* complete genome (Accession: GU001954, Passamonti et al. 2011) and to the previously published sequences of *cox1* gene region of *A. senhousia* in GenBank database, especially from Venice Lagoon, Italy (Passamonti 2007). Thus, the presence of a new invasive species in the Ponto-Caspian area was confirmed, both by morphological and molecular methods.

Discussion

The ongoing invasion process of Mytilopsis *species in the Ponto-Caspian region*

Our latest findings together with earlier records (Zhulidov et al. 2015, 2018; Mumladze et al. 2019) suggest that *M. leucophaeata* has successfully expanded into the inland sea basins of the Ponto-Caspian region (Figure 1). Different environmental factors driving dispersal of this species are discussed in detail in recent papers (Kennedy 2010; Zhulidov et al. 2015, 2018; Van der Gaag et al. 2016). Our findings highlight the following environmental points of interest. New records of *M. leucophaeata* in the Azov Sea and successful establishment in the Dniester Liman and Southern Bug basin which are locations fully exposed to winter freezing confirm the ability of this species to establish in waters with winter temperatures as low as 0 °C (Goptarev et al. 1991; Simonov and Altman 1991). This is a characteristic not previously apparent in its dispersal history. The water temperature in the south-eastern part of the Black Sea (see Figure 1, Locality #3 and #4) and in the middle part of the Caspian Sea (Figure 1, Locality #5) is within commonly known tolerance limits of *M. leucophaeata* (5–30 °C) (Simonov and Altman 1991; Terziev et al. 1992; Laine et al. 2006; unpublished data of the authors). However, mortality peaks at temperatures close to 0 °C, up to almost complete extinction of the population, were noted in experimental studies and field observations (Van der Gaag et al. 2016 and literature therein). Thus, our current finding could be viewed as indirect support for our earlier assumption that adaptive processes at the population level may be playing a role in distributions in the wild (Zhulidov et al. 2015).

Zhulidov et al. (2018) previously argued that *M. leucophaeata* was mostly collected in estuaries in the Azov-Black Sea region with variable and disturbed hydrological regimes. This was based on the fact that all living specimens from this region were collected in habitats that were subject to hydro-engineering and other human activities, which lead to larger changes in salinity than would naturally occur in Azov-Black Sea estuaries. We suggested that alterations of salinity regimes induced by such activities in Ponto-Caspian water bodies transform them into systems similar to tidal estuaries allowing for invasions of Atlantic brackish water species, such as *M. leucophaeata*. Such artificially transformed estuaries can be considered as potential stepping-stones for further dispersal. At the same time, these areas are zones used for the exchange of ballast water, which makes them the gateways for invasions (Zhulidov et al. 2018). These sites are especially important for the shipping corridor between the Sea of Azov and the Caspian Sea, which connects such zones and leads to an acceleration of the spread of alien species in both basins (Son et al. 2020).

Given these assumptions, most interesting are records of this species in Black Sea bays proximate to mouths of mountain rivers, viz Tuapse River (Zhulidov et al. 2018) and Mzymta River (this study), both flowing from the Caucasus. These rivers are largely rain-fed, and their hydrographs are characterized by a short lag time, high peak discharge, and steep rising and falling limbs, and therefore can exert similar impact on coastal zones adjacent to the river mouths facilitating the establishment of *M. leucophaeata*. The sea portion near the Muchka River flowing from Pontic Alps could be another example of such a situation if the presence of a self-sustained population of *M. leucophaeata* is confirmed. It should be noted that this type of Black Sea estuary has not been studied in detail, as compared to the estuaries of the “liman” type (a lagoon-type river estuary), and the specific features of their ecosystems are not well understood.

In the Netherlands, *M. leucophaeata* occurs in brackish water such as canals with saltwater intrusion via sluices with only slight daily fluctuations, but sometimes large annual fluctuations (Wolff 1969; Van der Gaag et al. 2016). Moreover, according to Wolff (1969) this species avoids brackish water estuaries with large daily fluctuations. It is evident now that the situation in the non-tidal Ponto-Caspian basin is generally similar to that in water bodies in the Netherlands where this species occurs. In all cases, there are irregular fluctuations with time intervals much larger than a day. The range of such oscillations can vary to a large extent, from very small in the freshened Taganrog Bay of the Azov Sea (Zhulidov et al. 2015), to values comparable with seawater salinity in the mouths of mountain tributaries of the Black Sea.

As stated previously by Zhulidov et al. (2018), the current distribution pattern of this species indicates jump dispersal with gradual expansion away from established populations. Most records of *M. leucophaeata* are associated with large seaports where invaders can be transported via seagoing vessels, both on ship hulls as fouling organisms and with ballast water as larvae. However, there are at least two locations where populations of *M. leucophaeata* occur, which have no port infrastructure, namely the Azov Sea near Primorsko-Akhtarsk and the Caspian Sea near Sulak. For these populations, we cannot exclude natural dispersal through coastal waters.

In the lower reaches of the Southern Bug and Ingul River within the city of Mykolaiv, where *M. leucophaeata* has already been recorded for one location (Heiler et al. 2010, Zhulidov et al. 2018) we have evidence by the growing number of localities that the population of *M. leucophaeata* is now well established. The finding of this species in areas with sand substrates is very interesting, in that *M. leucophaeata* formed druses with alien *Amphibalanus* sp. These druses are not attached to the sand and freely move with waves in the infralittoral zone. Therefore, in areas where hard substrates are not common, *M. leucophaeata* is able to occupy entirely new biotopes.

Taking into account the presence of *M. leucophaeata* both in the southernmost and northernmost parts of the Caspian Sea, one should also expect further records of this species in intermediate areas. First of all, the ports of Alyat (Baku City), Azerbaijan (the largest port in the Caspian Sea) and Turkmenbashi, Turkmenistan, could be of interest in this respect. Both ports are connected by railway ferry and have great importance as major transport hubs in the region. As was demonstrated earlier (Zhulidov et al. 2018), *M. leucophaeata* is able to successively colonize the areas of brackish-water ports by way of coastal shipping. Our new data confirm these preliminary results. Over a period of about two decades, the increasing number of this species' records around the Black and Caspian Seas as well as widespread occurrence of localities with suitable habitat indicates that this species has established populations in the region and probably has the potential for spreading even further.

Habitats of A. senhousia *in the Black Sea*

Our findings of *A. senhousia* provide additional information on dispersal of this species in the Black Sea. For Russia, this is its first recorded occurrence in the open coastal area of the Black Sea. The species was also earlier recorded in the Kerch strait that connects the Sea of Azov and the Black Sea (Kovalev et al. 2017). For Ukraine, this is the first record of this species in the country. Moreover, we cannot exclude the possibility that this species might have been present in the Ukraine earlier. We conducted interviews with local fish farm staff and amateur naturalists after this species was recorded and found that these locals noted multiple findings of unusual "oval" mytilids from the Odessa suburbs. However, although quite rare around Odessa, *Modiolus adriaticus* commonly occurs in shallow bays of the Black Sea (Semik and Shlyakhov 2009) and its juveniles potentially can be confused with *A. senhousia*.

In general, *A. senhousia* is usually considered a typical, opportunistic estuarine species, which occurs in shallow bays, estuaries and lagoons (Crooks 1996). It can form populations even in river estuaries with salinity ranges as low as 5–10 (Yamamuro et al. 2010). However, in the brackish Black Sea and its coastal water bodies it was found in localities where salinity values approach the maximum salinity of the Black Sea: 16.6 in the Kerch strait (Kovalev et al. 2017), 17.6 in the Burgas Bay (Chartosia et al. 2018), 15.0 in the Sukhoy Liman, and 16.4 in the Tuapse River bay. Soon after *A. senhousia* was recorded in the neighbourhood of Constanţa City in 1959, salinity levels of Romanian coastal waters have declined and unprecedentedly low levels have since been noted (10.1; Preda et al. 2012) and *A. senhousia* has not been further reported. It was shown that in the port water areas of the Sukhoy Liman salinity can be as low as 5.3, at the surface water layer while the bottom layer is relatively stable (circa 15) all the year round (natural hydrological regime of this estuary, the Sukhoy Liman, has been completely changed during the port construction process (Vinogradov et al. 2014). Earlier we have demonstrated the presence of *M. leucophaeata* near the Tuapse River mouth in October 2014 at locations with a salinity range of 4.7–10.2 (Zhulidov et al. 2018). Specimens of *A. senhousia* were found in the same area but somewhat farther off the shore where the freshening effect was negligible and salinity reached 16.4 (Table 1).

In this study, specimens of *A. senhousia* were found in localities with mixed sediments: sand, mud, and pebbles in the Tuapse River bay and mud, shell gravel, pebbles, and marine litter in the Sukhoy Liman. The first locality shows various substrates formed by the riverine influence. In the Sukhoy Liman, samples were taken at a site with limited water exchange between various artificial basins of harbours, with substrate composition sharply altered by man-made impact and a mosaic pattern of soft and hard substrates. Similar undisturbed parts of this estuary are represented by muds with an admixture of shelly gravel. All live specimens in this site were attached to pebbles or conglomerates of filamentous algae associated with bottom substrate (Figure 3).

Apart from the Constanţa Harbor record where the species was found on artificial constructions (Preda et al. 2012), the rest of findings reported for the Black Sea were also made on mixed sediments with dominating soft substrate in presence of shelly gravels or pebbles (Micu 2004; Karachle et al. 2017; Kovalev et al. 2017). Such substrates are less mobile than pure sands that likely makes it easier for fouling species to attach.

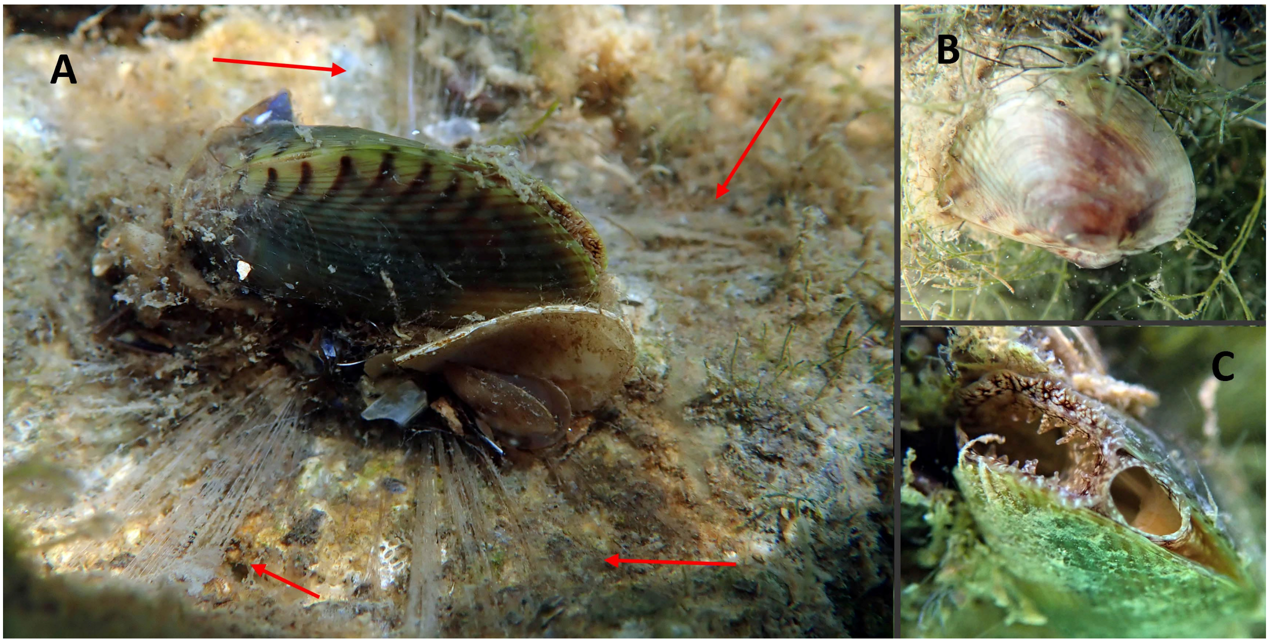

Figure 3. *Arcuatula senhousia* in the invaded habitat (the Sukhoy Liman): on a limestone gravel (arrows point to byssus filaments) (A); in a tangle of filamentous algae (B); in the process of filtration (C). Photos by M. Son.

Such bottom habitats designated as "A5.4. Sublittoral mixed sediments" according to EUNIS habitat classification (European Environment Agency 2012) are poorly populated by native byssus-attached bivalves in the Black Sea shallows. While native mytilids predominantly overgrow stones or large shells, *A. senhousia* seems to find a specific free niche. Overgrowing of the soft substrates is a specific feature of this species which constructs byssal mats for stabilization of the individuals within the sediment (Crooks 1996; Mistri 2002; Magni et al. 2006), however, it is also known to occur on hard substrates (Despalatović et al. 2013).

In general, in shallow waters of the Black Sea mixed type sediments are associated with estuarine conditions. However, large tributaries create in the brackish Black Sea and its limans extensive zones with salinities that are too low for *A. senhousia* to persist. Furthermore, many small bays, lagoons and limans (in particular, Sukhoy Liman and Burgas Bay) have estuarine origins and corresponding bottom substrates, although their river systems have currently much deteriorated and cannot form zones of decreased salinity due to negligible discharge. This is also typical for a number of larger shallow-water gulfs (namely Dzharylhach, Yahorlyk and Tendra gulfs), that have been formed in the areas of old Dnieper River estuaries and currently have no river discharge. Finally, mountain rivers such as the Tuapse River form mixed sediments in sea zones as a result of strong flash floods, but these zones are not subject to a permanent freshwater input.

Conclusions

Two invasive alien species, *M. leucophaeata* and *A. senhousia*, are in different phases of colonization within the Ponto-Caspian region. While

M. leucophaeata has established populations in the Black, Azov and Caspian Seas with nearly circumferential distribution in each sea basin, the latter species is just in the initial phase of its dispersal in the region. Due to different salinity tolerances, both species are distributed in coastal zones that are to a different extent subject to hydrological fluctuations (and have so far not been found together in the same habitat): *M. leucophaeata* occurs in more estuarine conditions where river discharges are more unstable, while *A. senhousia* seems to avoid shallows with substantial freshwater input. Furthermore, *M. leucophaeata* seems to have a broader range regarding various environmental factors including temperature, salinity and type of substrate. At the same time, both *M. leucophaeata* and *A. senhousia* in the Black Sea tend to settle and inhabit substrates that are not fully occupied by native byssus-attached bivalves. For now, the records of both species are limited mostly to surroundings of seaports or shipping canals where the biotopes associated with man-made infrastructure are widely occurring. It is quite likely that during further species expansion by means of natural spread the variety of the colonized biotopes can significantly increase.

Acknowledgements

Financial support for this work was provided by the South Russian Centre for Preparation and Implementation of International Projects Ltd. (CPPI-S), Rostov-on-Don, Russia. Halyna Morhun was awarded a travel grant for academic training on molecular taxonomy at the Department of Invertebrate Zoology and Hydrobiology (University of Lodz, Poland) by The Malacological Society of London. We are warmly grateful to Prof. Michał Grabowski and all the lab members for helping, teaching and encouraging during the fellowship. The molecular analysis was funded by the University of Lodz statutory funds. The publication of this research was funded by Radboud University, Nijmegen. We gratefully acknowledge Dr. Ekaterina Kalashnik (Institute of Marine Biology, National Academy of Sciences of Ukraine, Odessa) who kindly provided a sample of *A. senhousia*. We are indebted to Boris Linetskii (The Ukrainian Scientific Center of Ecology of the Sea) for his help in creating the map. Finally, we thank two anonymous reviewers for their constructive remarks by which the paper could be considerably improved.

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