

Research Article

The communities of microorganisms in the setae of invasive Chinese mitten crab (*Eriocheir sinensis*) in the southern Baltic catchment basin

Kinga Zatoń^{1,*}, Elżbieta Bogusławska-Wąs² and Przemysław Czerniejewski¹¹West Pomeranian University of Technology in Szczecin, Faculty of Food Sciences and Fisheries, Department of Fishery Management and Water Protection, Kazimierza Królewicza 4 Street, 71-550 Szczecin, Poland²West Pomeranian University of Technology in Szczecin, Faculty of Food Sciences and Fisheries, Department of Microbiology and Applied Biotechnology, Papieża Pawła VI Street, 71-459 Szczecin, PolandAuthor e-mails: Kinga.Zaton@zut.edu.pl (KZ), Elzbieta.Boguslawska-Was@zut.edu.pl (EBS), Przemyslaw.Czerniejewski@zut.edu.pl (PC)

*Corresponding author

Citation: Zatoń K, Bogusławska-Wąs E, Czerniejewski P (2019) The communities of microorganisms in the setae of invasive Chinese mitten crab (*Eriocheir sinensis*) in the southern Baltic catchment basin. *Aquatic Invasions* 14(4): 703–715, <https://doi.org/10.3391/ai.2019.14.4.09>

Received: 19 November 2018**Accepted:** 29 April 2019**Published:** 24 July 2019**Handling editor:** Maiju Lehtiniemi**Thematic editor:** Charles W. Martin**Copyright:** © Zatoń 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).

OPEN ACCESS**Abstract**

Chinese mitten crabs are one of the largest species of invasive crustaceans in the Baltic Sea catchment basin. Due to their catadromous migration, they can serve as a vector for small organisms, transporting them over distances greater than 500 km. Research on various crab species has shown their notable role in the spread of microorganisms to new aquatic environments. The aim of this study was to determine the diversity and abundance of microorganisms migrating with the Chinese mitten crab on the densely-distributed setae found on their claws. Our analysis also considered the potential differences in the number and genera of microorganisms between male and female Chinese mitten crabs. The study consisted of forty-eight (48) crabs (sex ratio 1:1) fished with a fyke net during their intensive downstream migration in Lake Dąbie (which is the southern part of the Oder River estuary) between November 8 and 11 2017. In these Chinese mitten crabs, a rich microbiological flora was found, which can be divided into three basic groups: heterotrophic bacteria, yeasts, and fungi. Microorganism genus biodiversity on claw setae was greater in males than in females, although the total aerobic microbial count (TAMC), total halophile count (THC), and total combined yeasts and molds count (TYMC) showed no statistically significant differences between sexes. Phylogenetic analysis of PCR products and genetic taxonomic analysis of dominating strains in samples from crab claw setae showed the dominance of *Bacillus tequilensis* bacterium. The presence of these microbiologically antagonistic bacteria, capable of adapting perfectly to different environmental conditions, indicates that the crabs are capable of introducing potentially dangerous microorganisms into new ecosystems.

Key words: invasive crab, non-native species, Baltic Sea, vectors, bacteria, migration, estuary

Introduction

Invasions by expansive non-native species are a major global problem and, according to the International Union for Conservation of Nature (IUCN), are one of the most significant threats to biodiversity due to the unpredictable effects the emergence of a new species can have on the environment (Dittel and Epifanio 2009). The introduction of non-native

species of plants and animals into new ecosystems are in most cases the result of deliberate human activity (intentional introduction), although there are many cases of accidental introductions related to the increasing shipping, trade, and exchange of products (Casal 2006).

In Polish waters, the largest group of alien species are invertebrates, namely crustaceans (Grabowski et al. 2006, 2007). For example, as stated by Jażdżewski et al. (2005), out of 56 species of higher crustaceans (Malacostraca) found on the Polish coast of the Baltic, nearly 20% are alien species (11 species). One of these is the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853), a species originating from Asian waters (Dittel and Epifanio 2009), currently the largest crustacean in the southern Baltic Sea basin, and simultaneously one of only two brachyuran species listed in the top 100 worst invasive species in the world. This species is also found in the List of Invasive Alien Species found in the Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species (Lowe et al. 2000; European Community 2014).

The Chinese mitten crab is a catadromous animal with a migration range of over 500 km in European waters (Dittel and Epifanio 2009; Herborg et al. 2003). Due to its migratory capabilities—up to 18.1 km per day (Herborg et al. 2003)—this foreign crab may potentially transport various smaller organisms on the setae of its claws over far distances (Normant et al. 2013). These microscopic organisms, including protozoa, bacteria, and fungi (some of which are parasitic), can spread unnoticed by humans and cause infections in other aquatic organisms (Schrimpf et al. 2014). This may pose a potential danger to new environments, especially during the period of autumn downstream migration (Dittel and Epifanio 2009; Czerniejewski and Wawrzyniak 2006), a threat exacerbated by the steady growth in biomass of Chinese mitten crabs in various European waters (Czerniejewski 2010).

The aim of this study was to determine the diversity and abundance of microorganisms transported by invasive Chinese mitten crabs on the setae found densely distributed over their claws and whether these organisms are sex-related.

Materials and methods

Chinese mitten crabs were caught with fyke nets between November 8 and 11 2017 during their intensive downstream migration (Czerniejewski and Wawrzyniak 2006) in Lake Dąbie. This reservoir is part of the estuary of the Oder River, in the catchment basin of the southern Baltic Sea (Figure 1). Eighty-nine (89) crabs were caught in fishing nets, of which 48 individuals with the largest carapaces and the most similar claw sizes were used for the research (24 females and 24 males). The captured specimens were weighed

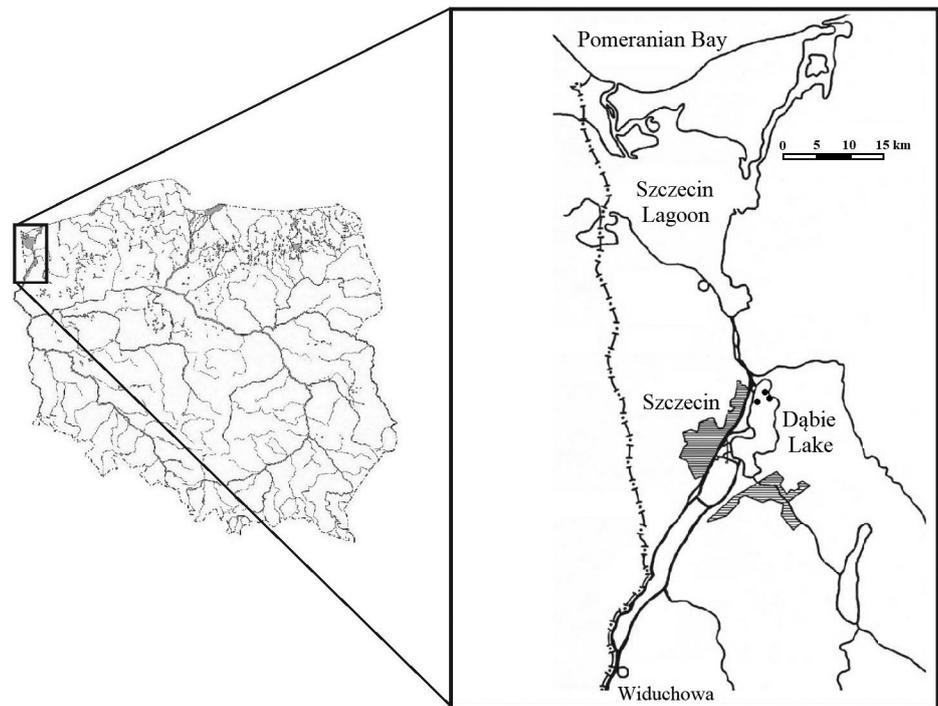


Figure 1. Location of the sites where the studied Chinese mitten crabs were collected.

to an accuracy of 0.1 g on an electronic weight type Axis 2000B. Then, using an electronic caliper coupled to a computer, measurements were taken to an accuracy of ± 0.01 mm of carapace width (CW) as well as length and width of each pair of claws. The claws with similar surface areas were used for further analyses.

Microbiological collection and analysis

Selected pairs of claws, labelled by sex (F, M) and individual (1–24), were placed in sterile resealable bags and transported in refrigerated conditions to the laboratory. In the next stage, claw setae were scraped and washed with 100 ml of sterile water.

The samples were subjected to microbiological culture analysis. Halophilic microorganisms (THC) were cultured on SeaWater medium (SW: 1.4% NaCl; 0.0385% KCl; 0.24% MgCl₂; 0.055% NaHCO₃; 0.175% MgSO₄; 5% beef extract; 0.5% peptone, 2% agar), heterotrophic bacteria (TAMC) were cultured on enriched agar (M-PA: 50% agar; 11.7% NaCl; 1.3% beef extract; 5.7% yeast extract; 13.3% peptone; 18% peptone K), prototrophic microorganisms (total prototrophic count – TPC) were cultured on an R2A medium (0.05% peptone; 0.05% sucrose; 0.05% glucose; 0.05% yeast extract; 0.05% casein hydrolysate; 2% agar), yeasts and molds (TYMC) were grown on a potato glucose agar (PDA: 1.7% agar; 2.0% glucose; 0.4% potato extract), and coliform bacteria (total fecal count - TFC) were grown on a LPB medium (1% peptone; 0.5% lactose; 0.5% NaCl, 0.005% bromocresol purple). Analysis was performed using serial dilution and spread plate techniques. Cultures were incubated under conditions suitable for each

group of microorganisms. Results were given as the total number of aerobic microorganisms, expressed in colony-forming units (cfu)/ml. For determination of coliforms, most probable number (MPN) index was used for readings. Through macroscopic analysis, dominant colonies were selected and sequenced.

Setae samples were analyzed for genotypic differentiation. To this end, DNA was sequenced according to the Genomic Mini AX protocol (A&A Biotechnology) using lyticase, lysozyme, and mutanolysin (Sigma-Aldrich). For amplification of the extracted bacterial DNA, the following primers were used – 338G: 5'-CGC CCG GGG CGC GCC GCC CCG GGG GGG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAGT-3' and RP534: 5'-ATT ACC GCG GCT GCT GG-3' (Mrazek et al. 2008). For the extraction of fungal DNA GCCATATCAATAAGCGGAGGAAAAG and LS2: ATTCCCAAACAACACTCGA primers were used. PCR was performed on a final volume of 25 μ L of reaction mixture with the following composition: 1.0 μ L MgCl₂, 2.5 μ L dNTP, 2.0 μ L of each primer, 1U Taq DNA polymerase (Eppendorf), 2.5 μ L polymerase buffer and 3.0 μ L of DNA matrix, all dissolved in water. PCR was carried out in a thermocycler (Eppendorf) with the following thermal profile conditions: denaturation 180 s / 94 °C, 35 cycles: 60 s / 94 °C, 30 s / 55 °C, 60 s / 72 °C and final elongation 10 min / 72 °C (Muyzer et al. 1993). The obtained amplification products were electrophoretically separated by DGGE method in 8% polyacrylamide gel and stained with ethidium bromide (Hesham et al. 2011). The results of electrophoretic separation were visualized with UV radiation using the GelDoc apparatus (Bio-Rad). Analysis of the similarity of the obtained amplification profiles was performed using the UPGMA method.

Sequencing

PCR products for dominant bacterial strains were obtained from the reaction performed on 50 μ L final volume with the composition: 5 μ L DNA, 25 μ L PCR Master Mix Plus High GC (A&A Biotechnology), 2 x 0.2 μ L primers: B-all for (GAG TTT GAT CCT GGC TCA G) and B-all Rev (ACG GCT ACC TTA CGA CTTT) and for fungi: ITS1 (TTC GTA GGT GAA CTT GCG G) and ITS 4 (TCC TCC GCT TAT TGA TAT GC). Following sequencing (Macrogen), the obtained DNA profiles were subjected to phylogenetic analysis. Comparisons of the obtained DNA sequences of the 16S rRNA subunit for bacteria and the ITS region for fungi were performed using the BLAST program. Confirmation of the species identification was performed by A&A Biotechnology Poland.

Statistical analysis

The degree of microbiota dispersion within the groups of males and females was determined on the basis of the coefficient of variation of a given trait, based on the mean and standard deviation. The obtained values

Table 1. Statistical comparison of the diversity of microorganisms isolated from setae.

Statistical evaluation parameter		THC	TAMC	TCP	TYMC*	TFC**
total microbiota	Mean	1.13E+06	6.35E+05	8.77E+05	1.37E+03	2.85E+03
	V_{cf}	168.3	62.8	49.0	91.4	119.2
F – females	Mean	1.44E+06	8.04E+05	1.04E+06	1.35E+03	5.70E+01
	V_{cf}	62.5	55.2	37.8	93.2	42.8
M – males	Mean	8.27E+05	4.66E+05	7.09E+05	1.39E+03	5.65E+03
	V_{cf}	42.3	57.3	42.7	93.1	47.5

are given as the coefficient of variation V (in %). Depending on the obtained V , the results were interpreted as having low variability ($V < 50\%$), moderate variability ($50\% < V < 100\%$), or high variability ($V > 100\%$). The level of similarity of the isolated microorganisms was determined by cluster analysis using the method of weighted arithmetic means according to the Euclidean distance. However, in the analysis of similarities for the amplification profiles obtained, the UPGMA method was used in the BioGene software. The levels of similarity of the obtained profiles (similarity index S) were determined by the Dice similarity coefficient = 3.0%

Linear regression analysis was performed to test if the size of the claws (length and width) accounts for the total number of bacteria and total number of fungi found on the claws. The normality of distribution of results was evaluated by the Kolmogorov-Smirnov test, while the equality of variances was assessed by the Levene's test. The results of both tests ($p > 0.05$ in each of them) indicate the possibility of using linear regression analysis. In addition, ANOVA and the Tukey's post hoc test were performed in order to biometric traits of the claws and carapaces of males and females, and compare the quantities of microorganisms. The analyses were performed using the Statistica 13.1 software.

Results

The study found no statistically significant differences ($p < 0.05$) in the number of isolated bacteria between females and males. The only exception were gram negative coliforms (TFC), which were three times more abundant in males than in females (Table 1). Moreover, it was confirmed that the crab claw microbiota consisted mainly of bacteria (10^5 – 10^6 cfu/ml) with yeasts showing significantly smaller numbers (10^3 cfu/ml) (Table 1).

A comparison of dispersion of the determined bacteria and fungi (V_{cf}) showed a high similarity between F and M, with the exception of halophilic bacteria (THC). This indicates that very similar mechanisms of regulating colonization by microorganisms are used in both sexes. For THC, TAMC, TYMC, and TFC, where $50\% < V_{cf} < 100\%$, the determined variability coefficients indicate a large dispersion of the microbiological profile. Differentiation of abundance determined by high V_{cf} (Table 1) was considered to reflect the determination of individual properties influencing the formation of a specific microbiota, in which the environmental microflora may play a significant role. Only bacteria determined as TPC were characterized by low

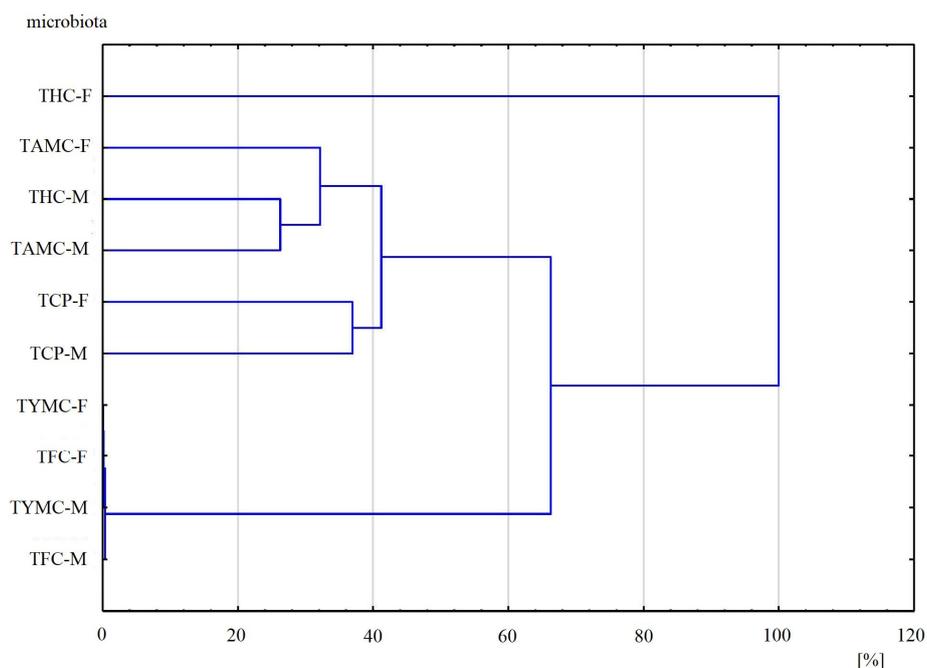


Figure 2. Cluster analysis (UPGMA) of the relation between microorganisms isolated from Chinese mitten crab setae. F – females, M – males, THC – total halophilic count, TAMC – total aerobic microbial count, TPC – total prototrophic count, TYMC – total yeast and mold count, TFC – total fecal count.

Table 2. Results of linear regression analysis between the total number of bacteria, total number of fungi, and the length and width of crab claws.

	Correlation (R)	R ²	t	p	b (intercept)	a (slope)
Length of claws						
Total number of bacteria	-0.14	0.02	-0.95	0.35	1302938.00	-147255.00
Total number of fungi	-0.11	0.01	-0.72	0.48	374635.00	-28638.00
Width of claws						
Total number of bacteria	-0.13	0.02	-0.89	0.38	1037141.00	-136753.00
Total number of fungi	-0.11	0.01	-0.76	0.45	333553.00	-29992.00

variability in the number of microorganisms ($V_{cf} < 50\%$), which may indicate their symbiotic relationship with the crabs.

Detailed statistical cluster analysis did not indicate significant differences between microbial groups (Table 1). The only exception was the halophilic microflora isolated from females, for which the measure of differences between the studied crabs (M + F) was determined at 100% (Figure 2).

Linear regression analysis was performed to test how the size of the claws (length and width) accounted for the total number of bacteria and total number of fungi found on the claws. However, linear regression analysis of relations between the total number of bacteria, total number of fungi, and size of claws (length and width) from which the test material was collected did not show statistically significant correlations (Table 2). In addition, ANOVA and the Tukey's post hoc test used to compare the biometric traits of claws and carapaces of males and females, showed statistically significant differences in the width of carapace, at $p < 0.0008$ (Table 3).

The profile of the DNA complex isolated from the claws of the tested Chinese mitten crabs indicated a slight microbiological differentiation

Table 3. Width of carapace and the length and width of claws (in mm) in Chinese mitten crab.

Traits	Males (mean ± SD)	Females (mean ± SD)	ANOVA, Tukey's post hoc test
Width of carapace	61.19* ± 10.28	79.27* ± 6.93	p = 0.0008
Length of the left claw	46.54 ± 10.96	47.75 ± 8.57	p = 0.6725
Width of the left claw	30.17 ± 10.74	32.83 ± 9.38	p = 0.3645
Length of the right claw	46.46 ± 11.48	47.46 ± 8.93	p = 0.7377
Width of the right claw	30.71 ± 11.36	31.21 ± 9.09	p = 0.8671

* – statistically significant difference between females and males ($p < 0.05$) according to the Tukey's post hoc test.

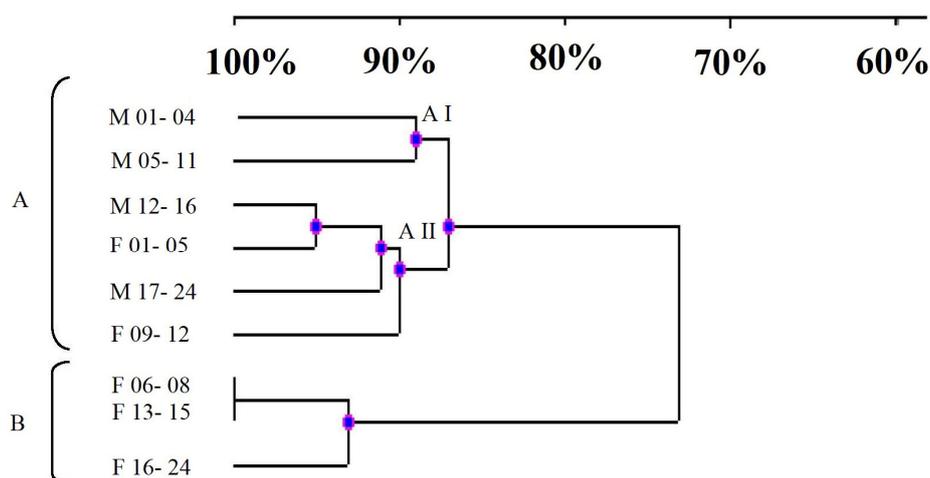


Figure 3. Cluster analysis of DGGE for the similarity of microbiological profile in crabs. F – females, M – males, 01–24 – numbers assigned to individuals from which material was sampled for phylogenetic analysis. % – level of phylogenetic similarity determined by the Dice similarity coefficient = 3%. Cluster analysis was performed using Quantity One software.

between the individuals. Both male and female samples showed the presence of populations with significant genetic similarity ($S > 70\%$); however, electrophoretic separation, shown as a dendrogram in Figure 3, made it possible to establish two independent groups—A and B—where B indicates the epibiota unique to females. In addition, the denaturing gradient gel electrophoresis (DGGE) profile for group B indicated high homogeneity ($S > 90\%$), which may reflect a predisposition in females for carrying certain bacterial taxa. Separated genotypes with high homogeneity were established in samples taken from 58% of the examined females (Figure 3).

Conversely, group A was characterized by much greater microbial diversity. Samples taken from the setae of all the examined individuals (both sexes) could be divided into two independent clusters with homogeneity at $90\% > S > 80\%$. Group A-I was formed exclusively by genotypes of microorganisms obtained from 46% of males. Microorganisms from the remaining 42% of females and 54% of males did not show any species specificity and constituted the combined A-II group (Figure 3).

The results of phylogenetic analysis of PCR products and genetic taxonomic analysis of bacterial strains in crab setae samples identify *Bacillus tequilensis* as the dominant bacterial species. In the case of fungal DNA sequencing results, *Aspergillus fumigatus* dominated.

Discussion

Depending on the inhabited area, Chinese mitten crabs live for an average of 1–5 years. Their life expectancy is closely linked to the time needed to reach full maturity and reproductive capacity (Veilleux and de Lafontaine 2007; Trivhkova et al. 2017). Due to their catadromous lifestyle, these crabs spend most of their lives in fresh and brackish waters. Brackish and marine waters are essential for its reproduction. The best habitats for this invasive species are river estuaries and lakes connected to them (Czerniejewski and Wawrzyniak 2006). Lake Dąbie perfectly fits these characteristics. This lake, located in a branched system of the lower Oder River, Roztoka Odrzanska, the Szczecin Lagoon, the Straits of Piana, Dziwna and Swina and a part of the Pomeranian Bay, is considered to be a part of the estuary of the Oder River.

It is also a highly complex hydrographic system with high change dynamics (mainly currents and water levels), which are the result of variability in the river inflow and sea level fluctuations, as well as high susceptibility of the system to these changes (Chojnacki 1999; Czerniejewski and Wawrzyniak 2006; Wiśniewski et al. 2007). The specificity of this Tertiary estuary combining the Oder River, Lake Dąbie, the Szczecin Lagoon and the Pomeranian Bay facilitates the smooth movement of this invasive species both within the catchment of the South Baltic Sea and in inland waters (Figure 1). Its food web interactions, including its tendency to feed on snails, mussels, and sometimes fish eggs, has created a significant threat to native species in the environments which it has invaded (Škraba et al. 2013). Additionally, its setae often contain numerous microorganisms and representatives of Nematoda and Bivalvia with potentially unknown effects on the new ecosystems (Normant et al. 2007). However, much more dangerous is the potential ability of these crabs to transmit *Aphanomyces astaci* (Oomycetes, Saprolegniales)—the mold responsible for the fatal crayfish plague—as observed by Svoboda et al. (2014).

The crab's setae are colonized by a wide spectrum of microorganisms (Figure 3). Their diversity reflects the species diversity of microorganisms with which the Chinese mitten crabs have been in contact during their migration. Statistical cluster analysis of the quantitative and qualitative structure of the isolated microflora supports the statement that females of this species are predisposed to transmit certain microorganisms, in this case halophilic bacteria (THC-F, Figure 2). It is difficult to unambiguously explain the reasons for this phenomenon. It may be suspected that this is due to the catadromous life cycle of these crabs and their tolerance to salinity at different stages of life. Young individuals use tidal currents to move into river systems. Most likely, the colonization effect of these microorganisms was already initiated during their stay in marine waters and the mature female Chinese mitten crabs may have been transported

back to the waters of the Lake Dąbie with the autumn Baltic Sea eddies. In addition, the periodic sea water inflows result in the general hydrochemical profile of the lake being influenced by the saltwater estuary of the Szczecin Lagoon (Stepanowska et al. 2009; Nędzarek and Tórz 2009). The halophilic microorganisms transported together with these waters co-create microbiocenoses in new habitats which include the Chinese mitten crabs (Normant et al. 2007).

Slower and less active females can serve as a reservoir for microorganisms as, during migration, less water rinsing the outgrowths of the cuticle claws allow for better adhesion and development of bacteria and fungi. However, total microorganism population is limited by the smaller number of setae on the females' claws.

In this study, microorganism genus biodiversity on claw setae of male Chinese mitten crabs was greater than in females (Figure 3). Although the total number of aerobic bacteria (TAMC), halophiles (THC) and fungi (TYMC) did not differ significantly between the sexes. Conversely, in intensively migrating males, their increased movement contributes to better filtration of organisms deposited on the setae, resulting in a similar number of microorganisms in both sexes despite the larger number of longer and more densely distributed setae in males.

An unambiguous assessment of the relationship between the microorganisms on *E. sinensis* is quite difficult. In most cases, its variability results from the fact that these crabs belong to the group of catadromous organisms, i.e. the variability of their environment (Najiah et al. 2010; Goffredi et al. 2014; Ding et al. 2017; Kim et al. 2017) is emphasized. Researchers indicate possible relation to climate change (Li et al. 2012; Liu et al. 2017; Ramachandrana et al. 2018), trophic conditions (Drotz et al. 2010; Normant et al. 2013), and food availability (Chen et al. 2015).

Due to the economic importance of *E. sinensis* related to its commercial use in China and the ecological threat associated its invasive character, its microflora is becoming increasingly important in studies of epidemiology and the transfer of potentially dangerous microorganisms to new aquatic environments (Gasparich 2010; Wang 2011).

Particularly worrisome are bacterial infections, which, although not common in crabs, may change the mineral content of their exoskeleton, resulting in the softening of the carapace and its eventual damage (Meng et al. 2010; Wang et al. 2011; Morado 2011; Zeng and Yeo 2018). Serological and biochemical studies have shown that the most common bacteria causing infections in both crabs and humans were pathogens of the genera *Vibrio*, *Bacillus*, *Acinetobacter*, and *Flavobacterium*. Some of these bacteria, especially those of the genus *Vibrio*, have shown strong pathogenicity over a wide temperature range. Additionally, infected individuals, especially migratory crab species, are able to transfer microorganisms to other remote

areas, posing an additional threat to native aquacultures (Collen et al. 2014; Lodge et al. 2012; Nghiem et al. 2013; Reynolds et al. 2013; Stahl et al. 2014; Yeo and Klaus 2014; Alamgir et al. 2015; Savvides et al. 2015; Patoka et al. 2016; Poesch et al. 2016). As a result, the danger posed by the presence of the Chinese mitten crab in the southern Baltic catchment basin is due not only to the expansion of this invasive species itself, but the accompanying migration of undetected microorganisms non-native to our waters.

Of the microorganisms identified in this study, particularly noteworthy is the Gram-positive bacterium *Bacillus tequilensis*, a regular resident of ballast tanks (a known transport vector bringing Chinese mitten crabs to new areas). This endosporous and *Bacillus subtilis*-related microorganism has shown an ability to adapt to prevailing conditions in numerous new environments (Aanniz et al. 2014). Similar to most bacteria of the genus *Bacillus* spp., it has the ability to produce many compounds with a germicidal effect.

The metabolism of *Bacillus tequilensis* gives it a significant advantage in regards to its ability to survive in novel environments (Said et al. 2014; Tiwari et al. 2014; Chen et al. 2015; Sharma et al. 2015; Khuro et al. 2016; Rani et al. 2016; Manohar et al. 2017). Like most bacteria of the genus *Bacillus* spp., it has an ability to produce many bactericidal compounds, which may contribute to its invasive character and antagonistic influence on the native microbiota of newly settled areas. Considering its adaptability and metabolism, detection of this bacterium in the samples studied indicates that the Chinese mitten crab may be a habitat of microbes with the new biological potential.

The dominance of this microorganism in the species structure of the crab microflora can be considered an effect of a competent strategy of protection against biological disease agents (Smith et al. 2012). This is even more important as this study showed the common presence of *Aspergillus fumigatus*, a representative of fungi classified as ubiquitous saprophytes, which in the environment play an important role in the recycling of carbon and nitrogen. Due to its wide range of enzymatic activities, mainly chitinase, these fungi are considered to be the main cause of aspergillosis in aquatic organisms (Higgins 2000).

The juxtaposition of dominant microorganisms allows for the determination of individual features of crabs which significantly influence the formation of their specific microbiota.

Summary

Non-native Chinese mitten crabs in the Baltic Sea catchment basin serve as vectors for various microorganisms. In this study, the observed number of bacteria and fungi was significantly higher than reported in studies involving other species of crabs. Despite the fact that males of this species

are more active, move much faster during migration, and have much longer and denser setae on their claws, the total number of bacteria and fungi on setae did not differ significantly between sexes. It is likely that the higher activity level of male crabs results in reduced number but higher microbiological diversity through the more intense flow of water. The presence of *Bacillus tequilensis* bacteria in the samples from crab claw setae, a microbiologically antagonistic bacterium capable of perfectly adapting to different environmental conditions, indicates that the Chinese mitten crabs may be a suitable habitat for microorganisms with unknown potential and environmental impacts in new ecosystems.

Acknowledgements

We would like to thank Maiju Lehtiniemi, Charles W. Martin and all reviewers for all their helpful comments on the manuscript.

References

- Aanniz T, Ouadghiri M, Melloul M, Swings J, Elfahime E, Ibijbjen J, Ismaili M, Amar M (2014) Thermophilic bacteria in Moroccan hot springs, salt marshes and desert soils. *Brazilian Journal of Microbiology* 46: 443–453, <https://doi.org/10.1590/S1517-838246220140219>
- Alamgir M, Mukul SA, Turton SM (2015) Modelling spatial distribution of critically endangered Asian elephant and Hoolock gibbon in Bangladesh forest ecosystems under a changing climate. *Applied Geography* 60: 10–19, <https://doi.org/10.1016/j.apgeog.2015.03.001>
- Casal CMV (2006) Global documentation of fish introductions: the growing crisis and recommendation for action. *Biological Invasions* 8: 3–11, <https://doi.org/10.1007/s10530-006-1833-0>
- Chen QQ, Liu B, Liu GH, Wang JP, Che JM (2015) Draft Genome Sequence *Bacillus tequilensis* Strain FJAT-14262a. *Genome Announcement* 3: e01317-15, <https://doi.org/10.1128/genomeA.01317-15>
- Chojnacki J (1999) Description of Ecosystem of the Lower Odra and the Odra Estuary. *Acta Hydrochimica et Hydrobiologica* 27: 257–267, [https://doi.org/10.1002/\(SICI\)1521-401X\(199911\)27:5<257::AID-AHEH257>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1521-401X(199911)27:5<257::AID-AHEH257>3.0.CO;2-V)
- Collen B, Whitton F, Dyer EE, Baillie JE, Cumberlidge N, Darwall WR, Pollock C, Richman NI, Soulsby AM, Böhm M (2014) Global patterns of freshwater species diversity, threat and endemism. *Global Ecology and Biogeography* 23: 40–51, <https://doi.org/10.1111/geb.12096>
- Czerniejewski P (2010) Changes in condition and in carapace length and width of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853) harvested in the Odra River estuary in 1999–2007. *Oceanological and Hydrobiological Studies* 39: 25–36, <https://doi.org/10.2478/v10009-010-0017-5>
- Czerniejewski P, Wawrzyniak W (2006) Seasonal changes in the population structure of the Chinese mitten crab, *Eriocheir sinensis* (H. Milne Edwards) in the Odra/Oder Estuary. *Crustaceana* 79: 1167–1179, <https://doi.org/10.1163/156854006778859588>
- Ding ZF, Cao MJ, Zhu XS, Xu GH, Wang RL (2017) Changes in the gut microbiome of the Chinese mitten crab (*Eriocheir sinensis*) in response to White spot syndrome virus (WSSV) infection. *Journal of Fish Diseases* 40: 1561–1571, <https://doi.org/10.1111/jfd.12624>
- Dittel AI, Epifanio CE (2009) Invasion biology of the Chinese mitten crab *Eriocheir sinensis*: a brief review. *Journal of Experimental Marine Biology and Ecology* 384: 79–92, <https://doi.org/10.1016/j.jembe.2009.04.012>
- Drotz MK, Berggren M, Lundberg S, Lundin K, von Proschwitz T (2010) Invasion routes, current and historical distribution of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853) in Sweden. *Aquatic Invasions* 5: 387–396, <https://doi.org/10.3391/ai.2010.5.4.08>
- European Community (2014) Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species (IAS). *Official Journal of the European Communities* L 317: 35–55
- Gasparich GE (2010) Spiroplasmas and phytoplasmas: Microbes associated with plant hosts. *Biologicals* 38: 193–203, <https://doi.org/10.1016/j.biologicals.2009.11.007>
- Goffredi SK, Gregory A, Jones WJ, Morella NM, Sakamoto RI (2014) Ontogenetic variation in epibiont community structure in the deep-sea yeti crab, *Kiwa puravida*: convergence among crustaceans. *Molecular Ecology* 23: 1457–1472, <https://doi.org/10.1111/mec.12439>
- Grabowski M, Konopacka A, Jażdżewski K, Janowska E (2006) Invasions of alien gammarid species and retreat of natives in the Vistula Lagoon (Baltic Sea, Poland). *Helgolander Marine Research* 60: 90–97, <https://doi.org/10.1007/s10152-006-0025-8>

- Grabowski M, Jażdżewski 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>
- Herborg LM, Rushton SP, Clare AS, Bentely MG (2003) Spread of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards) in Continental Europe: analysis of a historical dataset. *Hydrobiologia* 503: 21–28, <https://doi.org/10.1023/B:HYDR.0000008483.63314.3c>
- Hesham AEL, Qi R, Yang M (2011) Comparison of bacterial community structures in two systems of a sewage treatment plant using PCR-DGGE analysis. *Journal of Environmental Sciences* 23: 2049–2054, [https://doi.org/10.1016/S1001-0742\(10\)60647-X](https://doi.org/10.1016/S1001-0742(10)60647-X)
- Higgins R (2000) Bacteria and fungi of marine mammals: a review. *Canadian Veterinary Journal* 41: 105–116
- Jażdżewski K, Konopacka A, Grabowski M (2005) Native and alien malacostracan crustacea along the Polish Baltic Sea coast in the twentieth century. *Oceanological and Hydrobiological Studies* 34(Suppl. 1): 176–193
- Khuro A, Kaliyan BK, Al-Dhabi NA, Arasu MV, Agastian P (2016) Statistical optimization of thermo-alkali stable xylanase production from *Bacillus tequilensis* strain ARMATI. *Electronic Journal of Biotechnology* 22: 16–25, <https://doi.org/10.1016/j.ejbt.2016.04.002>
- Kim S, Lee MJ, Lee JJ, Choi SH, Kim BS (2017) Analysis of microbiota of the swimming crab (*Portunus trituberculatus*) in South Korea to identify risk markers for foodborne illness. *LWT - Food Science and Technology* 86: 483–491, <https://doi.org/10.1016/j.lwt.2017.08.043>
- Li S, Sun L, Wu H, Hu Z, Liu W, Li Y, Wen X (2012) The intestinal microbial diversity in mud crab (*Scylla paramamosain*) as determined by PCR-DGGE and clone library analysis. *Journal of Applied Microbiology* 113: 1341–1351, <https://doi.org/10.1111/jam.12008>
- Liu S, Chen G, Xu H, Zou W, Yan W, Wang Q, Deng H, Zhang H, Yu G, He J, Weng S (2017) Transcriptome analysis of mud crab (*Scylla paramamosain*) gills in response to Mud crab reovirus (MCRV). *Fish & Shellfish Immunology* 60: 545–553, <https://doi.org/10.1016/j.fsi.2016.07.033>
- Lodge DM, Deines A, Gherardi F, Yeo DCJ, Arcella T, Baldrige AK, Barnes M, Chadderton WL, Feder JL, Gantz CA, Howard GW, Jerde CL, Peters BW, Peters J, Reisinger LS, Turner CR, Wittmann ME, Zeng Y (2012) Global introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics* 43: 449–472, <https://doi.org/10.1146/annurev-ecolsys-111511-103919>
- Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12 pp. First published as special lift-out in *Aliens* 12, December 2000. Updated and reprinted version: November 2004. <http://www.issg.org/booklet.pdf>
- Manohar P, Gothandam KM, Kannan VR, Nachimuthu R (2017) Strengthened atylolytic activity of the intracellular aromatic alasis produced by *Bacillus tequilensis*. *Journal of Microbiology, Biotechnology and Food Sciences* 6: 1314–1318, <https://doi.org/10.15414/jmbfs.2017.6.6.1314-1318>
- Meng Q, Li W, Liang T, Jiang X, Gu W, Wang W (2010) Identification of adhesin-like protein ALP41 from *Spiroplasma eriocheiris* and induction immune response of *Eriocheir sinensis*. *Fish Shellfish Immunology* 55: 245–250, <https://doi.org/10.1016/j.fsi.2010.06.005>
- Morado JF (2011) Protistan diseases of commercially important crabs: A review. *Journal of Invertebrate Pathology* 106: 27–53, <https://doi.org/10.1016/j.jip.2010.09.014>
- Mrazek J, Strosova L, Fliegerova K, Kott T, Kopečný J (2008) Diversity of Insect Intestinal Microflora. *Folia Microbiologica* 53: 229–233, <https://doi.org/10.1007/s12223-008-0032-z>
- Muyzer G, De Waal EC, Ulterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied Environmental Microbiology* 59: 695–700
- Najiah M, Nadirah I, Sakri I, Harrison FS (2010) Bacteria with wild mud crab (*Scylla serrata*) from Setiu wetland, Malaysia with emphasis on antibiotic resistances. *Pakistan Journal of Biological Sciences* 13: 293–297, <https://doi.org/10.3923/pjbs.2010.293.297>
- Ńędzarek A, Tórz A (2009) The primary production of Tertiary eastuary (Dąbje Lake, Poland). *Limnological Review* 9(1): 17–26
- Nghiem LTP, Soliman T, Yeo DCJ, Tan HTW, Evans TA, Mumford JD, Keller RP, Baker RHA, Corlett RT, Carrasco LR (2013) Economic and environmental impacts of harmful non-indigenous species in Southeast Asia. *PLoS ONE* 8: e71255, <https://doi.org/10.1371/journal.pone.0071255>
- Normant MM, Korthals J, Szaniawska A (2007) Epibiota associated with setae on Chinese mitten crab claws (*Eriocheir sinensis* H. Milne-Edwards, 1853): a first record. *Oceanologia* 49(1): 137–143
- Normant M, Zawal A, Chatterjee T, Wójcik D (2013) Epibiotic mites associated with the invasive Chinese mitten crab *Eriocheir sinensis* - new records of Halacaridae from Poland. *Oceanologia* 55: 901–915, <https://doi.org/10.5697/oc.55-4.901>
- Patoka J, Wardiatno Y, Kuřiková YP, Petřtýl M, Kalous L (2016) *Cherax quadricarinatus* (von Martens) has invaded Indonesian territory west of the Wallace line: evidences from Java. *Knowledge and Management of Aquatic Ecosystems* 417: 1–6, <https://doi.org/10.1051/kmae/2016026>

- Poesch MS, Chavarie L, Chu C, Pandit SN, Tonn W (2016) Climate change impacts on freshwater fishes: a Canadian perspective. *Fisheries* 41: 385–391, <https://doi.org/10.1080/03632415.2016.1180285>
- Ramachandran P, Reeda E, Ottesena A (2018) Exploring the Microbiome of *Callinectes sapidus* (Maryland Blue Crab). *Genome Announcements* 6: 1–18, <https://doi.org/10.1128/genomeA.00466-18>
- Rani RP, Anandharaj M, Sabhapathy P, Ravindran AD (2016) Physiochemical and biological characterization of novel exopolysaccharide produces by *Bacillus tequilensis* FR9 isolated from chicken. *International Journal of Biological Macromolecules* 96: 1–10, <https://doi.org/10.1016/j.ijbiomac.2016.11.122>
- Reynolds JD, Souty-Grosset C, Richardson AMM (2013) Ecological roles of crayfish in freshwater and terrestrial habitats. *Freshwater Crayfish* 19: 197–218, <https://doi.org/10.5869/fc.2013.v19-2.197>
- Said M, Ezzahra AF, Jamal A, Mohammed R, Omar A (2014) Heterotrophic denitrification by Gram-positive bacteria: *Bacillus cereus* and *Bacillus tequilensis*. *International Journal of Scientific and Research Publications* 4: 1–5, <http://www.ijsrp.org/research-paper-0414/ijsrp-p2878.pdf>
- Savvides P, Louca V, Sfenthourakis S (2015) Competition for shelter occupancy between a native freshwater crab and an invasive crayfish. *Aquatic Ecology* 49: 273–278, <https://doi.org/10.1007/s10452-015-9522-7>
- Schrimpf A, Schmidt T, Schulz R (2014) Invasive Chinese mitten crab (*Eriocheir sinensis*) transmits crayfish plague pathogen (*Aphanomyces astaci*). *Aquatic Invasions* 9: 203–209, <https://doi.org/10.3391/ai.2014.9.2.09>
- Sharma A, Tewari R, Soni SK (2015) Application of Statistical Approach for Optimizing CMCase Production by *Bacillus tequilensis* S28 Strain via Submerged Fermentation Using Wheat Bran as Carbon Source. *International Scholarly and Scientific Research & Innovation* 9(1): 76–86
- Škraba D, Tošić AD, Miličić D, Nikolić V, Simonović P (2013) Invasiveness assessment of Chinese mitten crab *Eriocheir sinensis* (H. Milne Edwards, 1853) in the Serian section of the River Danube. *Archives of Biological Sciences* 65: 353–358, <https://doi.org/10.2298/ABS1301353S>
- Smith AL, Hamilton KM, Hirschle L, Wootton EC, Vogan CL, Pope EC, Eastwood DC, Rowley AF (2012) Characterization and Molecular Epidemiology of a Fungal Infection of Edible Crabs (*Cancer pagurus*) and Interaction of the Fungus with the Dinoflagellate Parasite *Hematodinium*. *Applied and Environmental Microbiology* 79: 783–793, <https://doi.org/10.1128/AEM.02945-12>
- Stahl MO, Tarek MH, Yeo DCJ, Badruzzaman ABM, Harvey CF (2014) Crab burrows as conduits for groundwater-surface water exchange in Bangladesh. *Geophysical Research Letters* 41: 8342–8347, <https://doi.org/10.1002/2014GL061626>
- Stepanowska K, Nędzarek A, Tórz A (2009) An assessment of the water quality of Lake Dąbie and the nutritive value of the Dąbie bream (*Abramis brama* L.). In: Gołdyn R, Klimaszuk P, Kuczyńska-Kippen N, Piotrowicz R (eds), The functioning and protection of water ecosystems. Wydawnictwo Naukowe Uniwersytetu im. Adama Mickiewicza 95–101
- Svoboda J, Mrugała A, Kozubíková-Balcarová E, Kouba A, Diéguez-Uribeondo J, Petrušek A (2014) Resistance to the crayfish plague pathogen, *Aphanomyces astaci*, in two freshwater shrimps. *Journal of Invertebrate Pathology* 121: 97–104, <https://doi.org/10.1016/j.jip.2014.07.004>
- Trivhkova T, Kutsarov Y, Todorov M, Puky M, Hubenov Z (2017) The Chinese Mitten Crab *Eriocheir sinensis* H. Milne Edwards, 1853 (Crustacea: Decapoda: Varunidae), a New Invasive Alien Species to the Bulgarian Fauna. *Acta Zoologica Bulgarica* Suppl. 9: 149–154
- Tiwari S, Shukla N, Mishra P, Gaur R (2014) Enhanced Production and Characterization of a Solvent Stable Amylase from Solvent Tolerant *Bacillus tequilensis* RG-01: Thermostable and Surfactant Resistant. *The Scientific World Journal* 2014: 972763, <https://doi.org/10.1155/2014/972763>
- Veilleux E, de Lafontaine Y (2007) Biological synopsis of the Chinese mitten crab (*Eriocheir sinensis*). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2812: vi + 45
- Wang W (2011) Bacterial diseases of crabs: a review. *Journal of Invertebrate Pathology* 106: 18–26, <https://doi.org/10.1016/j.jip.2010.09.018>
- Wang W, Gasparich EG, Wu W, Bi K, Ou J, Meng Q, Liang T, Feng Qi, Zhang J, Zhang Y (2011) *Spiroplasma eriocheiris* sp. nov., a novel species associated with mortalities in *Eriocheir sinensis*, Chinese mitten crab. *International Journal of Systematic and Evolutionary Microbiology* 61: 703–708, <https://doi.org/10.1099/ijs.0.020529-0>
- Wiśniewski B, Wolski T, Buchholz W, Kreft A (2007) Bilanse wód Jeziora Dąbie - THE Water balance of lake Dąbie. *Polska Akademia Nauk. Infrastruktura i Ekologia Terenów Wiejskich. Infrastructure and Ecology of Rural Areas* 4(2): 211–221
- Yeo DCJ, Klaus S (2014) Preface - freshwater decapod biology in the 21st century. In: Yeo DCJ, Cumberlidge N, Klaus S (eds), Advances in Freshwater Decapod Systematics and Biology. *Crustaceana Monographs* 19: 1–6, https://doi.org/10.1163/9789004207615_002
- Zeng Y, Yeo DCJ (2018) Assessing the aggregated risk of invasive crayfish and climate change to freshwater crabs: A Southeast Asian case study. *Biological Conservation* 223: 58–67, <https://doi.org/10.1016/j.biocon.2018.04.033>