Data in Brief 49 (2023) 109308

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Database on eukaryotic symbionts of native and invasive gammarids (Crustacea, Amphipoda) in the Baltic region of Poland with information on water parameters for sampling sites

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ARTICLE INFO

Article history:
Received 19 April 2023
Revised 25 April 2023
Accepted 5 June 2023
Available online 9 June 2023

Keywords:
Pomeranian region
Gammarus pulex
Gammarus zaddachi
Gammarus roeselii
Gammarus tigrinus
Dikerogammarus villosus
Pontogammarus robustoides
Echinogammarus ischnus

ABSTRACT

This dataset documents the diversity of eukaryotic endo- and epibiotic organisms from 612 host individuals of seven gammarid (Amphipoda) species (*Gammarus pulex, Gammarus zaddachi, Gammarus roeselii, Gammarus tigrinus, Dikerogammarus villosus, Pontogammarus robustoides, Echinogammarus ischnus*) of native and invasive origin in the Baltic region of Poland. We identify 60 symbiotic species of nine phyla from 16 localities of freshwater and brackish habitats. Twenty-nine symbiotic species belonged to the Ciliophora, 12 to Apicomplexa, 8 to Microsporidia, 3 to Platyhelminthes, 2 to Acanthocephala, 2 to Nematoda, 2 to Rotifera, 1 to Choanozoa and 1 to Nematomorha. The material in this Data in Brief paper is composed of three Microsoft® Excel files. The first file represents the raw data on the number of individuals (infrapopulation size) of each eukaryotic symbiont taxa recorded in

DOI of original article: 10.1016/j.ijpara.2023.02.006
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https://doi.org/10.1016/j.dib.2023.109308

each host individual and location. The data set contains information on the assemblage of symbionts per host individual in one table-matrix; macro- (host) and symbiont taxa name, host length, the date of collection, the geographic coordinates and locality name in columns; and amphipod host specimens in lines. The second file reports the symbiont species list (the species breakdown by phyla in spreadsheets) with information on host species, sampling date, locality and geographic coordinates, infection site, obtained sequences (if the case), brief morphological characteristics and microphotographs. The third file reports measured water parameters, habitat features and host density per sample. We generate the present dataset to evaluate the richness, diversity, population and community features of symbiotic organisms in native and invasive gammarid hosts in Poland.

Biological sciences: ParasitologyEnvironmental Science: Ecology; Hydrology and Water Quality

Specifications Table

Subject	1. Biological sciences: Parasitology 2. Environmental Science: Ecology; Hydrology and Water Quality
Specific subject area	Ciliophora, Apicomplexa, Microsporidia, Platyhelminthes, Acanthocephala, Nematoda, Rotifera, Choanozoa, Nematomorha; nitrogen, phosphorous and their derivatives.
Type of data	EXCEL files (.xlsx)
How the data were acquired	Hand net samples of gammarid hosts were taken in the field with subsequent laboratory surveys on symbiotic eucaryotic organisms. Amphipods were dissected under a stereomicroscope on an object glass. Each anatomical unit (body surface with muscles, intestine, body cavity organs, gills) was prepared and examined separately under a conventional light microscope. Symbionts were counted, registered and microphotographed. We identified symbiotic taxa based on morphologic and molecular data. The community richness and abundance of each symbiotic species per host individual were typed in the table. The water parameters were measured by using spectrophotometry, pH and conductometry and oxidation methods.
Data format	Raw
Description of data collection	We examined 612 host individuals of 7 species, including 2 native and 5 invasive gammarid species. Amphipods were collected from 16 sampling localities of the Baltic region of Poland in the cold season from October 2020 to April 2021. Crustaceans were transported to the laboratory alive and maintained in aerated aquaria under low temperatures (5–7 °C) before the examination. We surveyed the gammarids for symbiotic organisms as soon as they were sampled, usually within one working week after field collection. We performed measurements of water parameters in the field and laboratory settings.
Data source location	Poland, Pomerania, the Baltic Sea basin. Sixteen sample locations cover a range of rivers, streams, deltas and canals between the Oder and Vistula Rivers: 1) WS, Wisla Sobieszewska (54.314119, 18.931805); (2) PL, Port Lodolamaczy (54.308968,18.925215); (3) MW, Martwa Wisla (54.310727, 18.867225); (4) DP, Dębki Piasnicy (54.832288, 18.061855); (5) SL, Smoldzino Lupawa (54.662085, 17.212266); (6) RC, Rowy Canal (54.667288, 17.056982); (7) O, Orzechowa

(*continued on next page*)

	(54.598841, 16.918841); (8) SW, Wodnica Slupia (54.556568,16.875233); (9) SS, Słupsk Stream (54.475260, 17.042841); (10) LD, Lesny Dwor (54.358391, 17.155713); (11) KS, Krępa Słupska (54.403371, 17.047010); (12) DW, Darlowko Wieprza (54.433499, 16.389061); (13) JN, Jamiensky Nurt (54.281658, 16.135581); (14) DR, Dźwirzyno Regoujście (54.153051, 15.390534); (15) MP, Mrzeżyno Pera (54.140988, 15.284668); (16) WD, Wolin Dziwna (53.840308, 14.621854)
Data accessibility	Repository name: Mendeley Data identification number: DOI: 10.17632/c9fzyyzr52.1 Direct URL to data: https://data.mendeley.com/datasets/c9fzyyzr52 Google Maps information https://www.google.com/maps/d/viewer?mid=151D8nLF2GtY5Bj4SucBtqzqZ44GRxQU&usp=sharing
Related research article	V. Sarabeev, J.A. Balbuena, A. Jarosiewicz, N. Voronova, R.A. Sueiro, J.M. Leiro, M. Ovcharenko, Disentangling the determinants of symbiotic species richness in native and invasive gammarids (Crustacea, Amphipoda) of the Baltic region, Int. J. Parasitol. (2023) In press. https://doi.org/10.1016/j.ijpara.2023.02.006.

Value of the Data

- The dataset is the first report of a large variety of symbiotic organisms from native and invasive gammarid hosts in the Pomeranian region of Poland. These data are essential for testing ecological hypotheses on biological invasions and further phylogenetic and biogeographical studies; will assist to compare population and community patterns of symbionts from native and invasive hosts, different habitats and environmental conditions.
- Our database will be useful for parasitologits, general biologists, biogeographers and ecologists; as well as stakeholders who seek to protect the environment.
- These data could be used for evaluating the effect of biological invasions on the environment, predicting future scenarios of invasion (e.g. identifying species which are more likely to become invasive in the future) and assessing further environmental changes on symbiotic communities. Accumulated qualitative and quantitative data at the level of parasitism will aid to track host-parasite interactions change in the further invasion process.
- The database provides baseline data to use the recently proposed approach based on macroecological models [1] to assess the host-parasite relationships in invasive hosts and evaluate the repeatability of the yearly revealed patterns.

1. Objective

We generated the database in the framework of the project “Evaluating the effect of parasites and local community diversity on invasive gammarid species (Amphipoda) in the Pomeranian region of Poland”. The objective was to evaluate the role of parasites in maintaining ecosystem diversity and resilience, as effective regulators of invasive species. Our first papers prepared in the framework of this project provide a taxonomic overview of the revealed symbiotic species gammarid hosts and analyse their variability across different habitats and localities to shed light on the key factors determining the richness of microorganisms in native and invasive host species [2–4].

2. Data Description

The material in this Data in Brief paper is composed of three Microsoft® Excel files stored on the Mendeley Data repository [5].

File 1 contains one table-matrix with the raw data on the number of individuals (infrapopulation size) of eukaryotic symbiont taxa recorded in each host. The surveyed host species and

individuals are in rows, while the revealed symbiotic species are in columns. Host individuals are grouped by species, while symbiotic organisms are by phyla. We also provide sampling date, locality name and geographic coordinates, sample and host individual codes for each host individual.

File 2 comprises a symbiotic species list grouped by phyla in 9 tables. We supplied each symbiotic species with information on host species, sampling date, locality and geographic coordinates, infection site, obtained sequences (if the case), brief morphological characteristics and microphotographs, as well as used references for identification.

File 3 reports measured water parameters (temperature, pH, conductivity, concentration of chlorophyll-a (Cla), nitrogen (total nitrogen (N-tot), organic nitrogen (N-org), nitrates (N–NO3) and ammonium nitrogen (N–NH4)) and phosphorus (total phosphorus (P-tot), organic phosphorus (P-org) and mineral phosphorus (P-PO4)), habitat features (brackish or freshwater) and host density per sample.

3. Experimental Design, Materials and Methods

3.1. Material collection and processing

We sampled 7 gammarid species (Crustacea, Amphipoda), including 2 natives (*Gammarus pulex* (L.), *Gammarus zaddachi* Sexton, 1912) and 5 invasives (*Gammarus roeselii* (Gervais, 1835), *Gammarus tigrinus* Sexton, 1939, *Dikerogammarus villosus* (Sovinsky, 1894), *Pontogammarus robustoides* (G.O. Sars, 1894), *Echinogammarus ischnus* (Stebbing, 1899)), from 16 localities in the Pomeranian region of Poland [6]. The area of study covers a range of rivers, streams, deltas and canals between the Oder and Vistula Rivers. Amphipods were collected using a hand net in the cold season from October 2020 to April 2021, transferred to the laboratory and kept alive in aerated containers under low temperatures (5–7 °C) until they were examined for parasitic and symbiotic organisms. We complete host surveys within several days of capture, but not longer than one working week for the full sample.

Gammarid individuals were identified according to Eggers and Martens [7] and Dobson [8], measured (from the head to the third uropod), decapitated and dissected under a stereo microscope (SMZ-161 with digital camera Moticam BTU) on an object glass with distilled water. Each anatomical unit (body surface with muscles, intestine, hindgut, body cavity organs and gills) was prepared and examined separately under a compound microscope (Delta Optical Evolution 300). We surveyed 612 individuals of gammarids on epibiotic and endobiotic organisms. They were counted, registered and microphotographed using a digital camera Optikam B3.

Selected individuals of symbionts were isolated, fixed and prepared for morphological study according to their taxonomic group (staining, clearing, mounting, etc.), following well-established protocols [9–11]. All remains of each host individual were carefully transferred to tubes and fixed in 100% alcohol for subsequent molecular analysis. Parasitic microsporidians and digeneans were identified both morphologically and molecularly (via a partial sequence of the SSU rRNA gene), while all other symbiotic groups were defined only based on morphology. Taxonomic identification was attempted to the lowest possible level. Our database covers only symbiotic species belonging to metazoan parasites (Platyhelminthes, Nematoda, Nematomorha, Acanthocephala and Rotifera), protozoans (Ciliophora and Apicomplexa (Eugregarina)), fungi and fungi-like organisms (Microsporidia and Choanozoa, respectively).

3.2. Molecular identification

After visual identification of the microsporidian infection, alcohol-stored host individuals were dried with the subsequent mill in lysis buffer [12]. The gammarids were milled with glass balls (diameter 425–600 µm) using Retsch Mixer Mills (MM 400) and the maximal frequency of

30 Hz for 15 min and cooling tubes on the ice every 30 s of the mill. The extraction of DNA was performed following Leiro et al. [12] using lysis buffer and solution, phenol-chloroform-isoamylic alcohol extraction and ethanol precipitation. Microsporidians were detected using universal microsporidian primers, forward (V1f 5′-CACCAGGTTGATTCTGCCTGAC-3) and reverse (1492r 5′-GGTTACCTTGTTACGACTT-3′ or 1342R 5′-ACGGGCGGTGTGTACAAAGAACAG-3′) targeting the small rRNA subunit [13,14]. Digenean metacercariae were prepared for molecular identification as described by Sarabeev et al. (2022b). Briefly, a quick alkaline lysis protocol was used to extract DNA from excysted individuals of metacercaria [15,16]. The PCR was performed using universal eukaryotic primers F-566:5′- CAG CCG CGG TAA TTC C-3′ and R-1200:5′-CCC GTG TTG AGT CAA ATT AAG C-3′ to amplify V4 and V5 variable regions of SSU gene [17]. The PCR mixtures (25 µL) contained reaction buffer, 0.2 mM of each deoxynucleoside triphosphate (Nzytech, Portugal), 0.4 µM of each primer; 0.4 units of high fidelity NZYProof DNA polymerase (Nzytech) and genomic DNA. The reactions were run in an automatic thermocycler (T100^{TM} Thermal Cycler, BioRad, USA) as follows: initial denaturing at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, annealing at 57 °C or 62 °C (for digenea or microsporidia, respectively) for 45 s, and 72 °C for 1 min; and finally, a 7 min extension phase at 72 °C. We confirm the PCR products on 1.5% agarose gel to verify the presence of correct size bands under a variable-intensity 312 nm ultraviolet transilluminator (Spectroline, USA). The PCR product was sequenced in complementary directions using Sanger sequencing service (Eurofins Genomics, Germany). Forward and reverse sequences were assembled and visualised using MEGA-11 [18]. We compared the obtained sequences with GenBank entries by using BLAST platform [19] to establish their taxonomic position. The raw sequence data reported in this paper have been deposited in GenBank. The sequences accession numbers and morphological data for each species identified are compiled in File 2.

3.3. Water parameters estimation

The field observation also included measurements of water temperature, pH (CX-315, Elmetron) and conductivity (CC-401, Elmetron). A water sample from each locality was analysed in the laboratory settings of the Pomeranian academy in Slupsk with the aid of standard analytical procedures for the following parameters: the concentration of chlorophyll-a (Cla), nitrogen (total nitrogen (N-tot), nitrates (N–NO3) and ammonium nitrogen (N–NH4)) and phosphorus (total phosphorus (P-tot) and mineral phosphorus (P-PO4)). We determined spectrophotometrically CLa in cold water with 90% acetone [20]. N-tot and P-tot were determined after oxidation to nitrates and phosphates, respectively, by autoclaving the unfiltered water sample with a potassium persulfate solution, according to respective colourimetric methods. P-PO4 was measured spectrophotometrically (SP-830 plus Metertech) using the ascorbic acid method (890 nm). N–NO3 was determined at 410 nm, after a reaction with sodium salicylate. N–NH4 was measured at 690 nm, using the ammonium test spectroquant (Merck). We estimated the concentration of organic nitrogen (N-org) and organic phosphorus (P-org) as the difference between total nitrogen or phosphorus, and the sum of inorganic nitrogen (N-min = N–NO3 + N–NH4) or phosphates, respectively.

Ethics Statements

The present work meets the publisher ethical requirements (https://www.elsevier.com/authors/journal-authors/policies-and-ethics). The authors declare that the present work did not include experiments on human subjects and/or animals.

CRediT Author Statement

Volodimir Sarabeev: Field sampling, Morphological identification of symbiotic organisms, Data curation, Writing – original draft preparation; **Mykola Ovcharenko:** Supervision, Field sampling, Identification of Microsporidia, Editing; **Anna Jarosiewicz:** Water parameters estimation; **Abdulmalik Ahmed:** DNA extraction; **Rosa Ana Sueiro:** DNA amplification; **Jose Manuel Leiro:** BLASTing sequences.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The work was funded by the Polish National Agency for Academic Exchange (grant number PPN/ULM/2019/1/00177/U/00001). VS is partially funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V01-00017.

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