# SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

**Dr. Monika Mioduchowska**
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# Self-presentation

**1.** Name: **Monika Mioduchowska**

**2.** Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation.

**07.07.2009:** Master's degree in Biology, with specialization in Molecular Biology, obtained at the Faculty of Biology, University of Gdańsk. Title of the master's thesis: "Genetic diversity of Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum,1792)) from the western region of the Barents Sea"; supervisor: Dr. Barbara Wojtasik.

**09.12.2016:** Academic degree of Doctor of Biological Sciences in the field of biology, obtained at the Faculty of Biology, University of Gdańsk. Title of the doctoral dissertation: "Genetic structure of the endangered species, thick-shelled river mussel *Unio crassus* (Philipson, 1788), in Polish rivers"; supervisor: Dr. hab. Jerzy Sell, Prof. UG.

**3.** Information on employment in research institutes or faculties/departments or school of arts.

**10.2014–02.2017:** Assistant (research and teaching staff) at the Department of Genetics and Biosystematics, Faculty of Biology, University of Gdańsk (from December 2014 to May 2015 – medical leave).

**02.2017–until now:** Assistant professor (research and teaching staff) at the Department of Evolutionary Genetics and Biosystematics, Faculty of Biology, University of Gdańsk.

**08.2017–09.2018:** Specialist at the Department of Genetics and Biosystematics, Faculty of Biology, University of Gdańsk.

**10.2018:** Senior specialist at the Department of Genetics and Biosystematics, Faculty of Biology, University of Gdańsk.

**10.2019–09.2021:** Postdoctoral fellowship as a research assistant (post-doc position) at the Department of Marine Plankton Research, Institute of Oceanography, Faculty of Oceanography and Geography, University of Gdańsk, under of the OPUS project (funded by the National Science Center) led by Dr. hab. Agata Weydmann-Zwolicka, Prof. UG. Project title: "HIDEA – Hidden diversity of plankton in the European Arctic"; project number: UMO-2017/27/B/NZ8/01056.

**10.2021–06.2022:** Postdoctoral fellowship as a research assistant (post-doc position) at the Department of Invertebrate Zoology and Hydrobiology, Faculty of Biology and Environmental Protection, University of Łódź, under the OPUS project (funded by the National Science Center) led by Prof. Dr. hab. Magdalena Błażewicz. Project title: "Biodiversity Patterns and Scale: the case of Peracarid Crustacea from south-eastern Australia BIOPASS"; project number: UMO-2018/31/B/NZ8/03198.

**4.** Discussion of the achievements, set out in art. 219 para 1 point 2 of the Act of 20 July 2018 on Higher Education and Science (Journal of Laws of 2021, item 478, as amended), should encompass a substantive overview of the achievements and precisely determine the individual contribution to their creation, particularly in cases where a given achievement is a result of collaborative work, while considering the opportunity to indicate the professional career's entire body of work.

**a)** Title of Scientific Achievement :

**Molecular identification of (endo)symbiotic bacteria associated with freshwater Bivalvia, Crustacea and Eutardigrada, with a particular focus on bacteria from the genus *Wolbachia***

**b)** List of original publications constituting a scientific achievement.

The scientific achievement consists of a series of seven publications (original works) published between 2018 and 2023 in journals included in the Journal Citation Reports database.

Impact Factor (IF) are provided based on the year of publication of each work. Points of the Ministry of Science and Higher Education (MNiSW) / the Ministry of Education and Science (MEiN) are provided based on the year of publication and according to the latest list of scientific journals (as of July 17, 2023, https://www.gov.pl/web/edukacja-i-nauka/komunikat-ministra-edukacji-i-nauki-z-dnia-17-lipca-2023-r-w-sprawie-wykazu-czasopism-naukowych-i-recenzowanych-materialow-z-konferencji-miedzynarodowych).
The number of citations is according to: Web of Science Core Collection, Scopus oraz Google Scholar. In turn, quartiles are provided based on the Journal Citation Indicator (JCI) or Journal Impact Factor (JIF) Category.

The statements of the co-authors of all publications included in the habilitation thesis, specifying their individual contributions to each publication, are included in Appendix 5.

1) **Mioduchowska M.,** Czyż M.J., Gołdyn B., Kur J., Sell J. 2018a. Instances of erroneous DNA barcoding of metazoan invertebrates: are universal *cox1* gene primers too "universal"? *PLoS ONE*, 13(6): e0199609, DOI: 10.1371/journal.pone.0199609; $IF_{2018}$: 2.776; points of the $MNiSW_{2018}$: 40; points of the $MEiN_{2023}$: 140; number of citations: according to Web of Science Core Collection: 31, according to Scopus: 39, according to Google Scholar: 58; quartile (JCI Category): Q1.

   *My contribution to this publication included:*
   - participating in the development of research concepts;
   - performing all molecular laboratory work;
   - contributing to data analysis and interpretation: bioinformatics analysis and graphical visualization of the results;
   - reviewing and selecting relevant literature;
   - writing most of the text as the corresponding and lead author.

2) **Mioduchowska M.,** Czyż M.J., Gołdyn B., Kilikowska A., Namiotko T., Pinceel T., Łaciak M., Sell J. 2018b. Detection of bacterial endosymbionts in freshwater crustaceans: the applicability of non-degenerate primers to amplify the bacterial 16S rRNA gene. *PeerJ*, 6: e6039, DOI: 10.7717/peerj.6039; $IF_{2018}$: 2.486; points of the $MNiSW_{2018}$: 35; points of the

$MEiN_{2023}$: 100; number of citations: according to Web of Science Core Collection: 10, according to Scopus: 13, according to Google Scholar: 17; quartile (JCI Category): Q1.

*My contribution to this publication included:*

- development of research concepts;
- performing all molecular laboratory work;
- contributing to data analysis and interpretation: bioinformatics analysis and graphical visualization of the results;
- reviewing and selecting relevant literature;
- writing most of the text as the corresponding and lead author.

3) **Mioduchowska M.,** Zając K., Zając T., Sell J. 2020a. *Wolbachia* and *Cardinium* infection found in threatened unionid species: a new concern for conservation of freshwater mussels? *Conservation Genetics*, 21: 381–386, DOI: 10.1007/s10592-020-01255-9; $IF_{2020}$: 2.260; points of the $MNiSW_{2020}$: 70; points of the $MEiN_{2023}$: 70; number of citations: according to Web of Science Core Collection: 6, according to Scopus: 6, according to Google Scholar: 9; quartile (JCI Category): Q2.

*My contribution to this publication included:*

- development of research concepts;
- performing all molecular laboratory work;
- contributing to data analysis and interpretation: bioinformatics analysis and graphical visualization of the results;
- reviewing and selecting relevant literature;
- writing most of the text as the corresponding and lead author.

4) **Mioduchowska M.,** Zając K., Bartoszek K., Madanecki P., Kur J., Zając T. 2020b. 16S rRNA-based metagenomic analysis of the gut microbial community associated with the DUI species *Unio crassus* (Bivalvia: Unionidae). *Journal of Zoological Systematics and Evolutionary Research*, 58(2): 615–623, DOI: 10.1111/JZS.12377; $IF_{2020}$: 2.047; points of the $MNiSW_{2020}$: 70; points of the $MEiN_{2023}$: 70; number of citations: wg Web of Science Core Collection: 6, wg Scopus: 8, wg Google Scholar: 10; quartile (JCI Category): Q1.

*My contribution to this publication included:*

- development of research concepts;
- performing all molecular laboratory work;
- contributing to data analysis and interpretation: bioinformatics analysis and graphical visualization of the results;
- reviewing and selection relevant literature;
- writing most of the text as the lead author.

**5)** Kaczmarek Ł., Roszkowska M., Poprawa I., Janelt K., Kmita H., Gawlak M., Fiałkowska E., **Mioduchowska M.** 2020. Integrative description of bisexual *Paramacrobiotus experimentalis* sp. nov. (Macrobiotidae) from republic of Madagascar (Africa) with microbiome analysis. *Molecular Phylogenetics and Evolution*, 145: 106730, DOI:10.1016/j.ympev.2019.106730; $IF_{2020}$: 3.889; points of the $MNiSW_{2020}$: 140; points of the $MEiN_{2023}$: 140; number of citations: according to Web of Science Core Collection: 16, according to Scopus: 22, according to Google Scholar: 34; quartile (JCI Category): Q1.

*My contribution to this publication included:*

- developing research concepts for the analysis of the Tardigrade microbiome;
- performing all molecular laboratory work related to the analysis of the Tardigrade microbiome and the molecular component of the integrative taxonomy of the newly described Tardigrade species in this study;
- conducting analysis and interpretation of Tardigrade microbiome data and the molecular component of integrative taxonomy: bioinformatics analysis and graphical visualization of the results;
- reviewing and selecting relevant literature in the area of microbiome analysis and integrative taxonomy;
- as the last author, I being responsible for all work related to the Tardigrade microbiome and the molecular component of integrative taxonomy, and therefore, writing all manuscript sections related to these topics.

**6)** **Mioduchowska M.,** Nitkiewicz B., Roszkowska M., Kačarević U., Madanecki P., Pinceel T., Namiotko T., Gołdyn B., Kaczmarek Ł. 2021. Taxonomic classification of the bacterial endosymbiont *Wolbachia* based on next-generation sequencing: is there molecular evidence

for its presence in tardigrades? *Genome*, 64(10): 951–958, DOI: 10.1139/gen-2020-0036; $IF_{2021}$: 2.449; points of the $MEiN_{2021}$: 70; points of the $MEiN_{2023}$: 70; number of citations: according to Web of Science Core Collection: 5, according to Scopus: 5, according to Google Scholar: 8; quartile (JCI Category): Q3.

*My contribution to this publication included:*

- development of research concepts;
- performing all molecular laboratory work;
- contributing to bioinformatics analysis - classifyng obtained amplicons into Operational Taxonomic Units (OTUs);
- performing data analysis and interpretation: phylogenetic analysis and graphical visualization of the results;
- reviewing and selecting relevant literature;
- as the primary author and corresponding author, I wrote most of the text.

7) **Mioduchowska M.,** Konecka E., Gołdyn B., Pinceel T., Brendonck L., Lukić D., Kaczmarek Ł., Namiotko T., Zając K., Zając T., Jastrzębski J.P., Bartoszek K. 2023. Playing peekaboo with a master manipulator: metagenetic detection and phylogenetic analysis of *Wolbachia* supergroups in freshwater invertebrates. *International Journal od Molecular Sciences*, 24, 9400, DOI: org/10.3390/ ijms24119400; $IF_{2023}$: 5.600; points of the $MEiN_{2023}$: 140; kwartyl (JCI Category): Q1 (no citations are provided due to the recent publication date, i.e., May 28, 2023).

*My contribution to this publication included:*

- development of research concepts;
- performing all molecular laboratory work;
- contributing to data analysis and interpretation: bioinformatics analysis and graphical visualization of the results;
- reviewing and selecting relevant literature;
- as the primary author and corresponding author, I wrote most of the text.

***In summary:***

The total Impact Factor (IF): **21,507**

The total MNiSW/MEiN points: **565** (based on the year of publication); **730** (according to the latest MEiN scoring, as of July 17, 2023)

Number of citations according to Web of Science Core Collection: **74**

Number of citations according to Scopus: **93**

Number of citations according to Google Scholar: **136**

Quartile according to JCI or JIF Category: **Q1** – five publications, **Q2** – one publication, **Q3** – one publication

***Sources of funding for the publications described above:***

- **Publication 1**: co-financing of the conducted research – own funds;
- **Publication 2**: obtaining funds for molecular research as part of the project of which I was the project leader from 2018 to 2019; project title: "Application of designed primers for metagenomic sequencing of the gene encoding 16S rRNA: a new tool for identifying endosymbiotic bacteria in freshwater crustaceans (Crustacea: Branchiopoda)"; funding source: University of Gdańsk, Young Scientists competition; project number: 538-L260-B149-18.
- **Publications 3 and 4**: obtaining funds for molecular research as part of the project of which I was as the project leader from 2018 to 2019; project title: "Molecular identification of endosymbiotic bacterial species in the endangered freshwater mussel *Unio crassus* (Philipsson 1788): metagenomic analysis"; funding source: National Science Centre, Miniatura 1 competition; project number: 2017/01/X/NZ8/01873;
- **Publication 5**: co-financing of the conducted research – own funds;
- **Publication 6**: co-financing of the conducted research – own funds and implementation of work during a postdoctoral fellowship: "Coevolution of the endangered fairy shrimp *Branchipus schaefferi* (Branchiopoda, Anostraca) and intracellular endosymbiont *Wolbachia* bacteria"; funding source: European Molecular Biology Organization through the EMBO Short-Term Fellowship competition for research conducted at the Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Belgium; project number: 7862; project supervisor (2019);
- **Publication 7**: co-financing the conducted research within the projects: **i)** "Let's dry up and survive together: is anhydrobiosis in water bears (Tardigrades) modulated by a

specific microbiome community and does it depend on bacteria that survive desiccation together with them?"; funding source: National Science Centre, Sonata 17 competition; project number: 2021/43/D/NZ8/00344; project leader (since 2022, project currently ongoing); **ii)** "Dry out and survive": is the extraordinary ability of tardigrades (Tardigrada) to undergo anhydrobiosis a result of the presence of a specific microbiome and endosymbiotic bacterial infections?"; funding source: University of Gdańsk, UGrants-first competition within the Small Grants Program; project number: 1220/146/2021; project leader (2021); **iii)** "Coevolution of the endangered fairy shrimp *Branchipus schaefferi* (Branchiopoda, Anostraca) and intracellular endosymbiont *Wolbachia* bacteria"; funding source: European Molecular Biology Organization through the EMBO Short-Term Fellowship competition for research conducted at the Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Belgium; project number: 7862; project leader (2019); **iv)** "Application of designed primers for metagenomic sequencing of the gene encoding 16S rRNA: a new tool for identifying endosymbiotic bacteria in freshwater crustaceans (Crustacea: Branchiopoda)"; funding source: University of Gdańsk, Young Scientists competition; project number: 538-L260-B149-18; project leader (2018-2019); **v)** "Molecular identification of endosymbiotic bacterial species in the endangered freshwater mussel *Unio crassus* (Philipsson, 1788): metagenomic analysis"; funding source: National Science Centre, Miniatura 1 competition; project number: 2017/01/X/NZ8/01873; project leader (2018-2019).

**c)** Discussion of the scientific objective of the above work and the results.

The introduction outlines the research problem, discussing the existing literature on the identification of (endo)symbiotic bacteria in freshwater Crustacea, Bivalvia, and Eutardigrada, available prior to the research (until 2017). The summary of the results also mentioned the studies that emerged during the course of the research, concerning the discussed scope of identification of (endo)symbiotic bacteria in freshwater invertebrates. The term "(endo)symbiont" refers to symbiotic bacteria for which the intracellular occurrence within their host is not certain, as opposed to the commonly described obligate endosymbiotic bacteria, such as those from the genus *Wolbachia* or *Cardinium*. Bibliographic data of the cited works are included in the subsection "References".

The habilitation achievement comprises a series of seven scientific articles published in internationally renowned journals. The main theme of this series is the molecular identification of (endo)symbiotic bacteria, with particular emphasis on bacteria of the genus *Wolbachia*, associated with freshwater invertebrates belonging to three phyla within three classes: Crustacea (Arthropoda), Bivalvia (Mollusca), and Eutardigrada (Tardigrada).

***Outline of the research problem and scientific objective of the studies***

Symbiosis encompasses a wide range of relationships between organisms, including mutualism, commensalism and parasitism (Douglas, 1994). A broad analysis of symbiotic relationships revealed that the development of eukaryotic organisms originated from serial endosymbiosis, i.e., a form of intracellular symbiosis (Roger et al., 2017). It is now recognized that multicellular eukaryotic organisms engage in symbiotic relationships with various microorganisms, including Archaea, Bacteria, and Fungi (Oliver and Russell, 2016).

Symbiotic bacteria, both obligatory P-endosymbionts and facultative S-symbionts (Buchner, 1965), play a significant role in the biology of their hosts and influence microevolutionary processes (Toft and Andersson, 2010; Douglas, 2014). In terrestrial invertebrates, a wide range of symbiotic bacteria has been identified, and numerous obligatory associations of hosts and bacteria have been described (Chaston and Goodrich-Blair, 2010). Bacteria of the genus *Wolbachia* (belonging to the phylum α-Proteobacteria) are one of the most widespread endosymbiotic bacteria identified in terrestrial invertebrates, particularly in insects (Insecta). According to a meta-analysis conducted by Hilgenboecker et al. (2008), up to 66% of all terrestrial arthropod species (Arthropoda) are infected with *Wolbachia*. These bacteria are transmitted vertically (in the cytoplasm of infected female's eggs) and/or horizontally (via food). This endosymbiont plays a crucial role in evolutionary processes, shaping the sex ratio and reproduction of its hosts (Funkhouser and Bordenstein, 2013). *Wolbachia* infection can induce: i) cytoplasmic incompatibility (CI), i.e., inducing the death of embryos resulting from the fertilization of eggs by sperm from an infected male and an uninfected female's egg or infection of both sexes with different strains of this endosymbiont; ii) alter the population's sex structure by increasing the proportion of females at the expense of males through: inducing genetic male death (male-killing) or causing feminization (genetic males transforming into females); inducing parthenogenesis, i.e., development from unfertilized eggs (Charlat et al., 2003). Furthermore, bacteria from the genus *Wolbachia* can exert a beneficial effect on the functioning and lifespan of their hosts. They may also be essential in the process of embryogenesis and subsequent development of certain invertebrate species (Nikoh et al., 2014).

Similar to *Wolbachia*, the endosymbiotic bacteria from the genus *Cardinium* also influence the reproduction of their hosts through the induction of cytoplasmic incompatibility (CI) and causing sex ratio bias in favor of the offspring of infected females (Zhang et al., 2012). Initially, the impact of *Cardinium* on its host was considered to be harmful. However, there is increasing evidence indicating that the host also benefits from this symbiosis (Zug and Hammerstein, 2018). In 2008, it was reported that approximately 6-7% of all Arthropoda

species were infected with *Cardinium* (Duron et al., 2008). Nevertheless, further research on the identification of infections caused by this intracellular bacterium suggests that infections may be more widespread than previously anticipated (Dallai et al., 2011).

Despite the significant impact of symbiotic bacteria on the ecology and evolution of their hosts (Dattagupta et al., 2009), the presence of symbiotic bacteria in freshwater invertebrates remains poorly studied. Only isolated cases of vertically transmitted endosymbiotic bacterial infections to offspring have been documented (Perkins et al., 2005). Numerous hypotheses have been proposed regarding potential *Wolbachia* infections in freshwater invertebrates, which could result in endosymbiont-mediated manipulation of reproductive systems (e.g., Maniatsi et al., 2010). Furthermore, the possibility of *Wolbachia* transmission between phylogenetically close or distant hosts, as well as the potential for direct environmental infection (via food), is a premise for the existence of infections with this endosymbiont in freshwater invertebrates (Zhao et al., 2021).

i) Identification of *Wolbachia* in freshwater crustaceans (Crustacea):

Freshwater invertebrates inhabiting astatic water bodies show a high degree of specialization and adaptation to conditions of periodic habitat desiccation (Brendonck et al., 2017). This is due to their ability to enter cryptobiosis, form resistant cysts (Williams, 2006), short life cycles, and reproductive strategies that largely rely on parthenogenesis (Ford and Weeks, 2018). Despite being important and endangered components of astatic water bodies, the so-called “large branchiopods”, comprising taxa from the class Branchiopoda (Anostraca, Notostraca, Laevicaudata, Spinicaudata, and Cyclestherida), remain the least studied group of relatively large freshwater invertebrates (Colburn, 2004).

To date, there is no direct evidence indicating that bacterial endosymbionts manipulate the reproduction of freshwater crustaceans inhabiting astatic water bodies. However, there are hypotheses suggesting that the observed sex ratio imbalance (with a bias towards females) and diverse reproductive strategies, such as parthenogenesis, androdioecy, as well as masculinization and feminization, could be the result of endosymbiotic bacterial infections (Kageyama et al., 2012). In 2017, Sazama and colleagues estimated that *Wolbachia* infections occur in as many as 52% of the studied aquatic insect species. In freshwater crustaceans, *Wolbachia* infections have been found in only one species of isopods (Isopoda) (Bouchon et al., 1998) and four species of copepods (Copepoda) (Wiwatanaratanabutr, 2013). In 2010, an attempt was made to identify *Wolbachia* bacteria in both parthenogenetic and sexually reproducing populations of brine shrimp (*Artemia*) from lentic water bodies. The aim of the

study was to investigate whether *Wolbachia* were responsible for inducing parthenogenesis in these crustaceans (Maniatsi et al., 2010). However, the use of Sanger sequencing targeting the 16S rRNA fragments, and low-quality PCR products resulted in obtaining amplicons belonging to other bacterial species. In addition, specific primers designed for *Wolbachia* supergroups identified in terrestrial invertebrates, in which (endo)symbiotic bacterial infections are commonly detected, were used to amplify the selected molecular marker (Werren et al., 2008). Therefore, one reason for the lack of *Wolbachia* detection could be the use of inadequate molecular tools, including specific primers.

ii) Identification of bacteria belonging to the genus *Wolbachia* in freshwater mussels (Bivalvia)

Freshwater mussels represent a group of slowly evolving benthic organisms that are highly sensitive to changes in water chemistry, and thus serve as water purity indicators. As biofilters, they play a crucial role in water self-purification. Unfortunately, freshwater mussels, especially those from the family Unionidae, are currently among the most threatened aquatic invertebrates worldwide (Bogan, 2008). Separate sexes are observed in the life cycle of Unionidae, with a parasitic larval stage (glochidium) that colonizes specific species of fish (Wächtler et al., 2001).

For freshwater invertebrates, particularly mollusks (Mollusca), which represent the second-largest animal phylum in terms of species diversity, intracellular symbiosis is considered a rare phenomenon (Distel et al., 2011). In 1998, Schilthuizen and Gittenberger attempted to identify bacteria belonging to the genus *Wolbachia* in 38 species of Mollusca, including 24 species of land snails (Gastropoda), 11 species of freshwater snails (Gastropoda), and three species of freshwater mussels. The identification of *Wolbachia* bacteria was based on the *ftsZ* gene sequence, which encodes a cell division protein of the microorganism, using PCR techniques with specific primers. However, no *Wolbachia* infections were detected in the examined mollusks. Consequently, the study has been cited by other authors suggesting that *Wolbachia* bacteria are absent in mollusks (Lis et al., 2015) or emphasizing the need for further research on endosymbiont infection in this systematic group (Correa et al., 2016). Therefore, the question of the presence of *Wolbachia* and other endosymbiotic bacteria in mollusks remains unanswered even after 25 years. Hence, it is justified to undertake research aimed at comprehensive identification of bacterial endosymbionts in mollusks.

In 2016, Whelan and Strong pointed out that high mitochondrial heterogeneity affected the polyphyly of the studied Gastropoda belonging to the family Pleuroceridae, specifically

regarding mitochondrial DNA (mtDNA) markers. This phenomenon was particularly noticeable in mussels with doubly uniparental inheritance (DUI) of mtDNA. In addition, female-biased sex ratio was also observed within the family Pleuroceridae (Ciparis et al., 2012). Moreover, the observed incongruence between taxonomic classification based on morphological features and mtDNA markers resembled the linkage disequilibrium patterns seen in terrestrial invertebrates infected with *Wolbachia*. Considering the identification of bacteria belonging to the genus *Neorickettsia* (a bacteria related to *Wolbachia*) in freshwater snails of the families Pleuroceridae and Semisulcospiridae, the presence of such endosymbiont infections seems highly probable (Fredricks, 2006). Additionally, in marine mussels inhabiting extreme environments near hydrothermal vents, the presence of chemosynthetic symbiotic bacteria has been documented (Ikuta et al., 2016), along with vertically transmitted potential endosymbionts in the offspring of mussels from the family Vesicomyidae (Cary and Giovannoni, 1993).

iii) Identification of *Wolbachia* in tardigrades (Tardigrada)

Tardigrades possess a remarkable ability to survive in extreme environmental conditions due to their capability to enter a state of cryptobiosis. One form of cryptobiosis is anhydrobiosis, a state of desiccation in which tardigrades lose more than 90% of their water and are able to survive for several years (Bertolani et al., 2004; Guidetti et al., 2012). This unique adaptation makes tardigrades the most resilient invertebrates on Earth (Erdman and Kaczmarek, 2016). Depending on the species, tardigrades possess diverse reproductive systems, including separate sexes and parthenogenesis, although detailed reproductive mechanisms remain unknown for many species of this taxon (Altiero et al., 2019).

Despite their extraordinary cryptobiotic abilities, tardigrades remain one of the least understood group of animals. Until the start of my research in 2017, no targeted studies had been conducted on the identification of (endo)symbiotic bacteria in tardigrades. Only a few works mentioned tardigrade microbiomes as a marginal ecological aspect (Vecchi et al., 2016). Furthermore, another experimental study established a connection between tardigrades and the phytopathogenic bacterium *Xanthomonas* sp., as well as the human gut bacterium *Serratia marcescens* Bizio, 1823 (Krantz et al., 1999), so-called “bloody polenta”.

Previous difficulties in molecular identification of endosymbionts, particularly *Wolbachia* bacteria, in freshwater invertebrates can be attributed to:

• the use of nonspecific primers designed to identify endosymbionts in terrestrial arthropods (Marubayashi et al., 2014);

• variation in infection rates or prevalence among host species, where different strains of *Wolbachia* may infect only a small portion of rare host populations (Lorenzo-Carballa et al., 2019);

• insufficient taxonomic information on endosymbiotic bacteria, making their effective detection challenging (Flatau et al., 2021). This limitation particularly affects the amplification of all bacterial species in a given sample, even when using universal primers. This particularly applies to endosymbionts present in trace amounts (Muyzer et al., 1996);

• inability to cultivate bacterial endosymbionts on traditional microbiological media (so-called "uncultured bacteria"), applying molecular biology techniques as the main methods of species profiling (Conceição et al., 2021);

• horizontal gene transfer from endosymbiotic bacteria to the host genome (Cordaux and Gilbert, 2017);

• co-infection of the same host with genetically distinct bacterial strains (Shapoval et al., 2021).

Due to the lack of suitable molecular and bioinformatic tools for the identification of *Wolbachia* and/or other (endo)symbiotic bacteria associated with freshwater invertebrates, the presence of these infections remained in the realm of unconfirmed hypotheses. Therefore, the aim of my research was to verify the hypothesis of infection with these (endo)symbionts in representatives of three systematic groups of freshwater invertebrates: Bivalvia, Crustacea and Eutardigrada. To accomplish this, novel molecular and bioinformatic tools were employed to gain deeper insights into these associations and provide further understanding of these relationships.

***Below, the works comprising the habilitation thesis are discussed.***

**Publication 1:**

**Mioduchowska M.**, Czyż M.J., Gołdyn B., Kur J., Sell J. 2018a. Instances of erroneous DNA barcoding of metazoan invertebrates: are universal *cox1* gene primers too "universal"? *PLoS ONE*, 13(6): e0199609, DOI: 10.1371/journal.pone.0199609

This study focuses on the identification of erroneous sequences of cytochrome oxidase subunit I (*cox1*; a molecular marker also known as COI) in aquatic invertebrates. The analyses included COI sequences deposited in the GenBank (a database distributed by the National Center for Biotechnology Information, NCBI) and BOLD Systems (Barcode of Life Database) reference databases. Misidentified COI sequences were found to be mainly derived from bacterial contamination, including symbiotic bacteria.

In 1994, Folmer et al. described "universal" primers, namely forward-LCO1490 and reverse-HCO2198 (commonly known as Folmer primers), which enabled the amplification of the COI marker in invertebrates. These primers have been widely used to amplify barcode sequences, primarily for molecular identification of organisms and phylogenetic analyses. However, the number of misidentified COI sequences deposited in genetic databases, amplified using the Folmer primers, is increasing. Consequently, some COI sequences described as invertebrate sequences belong in fact to bacteria, including those derived from symbiotic bacteria of the host. Additionally, contamination with sequences of fungi, other invertebrates, as well as nuclear mitochondrial DNA segments (NUMTs) have also been identified in these COI sequences.

In this study, Folmer primers were used to amplify COI sequences of the freshwater crustacean *Branchipus schaefferi* (Fischer, 1834) (Crustacea: Branchiopoda), inhabiting astatic water bodies in the Poznań-Biedrusko military training ground. Unexpectedly, bacterial sequences were also detected in addition to *B. schaefferi* sequences. The contamination was caused by *Aeromonas* sp., a bacterium described as a symbiont of the medicinal leech *Hirudo medicinalis* Linnaeus, 1758 (Annelida: Clitellata) (Graf, 2000), which is also pathogenic to fish (Gudmundsdottir, 1998) and exerts toxic effects on certain species of nematodes Nematoda (Cauillault and Ewbank, 2002).

Applying appropriate bioinformatic tools, other misidentified invertebrate sequences were subsequently determined to be of bacterial origin. Specifically, the COI sequences previously attributed to the marine species (Quoy & Gaimard, 1834) (Mollusca: Gastropoda)

(Nakano and Ozawa, 2005) and *Tetranchyroderma* sp. 3 (Gastrotricha: Macrodasyida) (Todaro et al., 2011) were identified as sequences of the symbiotic bacterium *Endozoicomonas* sp. (Ding et al., 2016). Bacteria belonging to the genus *Endozoicomonas* have a positive impact on the host's vitality (Ding et al., 2016) and are commonly found in marine invertebrates, including *Elysia ornata* (Swainson, 1840) (Mollusca: Gastropoda) (Kurahashi and Yokota, 2007) and *Atrina pectinata* (Linnaeus, 1767) (Mollusca: Bivalvia) (Hyun et al., 2014).

Based on the statistical calculations performed, it was determined that the degree of contamination varied depending on the specific taxonomic group. The least erroneous sequences were found in the Mollusca and Arthropoda groups, accounting for less than 1% of the analyzed sequences (out of 300 deposited sequences for Mollusca and 300 deposited sequences for Arthropoda). In contrast, erroneous sequences constituted even 6.9% in the case of Gastrotricha (all 363 deposited sequences were included in the analysis).

The work also discusses the causes of erroneously amplified sequences, including the level of conservation and specificity of Folmer primers, as well as primer annealing temperatures in the Polymerase Chain Reaction (PCR) reactions. In addition, the implication associated with depositing such sequences in reference databases are discussed. It has also been demonstrated that misidentified sequences in metabarcoding analyses, which utilize Next-Generation Sequencing (NGS) technology, can result in an overestimation of biodiversity through the incorrect classification of Operational Taxonomic Units (OTUs) (Staats et al., 2016).

**Publication 2:**

**Mioduchowska M.**, Czyż M.J., Gołdyn B., Kilikowska A., Namiotko T., Pinceel T., Łaciak M., Sell J. 2018b. Detection of bacterial endosymbionts in freshwater crustaceans: the applicability of non-degenerate primers to amplify the bacterial 16S rRNA gene. *PeerJ*, 6: e6039, DOI: 10.7717/peerj.6039

This publication describes the original in silico design of undegraded primers: WOLBSL 5′-GCTAGTTGGTGGAGTAATAGCC-3′ and WOLBSR 5′ GACTACCAGGGTATCTAATCCTG-3′ for the amplification of the bacterial 16S rRNA gene fragment. The nucleotide sequences obtained using Sanger sequencing enabled pioneering discoveries of (endo)symbiotic bacteria associated with aquatic crustaceans.

The specificity of the designed primers for amplifying the 16S rRNA gene fragment of (endo)symbiotic bacteria was tested on representatives belonging to the class Ostracoda and species classified as large branchiopods Branchiopoda.

Using the designed primers, the following (endo)symbionts were discovered in freshwater crustaceans Crustacea: in *B. schaefferi* (Branchiopoda; populations from Poland): *Candidatus* Gortzia, *Methylophilus* sp., *Spirobacillus* sp., *Undibacterium* sp., *Wolbachia*; in *Streptocephalus cafer* (Lovén, 1847) (Branchiopoda; population from South Africa): *Wolbachia*; in *Moina macrocopa* (Straus 1820) (Branchiopoda; population from Poland): *Methylophilus* sp.; in *Heterocypris incongruens* (Ramdohr, 1808) (Ostracoda; populations from Poland and Italy): *Cardinium*, *Methylophilus* sp. The presence of uncultured bacterium was also observed in *B. schaefferi* (Branchiopoda; populations from Poland) and *Branchipodopsis wolfi* Daday, 1910 (Branchiopoda; population from South Africa). The work provides detailed descriptions of all identified (endo)symbiotic bacteria.

The process of identifying these bacteria presents a significant challenge for researchers. Appropriate molecular tools, such as specific primers, are crucial for the successful amplification of sequences of a specific (endo)symbiont. In this study, the amplification efficiency of bacterial sequences using the newly designed primers was 100%. However, when multiple bacteria were present in a single sample, the sequences were not readable even with high-quality PCR products. Therefore, while the designed primers are suitable for the initial stage of identification of (endo)symbiotic bacteria, subsequent use of species-specific primers is recommended.

**Publication 3:**

**Mioduchowska M.,** Zając K., Zając T., Sell J. 2020a. *Wolbachia* and *Cardinium* infection found in threatened unionid species: a new concern for conservation of freshwater mussels? *Conservation Genetics*, 21: 381–386, DOI: 10.1007/s10592-020-01255-9

The following work focuses on the identification of endosymbiotic bacteria, such as *Wolbachia* and *Cardinium*, associated with the endangered freshwater mussel *Unio crassus* Philipsson, 1788 from Poland. Samples for the study were collected with the permission of the General Directorate for Environmental Protection.

In the study by Mioduchowska et al. (2016), we described a unique model of mitochondrial DNA inheritance known as DUI in *U. crassus*. As a result of DUI, both somatic and gonadal tissues contained two types of mtDNA: type F (maternally inherited) and type M

(paternally inherited), with a varying distribution of the M mitotype. The sequences of both mtDNA genomes were published in the work by Burzyński et al. (2017). For the present study, individuals were selected based on the observed heteroplasmy of the M and F mtDNA genomes in somatic tissue (foot), low genetic diversity (partial results described in Kilikowska et al., 2020), as well as an imbalanced sex ratio, with a predominance of females, which could indicate the potential impact of endosymbiotic bacterial infections. Two molecular analysis techniques were employed: i) Next-Generation Sequencing of the highly variable V3-V4 region of the bacterial 16S rRNA gene to gain comprehensive insight into the microbiome profile; ii) molecular confirmation of the infection by potential endosymbiotic bacteria and analysis of their distribution in male and female individuals of the studied mussel. This involved a series of amplifications using specific primers followed by Sanger sequencing.

The analysis of the *U. crassus* microbiome identified endosymbiotic bacteria belonging to the genus *Cardinium* and sequences associated with bacteria of the order Rickettsiales. However, no OTU, classified at a lower taxonomic level specifically to the genus *Wolbachia* (which belongs to the order Rickettsiales), was found. The use of specific primers to amplify *Cardinium* and *Wolbachia* genes (Simőes et al., 2011; Singh et al., 2013; Mains et al., 2016) ultimately allowed the identification of these endosymbionts in *U. crassus* females. The infection rate among females was found to be 33%. No co-infection of *Wolbachia* and *Cardinium* was observed in the infected females.

Thus far, the patterns of genetic diversity and phylogeography of most freshwater mussel species have not been well characterized, and their phylogenetic relationships remain uncertain. The discovery of *Wolbachia* and *Cardinium* endosymbionts in *U. crassus* opens up new opportunities for further research, particularly in elucidating the correlation between endosymbiont-host mechanisms and their impact on the biology and ecology of this endangered mussel. As a result of further investigations, including proper identification of management units (MUs) and evolutionary significant units (ESUs), it will be possible to implement appropriate conservation measures for this threatened species.

**Publication 4:**

**Mioduchowska M.,** Zając K., Bartoszek K., Madanecki P., Kur J., Zając T. 2020b. 16S rRNA-based metagenomic analysis of the gut microbial community associated with the DUI species *Unio crassus* (Bivalvia: Unionidae). *Journal of Zoological Systematics and Evolutionary Research*, 58(2): 615–623, DOI: 10.1111/JZS.12377

This work presents a profile of the hepato-pancreatic microbiome of the mussel *Unio crassus* in the context of the host evolutionary lineages, sex, and the occurrence of populations in different habitat conditions. The conducted research is a continuation of the identification of (endo)symbiotic bacteria in this mussel species.

In the present study, metagenomic analysis of the bacterial profile was conducted using NGS amplicon sequencing targeting the highly variable V3-V4 region of the 16S rRNA gene, allowing identification of the domains Bacteria and Archaea. The research involved *U. crassus* populations inhabiting different ecological habitats in northern, central, and southern Poland, which allowed to correlate microbiome profile composition with environmental selection (Lokmer and Wegner, 2015). Additionally, populations representing different phylogeographic lineages, including both males and females, were selected based on previous studies (Mioduchowska et al., 2016).

The comprehensive analysis of the microbiome profile of *U. crassus* identified several bacterial species with different distributions in terms of population and sex. A common microbiome, consisting of 69 bacterial operational taxonomic units (OTUs), which accounted for 23% of all identified OTUs, was identified in all individuals studied. The most abundant OTUs were represented by bacterial types such as Proteobacteria (currently Pseudomonadonta), Bacteroidetes (currently Bacteroidota), and Firmicutes (currently Bacillota).

With respect to (endo)symbiotic bacteria, the presence of cellulose-degrading bacteria was observed, including *Bacillus* sp. (Pinheiro et al., 2015), *Flavobacterium* sp. (Dar et al., 2015), *Pseudomonas* sp. (Watkins and Simkiss, 1990) and *Stenotrophomonas* sp. (Pinheiro et al., 2015). Bacteria of the genus *Chryseobacterium* were also identified in one of the populations, and they occurred only in single specimens in the remaining populations (Van Horn et al., 2011). Previous studies have also revealed the presence of a specialized bacterial microbiota capable of digesting cellulose in various mollusk species (Charrier and Rouland, 1992). Moreover, the study revealed the evolutionary potential of associations with chemosymbiotic bacteria, enabling mollusks to inhabit extreme ecosystems (Taylor and Glover, 2010) and acquire rare xylotrophic abilities among animals (Distel et al., 2011).

A pioneering discovery was made of an endosymbiotic bacterium belonging to the genus *Candidatus* Xiphinematobacter, known for inducing parthenogenesis in its hosts, with an unknown transmission mechanism (vertical and/or horizontal) (Vandekerckhove et al., 2000). The endosymbiont was found in only one of the studied populations, infecting both males and females. *Candidatus* Xiphinematobacter has been previously identified in Nematodes, where a

coevolution between the host and the endosymbiont has been demonstrated (Vandekerckhove et al., 2000).

In summary, this study presented the microbiome profile of *U. crassus*. The variation in the bacterial composition of the hepatopancreas microbiota was found to be more dependent on the phylogeographic relationships between the studied populations rather than their different habitat conditions. No significant differences in the gut microbiome composition were observed between males and females of this species. Symbiotic bacteria have been identified that enable cellulose digestion, which is particularly important for organisms whose diet relies on this polysaccharide. The presence of the discovered endosymbiont *C.* Xiphinematobacter did not show a correlation with the host's sex. However, considering the widespread distribution of cellulolytic symbiotic bacteria, it was hypothesized that they were specific for the studied mussel species. Both the presence of the endosymbiont *C.* Xiphinematobacter and cellulolytic bacteria could be the result of horizontal transfer associated with food intake.

**Publication 5:**

Kaczmarek Ł., Roszkowska M., Poprawa I., Janelt K., Kmita H., Gawlak M., Fiałkowska E., **Mioduchowska M.** 2020. Integrative description of bisexual *Paramacrobiotus experimentalis* sp. nov. (Macrobiotidae) from republic of Madagascar (Africa) with microbiome analysis. *Molecular Phylogenetics and Evolution*, 145: 106730, DOI:10.1016/j.ympev.2019.106730

This interdisciplinary publication involved, among other things, the identification of (endo)symbiotic bacteria associated with a newly discovered tardigrade species called *Paramacrobiotus experimentalis*.

The research employed integrative taxonomy (i.e., combining morphological and genetic analysis), to describe this new tardigrade species *Paramacrobiotus experimentalis* Kaczmarek, Mioduchowska, Poprawa & Roszkowska, 2020 (in Kaczmarek et al., 2020) found in Madagascar. Through morphological, structural, and molecular analyses, it was determined that *Pam. experimentalis* is a dioecious species.

The microbiome profiles of two *Pam. experimentalis* populations were also described in this publication. These populations were maintained in laboratory culture for two years, and microbiome profiling was based on Next-Generation Sequencing of a bacterial fragment of the 16S rRNA gene. It was found that the examined populations shared 31 of the 86 identified bacterial OTUs. In contrast, when comparing the microbiome of *Pam. experimentalis* with the microbiome of the laboratory culture medium in which the cultures were maintained, only 16

of the 137 identified bacterial OTUs were shared. This has confirmed that the microbiome of tardigrades significantly differs from the bacterial composition of their environment. Additionally, metabolic profiles revealed that the analyzed microbiomes consisted of bacteria involved mainly in membrane transport, amino acid metabolism, and carbohydrate metabolism.

The identification of OTUs classified as potential endosymbiotic bacteria belonging to the order Rickettsiales was consistent with previous observations made by Vecchi et al. (2018) and Mioduchowska et al. (2019a). These bacteria share the features of obligatory intracellular symbiosis and horizontal transmission (Gilbert et al., 2012), leading to the designation of the order Rickettsiales as "host-dependent". However, certain exceptions have been reported, where vertical transmission occurs, and thus, these bacteria are referred to as "non-host-restricted" (Dale and Moran, 2006). It should also be emphasized that our research was conducted on populations of *Pam. experimentalis* maintained under laboratory conditions for two years. Therefore, the bacteria observed in the tardigrade species, which were absent in the culture medium, suggest a close association with their host. Additionally, the transmission of these bacteria to subsequent generations most likely occurs in a vertical manner. The absence of Rickettsiales OTUs in the environment of the studied species provides evidence that these bacteria are strongly associated with their host. However, similar to Vecchi et al. (2018), we were unable to confidently identify any OTUs that could be classified at a lower taxonomic level, such as the genus *Wolbachia*.

Furthermore, this work also identified the presence of an OTU belonging to the endosymbiotic genus *Polynucleobacter*. These bacteria have been described, among others, as obligate endosymbionts of freshwater ciliates (Ciliata) (Hoetzinger et al., 2019). Interestingly, a significant frequency of this endosymbiont was identified in the culture medium containing rotifers (Rotifera), which served as food for *Pam. experimentalis*. Additionally, a trace number of sequences originating from this bacterium was also observed in one population of the studied tardigrade. This suggests a probable horizontal transfer of the endosymbiont, which is associated with Rotifera.

Ultimately, referring to our previous work describing new molecular and bioinformatic tools, their successful application for the identification of endosymbionts in novel hosts, particularly water invertebrates, was demonstrated (**Publication 2**; Mioduchowska et al., 2019b).

**Publication 6:**

**Mioduchowska M.,** Nitkiewicz B., Roszkowska M., Kačarević U., Madanecki P., Pinceel T., Namiotko T., Gołdyn B., Kaczmarek Ł. 2021. Taxonomic classification of the bacterial endosymbiont *Wolbachia* based on Next-Generation Sequencing: is there molecular evidence for its presence in tardigrades? *Genome*, 64(10): 951–958, DOI: 10.1139/gen-2020-0036

This work verifies the hypothesis of the presence of the intracellular symbiont from the genus *Wolbachia* in the microbiome of tardigrades (Tardigrada).

High-throughput sequencing of a fragment of the bacterial 16S rRNA gene allowed insight into the microbiome profile of the following species belonging to Eutardigrada: *Hypsibius exemplaris* Gąsiorek, Stec, Morek, and Michalczyk, 2018 from the United Kingdom; *Macrobiotus polypiformis* Roszkowska, Ostrowska, Stec, Janko, and Kaczmarek, 2017 from Ecuador; *Paramacrobiotus fairbanksi* Schill, Förster, Dandekar, and Wolf, 2010 from Antarctica (including egg analysis); as well as two new undescribed species of the genus *Paramacrobiotus* from Poland. The freshwater crustacean *Streptocephalus cafer*, in which I detected *Wolbachia* infection (**Publication 2**), served as a positive control in this study. The classification of OTUs was performed using two reference databases that were utilized in previous studies concerning tardigrade microbiome analysis: i) the Greengenes database, which we applied in **Publication 5**, and ii) the SILVA database used by Vecchi et al. (2018).

Conducted bioinformatic analyses of the obtained amplicons based on the two reference sequence databases revealed significant differences in the number and taxonomy of OTUs. Sequences clustering into *Wolbachia* OTUs were identified only when the Greengenes database was applied. To confirm the validity of the obtained sequences, statistical comparisons were performed using BLAST against the GenBank nucleotide database.

The use of the Greengenes database allowed the identification of *Wolbachia* OTUs in the microbiomes of the studied adult specimens of tardigrade species, namely *Paramacrobiotus* sp. and *Mac. polypiformis*. On the other hand, bacteria belonging to the order Rickettsiales were found in the eggs of the parthenogenetic species *Pam. fairbanksi*, as well as in the microbiomes of sexually reproducing adult *Mac. polypiformis* and two taxa of *Paramacrobiotus* sp. Different results were obtained when the SILVA database was employed, where only Rickettsiales OTUs were found in the microbiome of one taxon, i.e., *Paramacrobiotus* sp. In the positive control (*S. cafer*), sequences clustering into Rickettsiales OTUs were obtained using both reference databases. Notably, no Rickettsiales or *Wolbachia* OTUs were identified in *Hys. exemplaris*

and *Pam. fairbanksi* (adult specimens). Additionally, no other endosymbionts were detected in the analyzed samples.

Phylogenetic analyses of all obtained *Wolbachia* and Rickettsiales OTUs were also carried out using both reference databases. Phylogenetic relationships were analyzed using: i) all Rickettsiales OTUs described in previous studies, including Vecchi et al. (2018) and **Publication 5** of this summary; ii) OTUs of potential endosymbionts belonging to Alphaproteobacteria, as described by Guidetti et al. (2020); iii) orthologous sequences identified through comparisons in the GenBank database; iv) selected *Wolbachia* OTUs from the Greengenes and SILVA databases. The analyses provided evidence for the correct classification of OTUs into the genus *Wolbachia* and the order Rickettsiales, as all aligned sequences clustered in the appropriate clades on the phylogenetic tree.

Bacteria belonging to the genus *Wolbachia* have been observed in a small number of tardigrade sequences, which may explain why such infections have not been detected using Sanger sequencing methods (Mee et al., 2015). As suggested in **Publication 2**, methodological optimization, including the use of specific primers for the amplification of bacterial molecular markers, may provide a solution to this problem. Therefore, in the case of the positive control (*S. cafer* isolate), in which *Wolbachia* was identified, only OTUs classified at the level of the order Rickettsiales were obtained. Thus, it is likely that among the sequences described as Rickettsiales OTUs, there were sequences belonging to bacteria of the genus *Wolbachia*.

Our research highlights the importance of selecting an appropriate reference database for accurate identification of bacterial OTUs, and its impact on further interpretation of results. Taxonomic assignment of OTUs should be accompanied by phylogenetic analyses, which serve as validation of the clustering. The discovery of bacteria belonging to the genus *Wolbachia* and and order Rickettsiales infections in several tardigrade species may initiate new directions in tardigrades research, including investigations into host-endosymbiont coevolution.

**Publication 7:**

**Mioduchowska M.,** Konecka E., Gołdyn B., Pinceel T., Brendonck L., Lukić D., Kaczmarek Ł., Namiotko T., Zając K., Zając T., Jastrzębski J.P., Bartoszek K. 2023. Playing peekaboo with a master manipulator: metagenetic detection and phylogenetic analysis of *Wolbachia* supergroups in freshwater invertebrates. *International Journal od Molecular Sciences*, 24, 9400, DOI: org/10.3390/ ijms24119400

In this subsequent work, which is part of the habilitation achievement, a new metagenomic method was developed, enabling the identification of *Wolbachia* bacteria in freshwater invertebrates within the taxa Crustacea, Bivalvia, and Eutardigrada. The aforementioned publication series demonstrated that Sanger sequencing was not sufficient to detect co-infections with different strains of *Wolbachia* in a single host (**Publication 2**). However, Next-Generation Sequencing, using universal and commonly applied primers to amplify the highly variable V3-V4 region of the bacterial 16S rRNA gene, provided insights into the microbiome profile and identification of individual (endo)symbionts (**Publications 3, 4, 5, 6**).

This study further expanded the scope by including both previously unstudied species and species that were utilized in the earlier publications discussed as part of the habilitation series. These include Crustacea (**Publications 1 and 2**), Bivalvia (**Publications 3 and 4**), and Eutardigrada (**Publications 5 and 6**).

In this work, the identification of *Wolbachia* bacteria was performed using multiple approaches:

i) Comparison of the metagenomic results obtained using designed primers amplifying the V3-V4 region of the 16S rRNA gene (described in **Publication 2**, which allowed the identification of various (endo)symbionts associated with Crustacea by Sanger sequencing) and commonly used primers for amplification of the bacterial gene. Additionally, the results obtained using Sanger sequencing and primers specific to individual *Wolbachia* strains were compared. The designed primers showed specificity to *Wolbachia* sequences, unlike the typically applied primers in microbiome analyses. However, due to their lower specificity towards other bacteria, the designed primers were not suitable for a comprehensive analysis of microbiome composition. On the other hand, Sanger sequencing enabled only limited detection of *Wolbachia* infections.

ii) Development of new bioinformatics tools, including a newly written Python script, which enabled the identification of *Wolbachia* sequences (based on Hamming distance values) from the analyzed microbiomes composed of bacterial V3-V4 16S rRNA amplicons.

iii) Phylogenetic analyses classified the obtained *Wolbachia* sequences into three supergroups: a) diverse supergroup A identified in Crustacea, Bivalvia, and Eutardigrada; b) supergroup E discovered in the Crustacea microbiome; and c) a newly identified supergroup V found in Crustacea and Bivalvia.

Endosymbiotic bacteria of the genus *Wolbachia* were identified in the following species/genus within the three studied taxonomic groups: a) Crustacea – *Wolbachia* infection

was confirmed in *B. schaefferi* and *S. cafer* (previously detected this endosymbiont using designed primers and Sanger sequencing, **Publication 2**). Additionally, *Wolbachia* was detected for the first time in *Artemia salina* (Linnaeus, 1758), *Artemia parthenogenetica* Bowen and Sterling, 1978, *Eulimnadia* sp., *Triops cancriformis* (Bosc, 1801), and *Chydorus* sp.; b) Bivalvia – *Wolbachia* infection was confirmed in *U. crassus* (previously detected using *Wolbachia*-specific primers and Sanger sequencing, **Publication 3**). In addition, *Wolbachia* was detected for the first time in *Dreissena polymorpha* (Pallas, 1771); c) Eutardigrada – *Wolbachia* was detected for the first time in *Pam. experimentalis* (previously only Rickettsiales infection was identified using metagenomics and amplification of the 16S rRNA gene with commonly used primers, **Publication 5**), as well as in *Macrobiotus basiatus* Nelson, Adkins Fletcher, Guidetti, Roszkowska, Grobys, and Kaczmarek, 2020. Detailed information regarding *Wolbachia* supergroups in individual species and the methodology used is described in this publication.

To date, due to the widespread occurrence of bacteria from the genus *Wolbachia* infections in terrestrial invertebrates and the rare occurrence in aquatic invertebrates, the hypothesis of the continental origin of this intracellular symbiont has been accepted (Bouchon et al., 1998). However, the presented results challenge this notion, as the oldest evolutionary clades of *Wolbachia* supergroups were represented by invertebrates known from both aquatic and terrestrial environments, while the evolutionarily youngest clades consisted of *Wolbachia* supergroups identified only in terrestrial invertebrates.

***Summary and conclusions of the presented scientific achievement.***

In conclusion, my research has significantly contributed to the identification of (endo)symbiotic bacteria associated with freshwater invertebrates. The main takeaway from the findings is that these infections are more widespread than previously believed, and their detection has been limited by the lack of suitable molecular and bioinformatic tools. The most significant achievement of my research is the numerous discoveries of *Wolbachia* bacteria in previously unexplored hosts belonging to Crustacea, Bivalvia, and Eutardigrada. Using various molecular and bioinformatics approaches, I have also made pioneering discoveries of infections with other (endo)symbiotic bacteria in these groups of freshwater invertebrates and the diagram of all discoveries is presented in Figure 1.

Pioneering discoveries of (endo)symbiotic bacteria described in subsequent publications are as follows:

- **Publications 1 and 2** – identification of (endo)symbionts in freshwater crustaceans Crustacea, namely (endo)symbionts associated with *Branchipus schaefferi* (Branchiopoda) – *Aeromonas* sp., *Candidatus* Gortzia, *Methylophilus* sp., *Spirobacillus* sp., *Wolbachia*; endosymbiont associated with *Streptocephalus cafer* (Branchiopoda) – *Wolbachia*; (endo)symbiont associated with *Moina macrocopa* (Branchiopoda) – *Methylophilus* sp.; (endo)symbiont associated with *Heterocypris incongruens* (Ostracoda) – *Cardinium*, *Methylophilus* sp.;
- **Publications 3 and 4** – identification of (endo)symbionts associated with the freshwater mussel *Unio crassus* (Bivalvia: Unionidae) – *Bacillus* sp., *Candidatus* Xiphinematobacter, *Cardinium*, *Chryseobacterium*, *Flavobacterium* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., *Wolbachia*;
- **Publications 5 and 6** – identification of symbionts in freshwater tardigrades Eutardigrada, namely *Wolbachia* endosymbiont associated with *Paramacrobiotus* sp. and *Macrobiotus polypiformis*, as well as probable endosymbionts from the order Rickettsiales and *Polynucleobacter* sp. associated with *Paramacrobiotus experimentalis*;
- **Publication 7** – new molecular and bioinformatic tool for the identification of endosymbiotic bacteria *Wolbachia* in three investigated systematic groups, i.e., i) Crustacea: *Artemia salina*, *Artemia parthenogenetica*, *Branchipus schaefferi*, *Chydorus* sp., *Eulimnadia* sp., *Streptocephalus cafer*, *Triops cancriformis*; ii) Bivalvia: *Dreissena polymorpha*, *Unio crassus*; iii) Eutardigrada: *Macrobiotus basiatus*, *Paramacrobiotus experimentalis*.

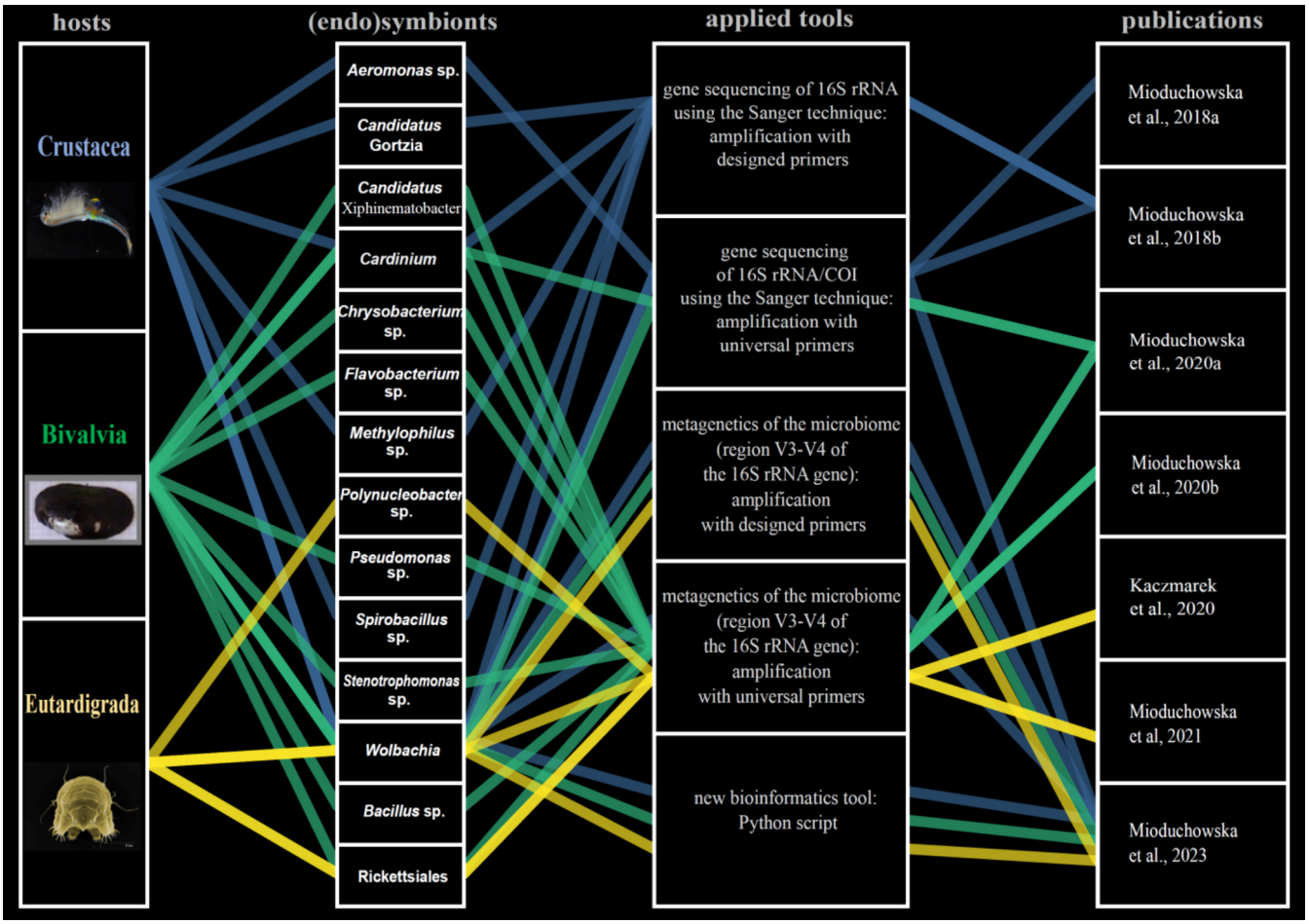


**Figure 1.** Diagram presents the molecular and bioinformatic tools used in each publication included in the series of my habilitation achievement, enabling the identification of (endo)symbiotic bacteria associated with Crustacea, Bivalvia, and Eutardigrada.

The novelty of this research is underscored by the absence of existing literature data on (endo)symbiotic bacteria associated with the studied species of freshwater invertebrates. The discoveries made in these studies provide a significant contribution to the field, shedding light on previously unexplored microbial associations of freshwater invertebrates. However, there have been several individual publications by other authors that confirm the presented findings:

- The discovery of *Wolbachia* in Tardigrada was confirmed by Tibbs-Cortes et al. (2022). Two earlier studies published between 2018 and 2020 (Vecchi et al., 2018; Guidetti et al., 2020) did not detect *Wolbachia* infection. However, the presence of bacteria belonging to the order Rickettsiales in the microbiome of the studied tardigrades led the authors to hypothesize the possibility of bacteria from the genus *Wolbachia* infection.

- The pioneering identification of the endosymbiotic bacteria from the genus *Cardinium* in *Heterocypris incongruens* (Ostracoda) was confirmed by Schön et al. (2018). The continuation of research on the frequency of *Cardinium* occurrence in different phylogenetically distant groups of freshwater Ostracoda, using specific primers designed by me, represents exciting direction of research conducted by a different team of researchers to which I belong (preliminary results were presented last year at the International Symposium on Ostracoda in Lyon: Prais K., Mioduchowska M., Namiotko T., Kilikowska A. 2022. Further evidence for widespread *Cardinium* infection in non-marine ostracod crustaceans. International Symposium on Ostracoda, Lyon, France).

I presented the results of the research contributing to the above-mentioned scientific achievement at seven international conferences [oral presentations (2), posters (7)] and five national conferences [oral presentations (3), posters (3)]. Detailed information is provided in Appendix 4.

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**5.** Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions.

My other scientific activities are outlined below. All cited publications resulting from my research are marked in bold font. References can be found at the end of this chapter. Other achievements, including internships, trainings, projects, and conferences are listed in Appendix 4, "List of scientific or artistic achievements that have significantly contributed to the development of a specific discipline."

In 2004, I began my studies in the field of Biology at the Faculty of Biology, Geography, and Oceanography at the University of Gdańsk. My scientific journey began during my undergraduate years. From 2006 to 2009, I served as the Chairwoman of the Student Scientific Club of Hydrobiology and Water Protection at the University of Gdańsk. During the years 2007-2009, I presented the results of my research at four national scientific conferences. The research results concerned the morphological analysis and identification of Copepoda, as well as the diversity of meiobenthic organisms in the Pomeranian region. These findings were published in the following publications: **Stolarska and Wojtasik (2008); Wojtasik et al. (2009); and Mioduchowska and Wojtasik (2009).**

In 2009, I defended my master's thesis entitled "Genetic diversity of Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum, 1792)) from the western Barents Sea region" (specialization – Molecular Biology, scope of the graduate seminar – Genetics). The master's thesis was conducted under the supervision of Dr. Barbara Wojtasik at the Department of Genetics and Cytology of the University of Gdańsk. The research included four populations of Greenland halibut collected from the Barents Sea during a commercial expedition on M/Tr Polaris, in collaboration with the Fisheries Department under the Ministry of Agriculture of the Republic of Lithuania and the Directorate of Fisheries (Norway). Financial support was provided by UAB "Seivalas" (Private Limited Liability Company (Uždaroji Akcinė Bendrovė); Klaipėda, Lithuania) The analysis of the genetic structure of Greenland halibut was based on seven polymorphic gene loci encoding enzymes. The literature indicated panmixia (random mating) of Greenland halibut in the study area, but the results obtained in my thesis unequivocally demonstrated the existence of two isolated populations. Consequently, the

commercial exploitation of Greenland halibut should be appropriately regulated to ensure effective management of fisheries. The findings of this study were published in the work of **Wojtasik et al. (2021)** and presented during the 1st Scientific Conference of Polish Sea Researchers.

***Research related to Bivalvia***

In 2009, directly after completing my master's studies, I was admitted to the first year of the third-degree doctoral studies in the field of Biology (in the Department of Genetics and Biosystematics), Ecology, and Microbiology at the Faculty of Biology of the University of Gdańsk. I obtained the highest rank in the recruitment process and began to continue my studies.

For my doctoral thesis titled "Genetic structure of the endangered species, the thick-shelled river mussel *Unio crassus* (Philipson, 1788), in Polish rivers", I conducted my research under the supervision of Dr. hab. Jerzy Sell, Prof. UG The research was funded under three scientific projects (the Ministry of Science and Higher Education grant (MNiSW), No. **3636/B/P01/2010/38N**, where I was a member of the research group (2009-2014); the Own Research program at the University of Gdańsk, No. **BW/1410-5-0376-8**, with my involvement in laboratory work (2009); and the Young Scientists competition at the University of Gdańsk, No. **538-L155-0803-12**, where I was the project leader (2012-2013)). The aim of the research was to assess the variability and genetic diversity of the endangered and protected freshwater mussel species *U. crassus* in Polish populations. I aimed to gain insight into the genetic structure of the species and understand the factors influencing its formation. Additionally, I conducted analyses to test the applicability of the mtDNA type M genome (M*cox1* fragment) in phylogeographic studies and to identify conservation units of this species in Poland.

In the doctoral thesis, I utilized both nuclear and mitochondrial DNA markers to achieve the research objectives outlined above. Due to the lack of literature data on microsatellite DNA in the thick-shelled river mussel, a genome scan was conducted using Next-Generation Sequencing, specifically the 454 pyrosequencing method. This genome scan enabled the design of primers complementary to the flanking regions of microsatellite DNA segments. The procedures related to the identification and characterization of polymorphic microsatellite loci in *U. crassus* were presented in the work of **Sell et al. (2013)**. In total, 154 most optimal primers were selected to amplify 77 loci with perfect repeat motifs, and 13 polymorphic microsatellite DNA loci were selected for further analysis.

Specific primers were designed for mitochondrial DNA (mtDNA) to amplify the ND3-ND2 region and the gene fragment encoding cytochrome c oxidase subunit I from both the

maternally (F*cox1*) and paternally inherited genomes (M*cox1*). This was related to the discovery of two types of mtDNA in the somatic tissues of male *U. crassus*, referred to as the F-type (maternal origin) and M-type (paternal origin) mtDNA. This mode of mtDNA inheritance is known as Doubly Uniparental Inheritance (DUI) and has so far only been observed in male Unionidae in gonadal tissues (Zouros, 2013). The distribution of DUI in somatic tissues of males was described in the publication by **Mioduchowska et al. (2016)**. Interestingly, this discovery has also enabled the development of a non-invasive molecular method for sex identification of this endangered mussel species **(Mioduchowska et al., 2016)**.

The analyses conducted using molecular markers of mitochondrial and nuclear DNA provided insights into the processes shaping the observed genetic variability of the studied populations. This analysis revealed a significant division of the populations into two distinct clades or evolutionary lineages, which likely originated from different glacial refugia. The isolation of the examined populations of *U. crassus* was evident, and the genetic variation observed in the species was consistent with the isolation-by-distance model (**Douda et al., 2014; Mioduchowska et al., 2016; Kilikowska et al., 2020**). The most critical outcome of this research was the identification of two main Evolutionary Significant Units (ESUs). The discovery of these distinct units can have profound implications for the conservation planning of this endangered species.

The results were presented at six national and five international scientific conferences (see Appendix 4 for details) and published in the aforementioned works. During the course of my doctoral studies, I also participated in three other projects related to *U. crassus*: i) POIS project, No. **POIS-05.02.00-00-084/08**, as a research project participant (2011-2012); ii) Young Scientists competition at the University of Gdańsk, No. **538-L155-0793-12**, as the project executor (2012-2013); iii) MNiSW project, No. **N304 328836**, as a research project participant (2013-2014). Furthermore, I conducted research on the identification of conservation units in Bulgarian populations of the European bullhead *Cottus gobio* (which is the host of the parasitic larval stage (glochidium) of the thick-shelled river mussel): the Own Research project at the University of Gdańsk, No. **L155-5-0416-0**, as the project executor (2009-2010).

Until now, I have conducted research related to the genetics of freshwater mussels. Some of the results obtained, concerning the identification of (endo)symbiotic bacteria associated with Bivalvia, have been described as part of my habilitation achievement (**Mioduchowska et al., 2020a,b; Mioduchowska et al., 2023**). Additionally, I participated in the pioneering sequencing of the F-type and M-type genomes of *U. crassus*. The sequenced genomes were published in the work of **Burzyński et al. (2017)**. Since 2019, I have been

actively involved as a member of the Management Committee (MC) of the international COST Action European Cooperation in Science and Technology, title of the project: "Conservation of freshwater mussels: a pan-European approach" (No. **CA18239**). Currently, joint scientific publications are being prepared based on the project conducted under the COST Action, including a monograph focusing on selected freshwater mussel species. In 2020, I participated in the applied project "Renaturalisation of the inland delta of the Nida River" (EU/NFOŚiGW/IOP, LIFE4DELTA applied project, No. **NAT/PL/000018**, participation in the research project). My task was to conduct phylogenetic analyses of individuals from a container culture of mussels. In 2022, I participated in the molecular identification of symbiotic ciliates (Ciliophora) associated with *U. crassus* (research conducted under the grant No. **2021/05/X/NZ2/01839** from the National Science Centre, participation in laboratory work). The developed methodology of high-throughput sequencing of Ciliophora enabled the identification of 98 taxa. The taxonomic composition was shown to vary between rivers and change throughout the year (publication is currently under review).

In total, the results from genetic analysis of *U. crassus* have been presented at six international and ten national scientific conferences (detailed data are provided in Appendix 4) and published in the following articles: **Sell et al., 2013; Douda et al., 2014; Mioduchowska et al., 2016; Burzyński et al., 2017; Kilikowska et al., 2020**. Some of the obtained findings, concerning the identification of (endo)symbiotic bacteria associated with Bivalvia, has been described as part of my main achievement: **Mioduchowska et al., 2020a,b; Mioduchowska et al., 2023**.

***Research related to Crustacea***

Since 2015, during the course of my doctoral work, my research in genetics has expanded to include taxonomic groups beyond Bivalvia. I developed an interest in freshwater crustaceans Crustacea, particularly the fairy shrimp *Branchipus schaefferi*. From 2015 to 2018, I conducted a metapopulation analysis of this species and investigated evolutionary mechanisms in isolated populations, focusing on both mitochondrial and nuclear DNA levels. I designed specific primers for amplifying barcode sequences (*cox1*) of *B. schaefferi* and identified polymorphic microsatellite DNA markers. My efforts in this area were rewarded when I secured three research projects related to *B. schaefferi* studies, taking on the role of project leader (Young Scientists competition at the University of Gdańsk, **No. 538-L155-B934-15, 538-L155-B249-16, 538-L260-B518-17-1M**). Some of the results were published in the

works of **Mioduchowska et al. (2018a,b,c)** and **Lukić et al. (2019)** (other manuscripts are currently in preparation).

In 2019, I completed an **internship at the Laboratory of Aquatic Ecology, Evolution, and Conservation at KU Leuven in Belgium**. The internship was conducted under **the supervision of Prof. Dr. Luc Brendonck**. During my stay, I conducted research as part of the "Coevolution of the endangered fairy shrimp *Branchipus schaefferi* (Branchiopoda, Anostraca) and intracellular endosymbiont *Wolbachia* bacteria" project funded by the European Molecular Biology Organization (EMBO) (project number **7862**). The internship allowed me to expand my laboratory skills in the area of amplification and analysis of molecular markers. I also learned new techniques in molecular biology and gained experience in conducting laboratory breeding of *B. schaefferi*. The culmination of the research conducted during the internship was the publication, which is included in my main achievement, namely, **Mioduchowska et al. (2023)**. Other results obtained in the laboratory of Prof. Dr. Brendonck will also be published soon. Furthermore, during my internship, I also had the opportunity to participate in a project on the genetic diversity of *Branchinecta ferox* (Milne-Edwards, 1840) and *B. orientalis* (Sars, 1901). The results of these studies were published in the work of **Lukić et al. (2021)**.

In addition to my research on freshwater crustaceans, I also participated in significant studies focusing on the colonization process of the Szmaragdowa Cave in the Kraków-Częstochowa Upland by invertebrates (**Kur et al., 2016a**). Moreover, I was involved in the analysis of the distribution of copepods (Copepoda) in various underground habitats in the southern regions of Poland, including caves and wells (**Kur et al., 2020**).

In total, the results of genetic analyses of Crustacea were presented at seven national and five international scientific conferences (see Appendix 4 for details) and published in the following articles: **Kur et al., 2016a,b; Mioduchowska et al., 2018a,b; Lukić et al., 2019; Kur et al., 2020; Lukić et al., 2021; Mioduchowska et al., 2023**. Some of the obtained results, concerning the identification of (endo)symbiotic bacteria associated with Crustacea, form part of my main achievement: **Mioduchowska et al., 2018a,b; Mioduchowska et al., 2023**.

***Research related Tardigrada***

In 2018, I have been actively involved in the field of integrative taxonomy of Tardigrada (commonly known as water bears). My primary area of interest lies in the molecular aspect of integrative taxonomy, which involves obtaining sequences of mitochondrial (COI) and nuclear DNA (ITS2, 18S rRNA, 28S rRNA) suitable for a comprehensive description of the studied species completing morphological analyses. Additionally, I perform appropriate bioinformatic

analyses of the obtained nucleotide sequences, including phylogenetic analyses of the studied species, to confirm or exclude their clustering in the generated phylogenetic tree.

As a result of my work, I have been involved in co-authoring the description of seven new Tardigrada species:

- *Macrobiotus wandae* Kayastha, Berdi, Mioduchowska, Gawlak, Łukasiewicz, Gołdyn & Kaczmarek, 2020 from Nepal (**Kayastha et al., 2020a**),
- *Richtersius ziemowiti* Kayastha, Berdi, Mioduchowska, Gawlak, Łukasiewicz, Gołdyn, Jędrzejewski & Kaczmarek, 2020 from Nepal (**Kayastha et al., 2020b**),
- *Macrobiotus porifini* Kuzdrowska, Mioduchowska, Gawlak, Bartylak, A. Kepel, M. Kepel & Kaczmarek, 2021 from Madagascar (**Kuzdrowska et al., 2021**),
- *Echiniscoides ritavargasae* Bartels, Fontoura, Mioduchowska & Kaczmarek, 2021 from Costa Rica (**Bartels et al., 2021**),
- *Macrobiotus birendrai* Kayastha, Roszkowska, Mioduchowska, Gawlak & Kaczmarek, 2021 and *Bryodelphax mareki* Kayastha, Roszkowska, Mioduchowska, Gawlak & Kaczmarek, 2021 from Canada (**Kayastha et al., 2021**),
- *Macrobiotus kosmali* Kayastha, Mioduchowska, Gawlak, Sługocki, Araújo, Gonçalves & Kaczmarek, 2023 from Madeira (Portugalia) (**Kayastha et al., 2023a**).

Additionally, I have completed descriptions of several other new species at the genetic-level, which will soon be published.

In addition to describing new species, I have also contributed to the redescription of already characterized species:

- *Dastychius improvisus* (Dastych, 1984) from Antarctica (**Mioduchowska et al., 2021a**);
- *Diploechiniscus oihonnae* (Richters, 1903) from Norway (**Kaczmarek et al., 2021**);
- *Minibiotus intermedius* (Plate, 1888) from Germany (**Kaczmarek et al., 2022a**);
- *Echiniscus quadrispinosus quadrispinosus* Richters, 1902 from Germany (**Kaczmarek et al., 2022b**).

Furthermore, my research extended to investigating the genetic diversity of the cosmopolitan species *Paramacrobiotus fairbanksi* Schill, Förster, Dandekar & Wolf, 2010 (**Kaczmarek et al., 2020a**), as well as other species from the genus *Paramacrobiotus* (Eutardigrada: Macrobiotidae) (**Kayastha et al., 2023b**). Other publications related to the identification of (endo)symbiotic bacteria associated with Tardigrada have been described as part of my main achievement (**Kaczmarek et al., 2020b; Mioduchowska et al., 2021b; Mioduchowska et al., 2023**). In total, the results from genetic analyses of Tardigrada were published in 15 scientific articles mentioned above and presented at four international and one national scientific conferences (see Appendix 4 for details).

***The research related to marine organisms***

My current scientific interests also focus on marine organisms. **From 2019 to 2021**, I completed a **postdoctoral internship** as a research assistant (postdoctoral researcher) at **the Laboratory of Marine Plankton Studies at the Institute of Oceanography, Faculty of Oceanography and Geography, University of Gdańsk**. This internship was part of the OPUS project "HIDEA – Hidden diversity of plankton in the European Arctic", funded by the National Science Centre and led by Dr. hab. Agata Weydmann-Zwolicka, Prof. UG; project number: **UMO-2017/27/B/NZ8/01056**. The main aim of the research was to assess the impact of climate change and Arctic atlantification on the biodiversity of bacterioplankton, zooplankton, and phytoplankton. The project also involved the functioning of the pelagic trophic network in the area influenced by the West Spitsbergen Current. Samples were collected from the Norwegian Sea, Greenland Sea, and Arctic Ocean during three scientific cruises (2019–2021) on the research vessel *Oceania* (IOPAN). The results of the research were presented at two international scientific conferences. Preliminary results involving laboratory and bioinformatic procedures of the studied marine microbiome were published in the work of **Mioduchowska et al. (2022)**. Other publications are currently under review or in preparation.

In 2020, as part of a postdoctoral internship, I attended a training course organized by Physalia titled "16S/ITS metabarcoding of microbial communities". Additionally, in the years 2020-2021, I participated in three scientific research cruises conducted on the specialized research vessel *Oceanograf* in the Gulf of Gdańsk. The purpose of these cruises was to collect plankton samples for the analysis of their genetic diversity.

Thanks to my postdoctoral internship, I significantly expanded my laboratory skills and acquired new abilities in the analysis of bioinformatic metadata obtained by NGS sequencing. I also became familiar with sampling techniques using specialized equipment on the research

vessel *Oceanograf*. Moreover, newly acquired skills allowed me to become a member of the "MetaZooGene-ICE research group" through a collaboration with Professor Ann Bucklin from the Department of Marine Sciences, University of Connecticut, USA. This collaboration involved conducting research and optimizing laboratory procedures related to marine zooplankton metabarcoding (publication in preparation).

I have published the following publications affiliated with the Laboratory of Marine Plankton Studies, Institute of Oceanography, Faculty of Oceanography and Geography, University of Gdańsk: **Kaczmarek et al., 2020a; Kayastha et al., 2020a,b; Kilikowska et al., 2020; Kur et al., 2020; Bartels et al., 2021; Kaczmarek et al., 2021; Kayastha et al., 2021; Kuzdrowska et al., 2021; Lukić et al., 2021; Mioduchowska et al., 2021a,b; Wojtasik et al., 2021; Kaczmarek et al., 2022a; Mioduchowska et al., 2022; Mioduchowska et al., 2023.**

In the years **2021-2022**, I embarked on another **post-doctoral internship** as a research assistant at **the Department of Invertebrate Zoology and Hydrobiology, Faculty of Biology and Environmental Protection, University of Łódź** as part of the OPUS project (funded by the National Science Centre) led by Prof. Dr. hab. Magdalena Błażewicz; project title: "Biodiversity Patterns and Scale: the case of Peracarids Crustacea from south-eastern Australia BIOPASS"; project number: **UMO-2018/31/B/NZ8/03198**. During the internship, I studied the genetic diversity of marine crustaceans Tanaidacea collected off the coast of Australia. Additionally, I conducted analyses of the microbiome of selected Tanaidacea species and identified a previously undescribed parasitic species of ciliates (Ciliophora) associated with one of the Tanaidacea species (for this purpose, I designed specific primers). Through these research activities, I expanded my knowledge and laboratory skills by incorporating new molecular markers. I also gained valuable experience in studying the taxonomically challenging group of Tanaidacea and optimized some laboratory procedures. Publications based on the obtained results are currently being prepared.

I have published the following publications affiliated with the Department of Invertebrate Zoology and Hydrobiology, Faculty of Biology and Environmental Protection, University of Łódź: **Kur i in., 2021; Lukić et al., 2021; Mioduchowska et al., 2021b; Kaczmarek et al., 2022a; Mioduchowska et al., 2023.**

***Summary of my scientific achievements***

- I conducted research within the framework of nine projects funded as a result of competitions from external sources, as well as ten projects funded through competitions at the University of Gdańsk. Currently, I am working on two projects funded through competitions from external sources.
- I completed three postdoctoral internships at three institutions, including one abroad.
- I was a co-author of 24 presentations (oral or poster) at international conferences and 38 national conferences (oral or poster).
- The research results have been published in 38 scientific publications.
- The total Impact Factor (IF) of all published articles is 73.594 (considering the publication year of the articles).
- The total MNiSW/MEiN points: 2472 (based on the year of publication) / 3110 (according to the latest MEiN scoring, as of July 17, 2023).
- The total number of citations for all publications is: according to Google Scholar: 418, according to Scopus: 258, and according to the Web of Science Core Collection: 241.
- My Hirsch Index is: according to Google Scholar: 11, according to Scopus: 9, and according to the Web of Science Core Collection: 8.

Detailed information is provided in Appendix 4.

***Future plans***

In 2022, I received funding from the National Science Centre (SONATA 17 competition) to carry out the project titled "Let's dry up and survive together": is anhydrobiosis in water bears (Tardigrada) modulated by a specific microbiome community and does it depend on bacteria that survive desiccation together with them?" (project number: **2021/43/D/NZ8/00344**, project leader). A year earlier, I also received funding from the UGrants-first program at the University of Gdańsk (project number: **1220/146/2021**), which provided support in the preparation of the above-mentioned project. The mechanisms that enable tardigrades to survive through anhydrobiosis (a state of dehydration in which tardigrades lose more than 90% of their water and can survive for several years) are not sufficiently understood. Until now, it has not been investigated whether this ability could be attributed to a specific microbiome inhabiting their bodies, and which bacteria could potentially play a significant role in this process. The aim of this project is a comprehensive analysis of the microbiome at various stages of tardigrade development, including eggs and adult individuals

before, during, and after anhydrobiosis. The microbiome profile will be analyzed using NGS sequencing. To confirm the role of bacteria in tardigrade anhydrobiosis, an experiment will be conducted in which microbiomes will be exchanged between tardigrade species with anhydrobiotic abilities and those lacking this characteristic. The hypothesis assumes that the exchange of microbiomes between tardigrades could result in a loss or gain of the ability to undergo anhydrobiosis. The experiment will involve analysis by means of shotgun metagenomics of the microbiome. If the hypothesis regarding the significant role of specific bacteria in tardigrade anhydrobiosis is confirmed, the research will be a breakthrough in understanding this phenomenon.

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**6.** Presentation of teaching and organizational achievements as well as achievements in popularization of science or art.

**I have conducted educational activities at the Department of Evolutionary Genetics and Biosystematics, Faculty of Biology, University of Gdańsk** (however, due to the postdoctoral internships in the period from April 2019 to July 2022, I did not engage in any teaching activities during that period).

**1)** Subjects taught in the field of Biology:

- Genetics (laboratory classes);
- Intricacies of sex determination processes (series of original optional lectures);
- Modern research methods in animal taxonomy (bioinformatic workshops);
- Molecular Biology (lecture, participation);
- Genetics (lecture, participation);
- Specialization laboratory;
- Diploma laboratory.

**2)** Subjects taught in the field of Medical Biology:

- Basics of Genetics (laboratory classes);
- Intricacies of sex determination processes (series of original optional lectures);
- Specialization laboratory;
- Diploma laboratory.

**3)** Subjects taught in the field of Genetics and Experimental Biology:

- Specialization laboratory;
- Diploma laboratory.

**4)** Subjects taught on the Nature Resources Conservation course:

- Molecular Ecology (series of original obligatory lectures).

**5)** Subjects taught in the field of Bioinformatics:

- Genes and Populations in Time and Space (bioinformatic workshops).

**6)** From 2009 to 2016, I was involved in the laboratory and content supervision of the preparation of twelve master's theses in the Student Part-Time Laboratory.

**7)** Since 2016: supervisor of four bachelor's theses and four master's theses.

**8)** Since 2023: assistant supervisor of the doctoral thesis of Ms. Pushpalata Kayastha, conducted at the Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań. The title of the dissertation is "The genus *Paramacrobiotus* (Tardigrada): integrative taxonomy, biogeography, and effects of stress factors on the selected species". The part involving genetics of tardigrades of the genus *Paramacrobiotus* is carried out under my supervision.

**Other educational activities:**

**1)** Supervisor of the Student Genetic Club, University of Gdańsk (2014 – 2015);

**2)** Supervisor the Student Scientific Club of Hydrobiology and Water Conservation, University of Gdańsk (2015 – 2019);

**3)** Conducting workshops for members of the OIKOS Scientific Club in Olsztyn as part of hydrobiological research carried out by the Student Scientific Club of Hydrobiology and Water Conservation at the University of Gdańsk, of which I was the Scientific Supervisor (16-17.08.2015);

**4)** Coordination of laboratory research conducted as part of the Biological Olympiad by a student from the Academic High School "Lingwista" in Gdańsk (academic year 2015/2016);

**5)** Lecture delivered at the Laboratory of Aquatic Ecology, Evolution, and Conservation, KU Leuven, Belgium; title: "Coevolution of the endangered fairy shrimp *Branchipus schaefferi* (Brianchopoda, Anostraca) and intracellular endosymbiont *Wolbachia* bacteria" (10.05.2019).

**Organizational activities:**

**1)** Chairwoman of the Student Scientific Club of Hydrobiology and Water Conservation at the University of Gdańsk (2006 – 2009);

**2)** Representative of the Doctoral Student Self-governing Body in the Disciplinary Appeals Committee for Doctoral Students (2010 – 2012);

**3)** Member of the Faculty Scholarship Committee of the University of Gdańsk (2011 –2014);

**4)** Treasurer of the Council of the Faculty of Doctoral Students of the University of Gdańsk (2012 – 2014).

**Science popularization:**

**1)** Participation in organizing a student scientific camp in Niedzica (14-20.07.2007). Collaboration with the Department of Genetics and Cytology at the University of Gdańsk and the Department of Pojezierza Geography at the University of Gdańsk;

**2)** Participation in nine editions of the Baltic Festival of Science (years: 2007-2017) and 9th and 12th Science Picnic (years: 2008 and 2011);

**3)** Preparation of a presentation on the activities of the Student Scientific Circle of Hydrobiology and Water Conservation at the University of Gdańsk (of which I was the chairperson) for the University of Gdańsk Gazette (04.2009);

**4)** Workshops in the Universal Science Zone at Galeria Bałtycka (years: 2010; 2015; 2016);

**5)** Conducting educational workshops for high schools titled "Discover the Work of a Biologist". Workshop topic: "You Share Your Fruits with Us - Genetic Model" (years: 2010-2019);

**6)** Preparation and conduct of workshops during the Night of Biologists (years: 2012; 2014-2018-2019, 2021);

**7)** The 10th Picnic "Biodiversity – Discover to Preserve" in R. Reagan Park in Gdańsk (19.05.2018) and the 5th Picnic "Island of Naturalists" at the Biological Station of the University of Gdańsk on Sobieszewo Island (07.07.2018).

**7.** Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.

**Research Scholarships:**

**1)** Scientific Scholarship awarded by the University of Gdańsk to the best students in the academic year 2005-2009;

**2)** Scholarship from the Ministry of Science and Higher Education in the academic year 2008/2009;

**3)** Scientific Scholarship awarded by the University of Gdańsk to the best doctoral students in the academic year 2009-2015;

4) Doctoral Scholarship from institutional funding to support high-quality tasks in the academic year 2011-2015;
5) Scholarship awarded in the academic year 2014 as part of the project: "We Educate the Best – A Comprehensive Development Program for Doctoral Students, Young Doctors, and Academic Teaching Staff of the University of Gdańsk". The project was implemented under the Operational Program Human Capital, Priority IV, Action 4.1, Sub-measure 4.1.1. Strengthening the educational potential of the university, financed by the European Social Fund;
6) Scholarship awarded in 2019 by the European Molecular Biology Organization (EMBO) for the Short-Term Fellowship to conduct research at the Laboratory of Aquatic Ecology, Evolution, and Conservation, KU Leuven, Belgium.

**Prizes, awards:**

1) Nomination by the Rector of the University of Gdańsk in the competition for the "Red Rose" Award for the Best Student Science Club (Gdańsk, 18.05.2009);
2) Honorable mention in the Contest for the Best Student in Poland - Student Nobel Prize 2009;
3) In recognition of outstanding scientific achievements during my studies in the field of Biology, I received a Graduate Diploma awarded on behalf of the Faculty of Biology, University of Gdańsk (10.2010);
4) Second-degree award for the poster presented at the IV Polish Congress of Genetics (Poznań, 13.09.2013);
5) Award for the best presentation at the XXXII Malacological Seminar (Spała, 13-15.10.2016);
6) Publication 1 (Mioduchowska et al. 2018a), part of a series of seven publications representing my scientific achievement, was among the top 10% most cited articles published in the PlosOne journal in 2018;
7) In 2020, I received a nomination from renowned malacologists Dr. Manuela Lopes-Lima and Dr. hab. Tadeusz Zając, Prof. IOP PAN for the "Diversity Young Investigator Award";
8) Individual Rector's Award, Second Degree Award in 2021 for a series of publications titled "Application of integrative taxonomy and molecular identification of symbiotic bacteria in aquatic invertebrates".

**Manuscript reviews in scientific journals:**

Animals; Biologia, Section Zoology; Current Microbiology; Diversity; Hydrobiologia; International Journal of Molecular Science; Journal of Invertebrate Pathology; Limnology; Marine Biodiversity; Marine Biology; Molecular Ecology; Molecular Ecology Resources; PeerJ; PlosOne; Science of the Total Environment; Scientific Reports; Systamatic Entomology.

**Editorial boards, organizing committees, and scientific societies:**

1) Since September 2019, I have been a member of the Management Committee (MC) for the international COST action – European Cooperation in Science & Technology, within the project "Conservation of freshwater mussels: a pan-European approach". The MC nomination was granted in 2019 by the Minister of Science and Higher Education;
2) On July 19-22, 2019, I co-organized the 9th European Ostracodologists Meeting, an international conference held in Gdańsk;
3) Since January 2021 to July 2023, I was the Guest Editor for the journal Diversity, in a special issue titled "Bacterial Symbionts of Invertebrates: Diversity, Transmission, and Impacts" (https://www.mdpi.com/journal/diversity/special_issues/bacterial_symbionts_invertebrates).

Detailed information regarding other scientific achievements, including a list of participation in research teams conducting projects funded through national or international competitions, is provided in Appendix 4.

Monika Mioduchowska

(Applicant's signature)