ATTACHMENT 3

Summary of professional accomplishments

DANIEL STEC, PhD

Institute of Systematics and Evolution of Animals

Polish Academy of Sciences

Kraków 2022

ATTACHMENT 3

1. Name.

   Daniel Stec

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

   - **2021** – PhD in Biology, Jagiellonian University (Institute of Zoology and Biomedical Research). Title of the PhD dissertation: "Taxonomy and evolution of the *Macrobiotus hufelandi* complex (Tardigrada: Macrobiotidae)".
     Advisor: dr hab. Łukasz Michalczyk.
     PhD degree conferment date: 29.06.2021.
   - **2015** – MSc in Biology, Jagiellonian University (Institute of Zoology). Title of the MSc thesis: „An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya, with notes on the Kenyan tardigrade fauna".
     Advisor: dr Łukasz Michalczyk.
     MSc title conferment date: 12.06.2015.

3. Information on employment in research institutes or faculties/departments.

   - 2021-07-22 – currently; **adjunct**, Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences.

4. Description of the achievements, set put in art. 2019 para 1 point 2 of the Act.

   A. Achievement title

      Modern taxonomic studies in microscopic invertebrates – Tardigrada

   B. Publications included in the achievement, with the *impact factor* (IF) of the journal according to JCR and the number of ministerial points, according to lists for the publication year or the newest available listing

(I) **Stec D.** 2022. An integrative description of two new *Mesobiotus* species (Tardigrada: Eutardigrada: Macrobiotidae) with updated genus phylogeny. *Zoological Studies* 61: 85. https://doi.org/10.6620/ZS.2022.61-85
IF_{2021} = 1.904. $MEiN_{2022}$ = 70.

(II) **Stec D.**, Morek W. 2022. Reaching the monophyly: re-evaluation of the enigmatic species *Tenuibiotus hyperonyx* (Maucci, 1983) and the genus *Tenuibiotus* (Eutardigrada). *Animals* 12(3): 404. https://doi.org/10.3390/ani12030404
IF_{2021} = 3.231. $MEiN_{2022}$ = 100.

(III) **Stec D.** 2022. Integrative taxonomy helps to revise systematics and questions the purported cosmopolitan nature of the type species within the genus *Diaforobiotus* (Eutardigrada: Richtersiusidae). *Organisms Diversity & Evolution*. https://doi.org/10.1007/s13127-022-00592-6
IF_{2021} = 2.663. $MEiN_{2022}$ = 100.

(IV) **Stec D.**, Vecchi M., Dudziak M., Bartels P.J., Calhim S., Michalczyk Ł. 2021. Integrative taxonomy resolves species identities within the *Macrobiotus pallarii* complex (Eutardigrada: Macrobiotidae). *Zoological Letters* 7: 9. https://doi.org/10.1186/s40851-021-00176-w
IF_{2021} = 3.157. $MEiN_{2021}$ = 140.

(V) Kiosya Y., **Stec D.** 2022. New species of the genus *Richtersius* Pilato & Binda, 1989 (Tardigrada: Eutardigrada: Richtersiusidae) from Uzbekistan. *Folia Biologica (Kraków)* 70: 141-150. https://doi.org/10.3409/fb_70-4.18
IF_{2021} = 0.714. $MEiN_{2022}$ = 100.

C. <u>Description of the aim and obtained results</u>

BACKGROUND

Knowledge of and the ability to recognize the organisms that surround us constitute the basis in many scientific disciplines, such as ecology, agriculture, medicine,

evolutionary biology, physiology, and in studies on biodiversity (GRAY & CAVERS 2014, COWIE et al. 2022). Today's understanding of natural history was shaped during the Renaissance, and its main goal to this day is to discover and present all organisms on the Earth. As part of the study of natural history, taxonomy deals with the discovery, naming, and identification of organisms, which allows us to recognize the differences and relationships between them, showing us also a comprehensive and comprehensive picture of evolution and ecology (PFENNINGER et al. 2014, ZAMANI et al. 2022). From the beginning, morphology was and still is the gold standard in taxonomic research. Therefore, some of the groups of microscopic invertebrates are considered to be extremely demanding due to not only the small size of their body but, above all, the small number of characters that are valuable in the context of their taxonomy. As an often-observed result of this state of affairs, several similar-looking species are classified as one. Thus, the existence of a species complex is masked. Consequently, a conglomeration of many species recognized as a single species has a significantly overestimated geographical and ecological range. The scale of such overestimations may be at the level of the entire globe, and examples of this are known in tardigrades (Tardigrada; e.g., STEC et al. 2018, STEC et al. 2020a, MOREK & MICHALCZYK 2020). Tardigrades are microscopic organisms and in their case, scientists often struggle with the problem of species complexes, which group species that are difficult to identify. These animals are found all over the world in terrestrial and aquatic environments (freshwater and marine). Although some representatives of Tardigrada can live on land, they are only active when they are surrounded by at least a thin layer of water. When it dries out, tardigrades can survive drought periods due to their ability to enter the state called cryptobiosis, which enables them to survive unfavourable environmental conditions (KACZMAREK et al. 2019).

Today, in the time of global biodiversity crisis and accelerated species extinctions, the field of taxonomy is under pressure to accelerate the discovery, description, and naming of new species before they disappear unnoticed (COWIE et al. 2022). In the early 2000s, this pressure led to heated debates over the usefulness and limitations of DNA barcoding in taxonomy. One answer to this debate is integrative taxonomy as a means of incorporating DNA sequences into species discovery and identification while maintaining the "traditional" ways of doing taxonomic research, which are fundamental to understanding species diversity. An

integrative approach to taxonomy should combine the widest possible spectrum of different types of data, such as morphological, molecular, or ecological (DAYRAT 2005, VINARSKI 2020). Thanks to this, it is possible to draw more reliable conclusions about species hypotheses, relationships between species, and their evolution (STEC et al. 2022a).

The taxonomy of tardigrades, like most groups of organisms, has always been based on their morphology and anatomy (RAMAZZOTTI & MAUCCI 1983). It was only less than 20 years ago that research on Tardigrada began to use molecular methods to solve doubts related to the classification of taxa within this type of animals (e.g. JØRGENSEN et al. 2019). However, it has only been in the last eight years or so that tardigrade taxonomy has been revolutionized by an interactive approach that has contributed to a much better understanding of the evolution and phylogenetic relationships between major and minor taxonomic groups. The study of tardigrade species diversity, like studies on many other meiofauna groups, is considered a difficult activity not only because of the microscopic size of these animals but in particular because most genera have a very poor set of taxonomically valuable characteristics, which are also often not easy to diagnose (e.g. KOSZTYŁA et al. 2016, MOREK et al. 2016, SCHENK & FONTANETO 2020). The main result of this state of affairs is the erroneous recognition of many species of Tardigrada as widely distributed and occurring on many continents, while integrative analyses indicate the existence of complexes of morphologically similar, but genetically distinct species (e.g. BERTOLANI et al. 2011, STEC et al. 2018, GUIDETTI et al. 2019). Phenotypic diversity does not always evolve in line with genetic diversification, which results in the existence of species complexes grouping taxa that are difficult to identify, and as such constitute the main challenge for documenting real biodiversity. Therefore, disentangling such complexes is of great importance for the knowledge, documentation, and classification of living beings on our planet.

My research can be considered unique as I use a wide range of integrative taxonomy methods to analyse data from tardigrade populations located around the world. Many of these studies have attempted to answer the question about detailed phylogenetic relationships within species complexes, genera, or higher taxonomic units. In addition, my detailed studies on the morphology and morphometry of tardigrades have contributed to the identification of reliable and taxonomically informative characters or sets of characters that are useful for distinguishing species

within this animal group. In my scientific work so far, I have focused in particular on tardigrades belonging to the superfamily Macrobiotoidea, but my achievements also include many scientific works on the taxonomy of other tardigrade groups (e.g. STEC et al. 2018, MOREK et al. 2019, GĄSIOREK et al. 2019, ZAWIERUCHA et al. 2023), experimental or ecological studies on these animals (e.g. STEC et al. 2016, BRYNDOVÁ et al. 2020, TŮMOVÁ et al. 2022, VECCHI et al. 2022) as well as several publications on other groups of invertebrates (e.g. STEC & KUSZEWSKA 2020, MILER et al. 2020, OSTAP-CHEC et al. 2021, ZAJĄC & STEC 2020).

My scientific achievement submitted here to obtain the habilitated doctor degree includes five original scientific papers devoted to the taxonomy of tardigrades. These are works that illustrate solutions to some problems that this field of knowledge is facing today. I will briefly discuss each of these publications below.

PUBLICATION I

The first work included in my habilitation achievement concerns the genus *Mesobiotus* Vecchi et al., 2016 and shows the most common problem in taxonomy, which is the distinction and formal description of a species new to science. The genus I studied can be considered a relatively large group of tardigrades, currently comprising 75 species, of which four are designated as *nomina inquirenda* (KACZMAREK et al. 2020, DEGMA & GUIDETTI 2022). The genus *Mesobiotus* was elevated to its systematic rank based on morphological and genetic data (VECCHI et al. 2016). The analyzes carried out in the cited studies showed that the two previously recognized informal species groups within the genus *Macrobiotus* Schultze, 1934 (the *Macrobiotus harmsworthi* group and the *Macrobiotus furciger* group) form a monophyletic clade, grouping species phenotypically different from other genera distinguished in the family Macrobiotidae and as such deserving the rank of the genus *Mesobiotus*. However, the first study on more detailed phylogenetic relationships among species of the genus *Mesobiotus* was carried out by KACZMARK et al. (2018), which was then supported by subsequent studies dealing directly or indirectly with this topic (KACZMAREK et al. 2020, STEC 2021, STEC et al. 2021, STEC et al. 2022b, SHORT et al. 2022). All of the studies mentioned above dealing with phylogeny at the family level confirmed the monophyly of the genus, while all also showed a lack of congruence between morphology and genetics within this tardigrade group. Moreover, both informal morphologically

differentiated groups (*harmsworthi* and *furciger*) within *Mesobiotus* are not monophyletic, as representatives of both of them are intermixed on the phylogenetic tree. The distinction of these groups of species within the genus is vaguely based on the morphology of the egg shells, which has recently been criticized by SHORT et al. (2022). In their research, the authors showed a large divergence between the Antarctic and non-Antarctic *Mesobiotus* taxa, proposing to abandon the use of these informal species groups within the genus. The authors reasoned that these groups have no systematic value (they are not monophyletic) and their use masks evolutionary links and biogeographical patterns between species.

In my work (PUBLICATION I) I described two new species of the genus *Mesobiotus*. These descriptions are integrative as, apart from detailed morphological and morphometric data collected in a light microscope and a scanning electron microscope, they also contain genetic data in the form of DNA sequences. These descriptions are also a good example of the standards currently prevailing in tardigrade taxonomic studies, where the combination of phenotypic and molecular data allows for the reliable testing of hypotheses regarding the identification of new taxa. Furthermore, in PUBLICATION I, I conducted a multigene phylogenetic analysis, which indicated the position of the studied species within the genus *Mesobiotus*, as well as the relationships between its individual taxa. The main conclusions of this analysis are in line with the conclusions of the previous studies mentioned above, that the species representing the *furciger* and *hramsworthi* groups do not form monophyletic clusters. In addition to the populations of the new species in PUBLICATION I, I also studied the population of the already known but rare Greenlandic species *Mesobiotus peterseni* (Maucci, 1991), which is characterized by unique globular processes on its eggs. Bearing in mind the criticism of distinguishing informal morphological groups within the genus due to too ambiguous criteria relating to the characteristics of egg shells (SHORT et al. 2022), I proposed to clarify them. An in-depth comparative analysis of egg shell ornamentation in *Mesobiotus* taxa led me to distinguish not two but three morphological groups within the genus, which I defined in detail in PUBLICATION I. Moreover, I found that maintaining the use of morphological groups within the genus *Mesobiotus*, although they are not monophyletic, is of great practical value to both taxonomists and other name users, facilitating navigation, identification, and communication regarding the taxonomy and systematics of this tardigrade group. A

comprehensive reexamination of the morphological diversity within the genus *Mesobiotus* (in particular, the huge variety of egg shell ornaments) led me to update and improve the taxonomic key that I prepared with my coauthors 2 years ago (KACZMAREK et al. 2020). The presented key contains 71 nominal *Mesobiotus* species (excluding four species designated as *nomina inquirenda*) and is a valuable tool for other researchers to help identify tardigrades belonging to this genus.

PUBLICATION II and III

The studies presented in PUBLICATIONS II and III concern the genus *Diaforobiotus* Guidetti et al., 2016, which until recently had only two formally recognized subspecies (DEGMA & GUIDETTI 2022). This research also clearly shows that although descriptions of species new to science are an extremely important contribution to the taxonomy of a given group, in many cases integrative revisions or redescriptions of individual species or their groups can play an even more important role (MEIER & DIKOW 2004, SIGWART 2018). They provide updated information on taxa described in the past, which, due to inaccurate characterization, constitute often a big problem today because they prevent detailed comparisons with available and new nomina (VINARSKI 2020). Tardigrades are a taxonomically challenging group due to the frequent occurrence of cryptic species, inaccurate and questionable descriptions of species made in the past, and due to the frequent lack of type material. Although this problem affects all nominal species, its greatest impact on the correct identification of tardigrade diversity is visible in the case of ambiguous descriptions of type species for larger groups, such as families, genera, or species complexes. Such insufficient knowledge about the appearance of individual species (in particular the aforementioned type species) most often results in a huge overestimation of their ranges and a significant underestimation of true species diversity (e.g. KACZMAREK et al. 2015, KACZMAREK et al. 2016, MCINNES et al. 2017).

In the first of the papers discussed here (PUBLICATION II), I revised the enigmatic species *Tenuibiotus hyperonyx* (Maucci, 1983). Like PUBLICATION I, this work also provided detailed phenotypic and genetic data for the newly discovered topotypic population. The multilocus phylogenetic analysis clearly showed that the examined species belongs to the genus *Diaforobiotus* and not *Tenuibiotus* Pilato and Lisi, 2011. The morphological analysis was consistent with

the genetic results; therefore, I formally transferred the examined species to the first of the mentioned genera. The exclusion of *Diaforobiotus hyperonyx* (Maucci, 1983) from the genus *Tenuibiotus* allowed me to analyze the species composition of the latter genus in detail. Comparative analysis of the morphological characteristics of *Tenuibiotus* taxa led me to the improvement of the diagnosis of *Tenuibiotus willardi* (Pilato, 1977) and *Tenuibiotus bozhkae* Pilato et al., 2011, which also enabled me to correct and unify the diagnosis of the entire genus, which was previously reported as polyphyletic (STEC et al. 2021). Importantly, the thorough reexamination of *D. hyperonyx* and the demonstration of its phylogenetic position showed another example of convergent evolution in the claws elongation on legs of tardigrades living in moist and icy environments. In addition to species from the *Macrobiotus ariekammensis* complex and *Mesobiotus barabanovi* (Tumanov, 2005), *D. hyperonyx* illustrates the third case of claw phenotypic convergence within the superfamily Macrobiotoidea (STEC et al. 2022b, PUBLICATION II).

In PUBLICATION III I present a formal integrative redescription of the type species for the genus *Diaforobiotus*, namely *Diaforobiotus islandicus* (Richters, 1904), and an integrative description of a new species from the same genus. Genetic data for both populations studied have already been used in my previous work for phylogenetic analyzes (STEC et al. 2020b, PUBLICATION II), but without detailed reference to their morphology. In PUBLICATION III, based on the data obtained in a phase-contrast light microscope as well as in a scanning electron microscope, I provided a detailed morphological and morphometric characterization of the studied populations. As the original description of *D. islandicus* is inaccurate and vague, it would be impossible to describe a new species without redescribing *D. islandicus* first. Therefore, PUBLICATION III can be seen as a necessary first step towards describing the species diversity of the genus *Diaforobiotus*, which until now has been hidden by the ambiguous original description of the type species and the lack of type specimens. My redescription and neotype designation stabilize the taxonomy within the genus. In addition, the comparative analysis allowed me to discuss in detail the species composition of the genus *Diaforobiotus*. Thanks to that, I designated *D. islandicus nicaraguensis* (Séméria, 1985) as a *nomen inquirendum*, improved the diagnosis of the family Richtersiusidae, and prepared a taxonomic key for four (undisputed) *Diaforobiotus* taxa. In conclusion, PUBLICATIONS II and III constitute an integrative revision of the genus, removing pre-existing obstacles and

thus providing a solid basis for further taxonomic and faunistic research on this tardigrade group.

PUBLICATION IV

The total number of species living on Earth is still not precisely estimated, and it ranges from 3 to 15 million species (COSTELLO et al. 2013, MORA et al. 2011). In addition, as mentioned above, biodiversity also seems to be significantly underestimated due to the presence of unrecognized cryptic species, the identification of which is possible mainly based on genetic data (HEBERT et al. 2003). We now know that cryptic species are common in all groups of organisms on our planet (PFENNINGER & SCHWENK 2007) and that further study on them is desperately needed as the existence of such species can have serious implications for our understanding of biodiversity and conservation management (BICKFORD et al. 2007).

The commonness of cryptic or pseudo-cryptic species is also a significant problem in Tardigrada research. PUBLICATION IV is an example of a comprehensive disentanglement of the taxonomy and systematics in the *Macrobiotus pallarii* species complex. It groups closely related and extremely similar species that could be considered cryptic, or at least pseudo-cryptic, i.e. practically indistinguishable. In such cases, an integrative approach using genetic data and detailed morphological characterization is extremely important. In this paper, I provided an integrative revision of the type species for the studied complex, i.e. *Macrobiotus pallarii* Maucci, 1954, based on the newly discovered topotypic population. Updated data on this key species allowed for thorough genetic and morphological comparisons with four additional studied populations tentatively classified only as *M.* cf. *pallarii*. The conducted analyses of the molecular species delimitation and a detailed morphological and morphometric comparative analysis showed the existence of four separate species, three of which were formally described in my work as new to science. In this work, I also presented the taxonomic key for six species that I included in the *M. pallarii* species complex. PUBLICATION IV can constitute a model for research on other complexes of closely related and similar species. The presented integrative revision will certainly benefit other researchers and will be helpful in further research on the diversity of the genus *Macrobiotus* and the complex itself. This is evidenced by the recent

discovery and description of another new species of the *M. pallarii* complex from China (Yuan et al. 2022). It should be noted that the authors would probably not be able to distinguish the new species *Macrobiotus hupingensis* Yuan et al., 2022 without the results presented in PUBLICATION IV.

PUBLICATION V

Modern systematics is a dynamic and exciting field of science that, thanks to advances in molecular biology, is attracting increasing attention from taxonomists and evolutionary biologists. The inclusion of genetic information within species descriptions, e.g. in the form of sequences of specific DNA fragments, is a visible trend. As a result, systematics has at its disposal more and more data resources allowing for an accurate distinction of species and the construction of reliable phylogenies. Because of this, it is possible to discover relationships between species in a group that is of interest to specific researchers. However, in the vast majority of organism groups, phenotypic characters are still a solid and persistent basis for describing a given taxon, where morphology is usually considered the gold standard (PANTE et al. 2015, GOULDING & DAYRAT 2016).

Taking into consideration papers on tardigrade taxonomy published over the last decade, it is easy to see that integrative taxonomy has revolutionized the thinking and understanding of systematic classification and evolution in the phylum Tardigrada. However, despite this, the fifth paper included in the habilitation achievement presented here (PUBLICATION V) concerns the classical description of a new tardigrade species based only on morphology. The new species described there (*Richtersius mazepi* Kiosya & Stec, 2022) is the fourth formally described species in the genus *Richtersius* Pilato & Binda, 1989. It differs from the other three species mainly by the specific morphology of the egg shells. In a recent publication devoted to the redescription of the type species for the genus *Richtersius*, STEC et al. (2020c) expressed the hope that all subsequent descriptions of new species in this genus will be integrative. Contrary to this, PUBLICATION V presents a new species as a working hypothesis (DE QUEIROZ 2007). Many forms of evidence such as morphological, genetic, ecological, reproductive, geographic, and combinations of these can be used to define and delimit a species. In the case of PUBLICATION V, the elevation of a new species is supported only by morphological and morphometric data, which clearly show that the studied population is a species new to science. In

other words, the phenotypic evidence here is sufficient to distinguish this species from other already known taxa. As a co-author of the aforementioned recommendation regarding the value of integrative descriptions, I strongly support this type of research in the taxonomy of tardigrades, especially considering their usefulness in cases similar to those presented in previous publications of my habilitation achievement, e.g., when dealing with cryptic or pseudo-cryptic species. However, situations where some types of evidence will be omitted or impossible to present in species descriptions (e.g. DNA, SEM, physiology) will certainly arise, and the mere inability to consider all possible forms of evidence does not automatically invalidate the establishment of a new species. The prohibition of formulating sufficient and reliable species hypotheses based only on certain types of evidence, proposed in the recent work by GĄSIOREK et al. (2021), is not warranted. As I have written above, it is true that inaccurate and outdated species descriptions do indeed present a serious obstacle to taxonomic research. However, such obstacles cannot be overcome by establishing a single rule that identifies certain data configurations as the only correct solution. Additionally, revisions and redescriptions are relatively readily available tools for taxonomists when species descriptions prove to be insufficient and unsatisfactory. Preventing species descriptions that do not include some arbitrarily selected types of data can seriously hamper our understanding of biodiversity. In the case of the example from PUBLICATION V, without a new description of the species, knowledge about the morphological diversity of egg ornamentation in the genus *Richtersius* would be much more limited. Nomenclature and organisms naming allow species to be cataloged and ensure that they will be included in future taxonomic studies (SEIFERT 2017). The danger associated with difficulties while inventorying live fauna and flora is all the more worrying now, when we live in the times of the so-called sixth mass extinction, and the rate of description of new species is extremely slow (FONTAINE et al. 2012). Since there is no one-size-fits-all solution and no rule that indicates specific data types and thus governs the formal identification of organisms, the responsibility for assigning a name to a given organism rests solely with the authors, who should always try to provide the best possible evidence when testing species hypotheses. The problem is not trivial because species play a key role in biology, and species names have a huge impact on how we assess and treat these fundamental elements of biodiversity, their conservation, and evolution.

FINAL REMARKS

The presented publications are an important contribution to our understanding of the taxonomy, systematics, and evolution of tardigrades. Thus, PUBLICATION I shows the basic problem of taxonomy, which is the formal description of a species new to science, using an integrative approach. PUBLICATIONS II and III are an example of an integrative revision of a higher taxonomic unit using as an example the genus *Diaforobiotus*, while PUBLICATION IV demonstrates a detailed revision of the species complex and the problem of crypticity/pseudo-crypticity in tardigrades. In turn, PUBLICATION V is an example of a classic description of a new species based solely on morphological and morphometric features, also discussing the legitimacy of the so-called traditional approach to taxonomic research. In other words, the presented series of works, which constitute my habilitation achievement, demonstrates the tools and ways of solving problems encountered in the taxonomy and systematics of tardigrades. Tardigrada is not the only group of organisms that struggle with cumbersome and difficult taxonomy, therefore, my research can be used as a model for other groups of microscopic invertebrates, such as rotifers, nematodes, gastrotrichs, and many others.

Four of the works included here were made during my employment at the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, while one of them was done at the end of my doctoral studies at the Jagiellonian University. In two of the presented works, I am the sole author, whereas in the others, I am the leading author. Based on the contribution statements included directly in each coauthor publication, it is clear that my role was crucial. However, I would like to emphasize here that I appreciate the contribution of my coauthors, whose help was very important to me and without whom the creation of this series of works would be much more difficult.

LITERATURE CITED

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D. Individual contribution of the applicant in each publication

My contribution to each of the works included in the achievement was similar and leading. This contribution is confirmed by my separate declaration (attachment 5) and the declarations in the relevant sections included directly in each multiauthor publication.

5. Information on any other scientific or research activity.

A. INTRODUCTION

I decided to apply for the conferment of a post-doctoral degree of habilitated doctor in the subject of tardigrade taxonomy because, in my opinion, it is a subject that perfectly reflects my scientific independence. However, my research so far is by no means limited to this topic. Below, I will briefly discuss my broader scientific and research activity, broken down into the period before and after obtaining a doctoral degree. In this discussion, I will repeatedly refer to the scientific articles indicated in the complete detailed list of achievements (attachment 4).

B. PERIOD BEFORE THE DOCTORAL DEGREE

My first experience with practicing science falls during my MSc studies when I was a scholarship holder and investigator in a scientific project financed by the Foundation for Polish Science. The project concerned experimental research on the phenotypic plasticity of tardigrade traits important to their taxonomy. During my work on the project, I gained unique experience in culturing tardigrades in laboratory conditions and planning experimental research. The results obtained in the conducted research have been published in prestigious scientific journals (e.g. articles 52, 54-56). During my MSc studies, I also became interested in the taxonomy of tardigrades, and my thesis concerned the description of a new to science tardigrade species belonging to the *Macrobiotus hufelandi* complex, which was also published (article 60).

I especially loved the aforementioned species complex of tardigrades. Therefore, during my own research leading to obtaining a doctoral degree, I focused on the taxonomy and evolution of the *M. hufelandi* species complex. My doctoral dissertation consisted of four scientific publications, which presented research that significantly expanded our knowledge about the diversity of the complex (articles 3, 25, 35, 37). However, I also published a number of other original scientific papers about this subject (e.g., articles 2, 7, 16, 24, 33, 36), which, together with the articles included in my PhD thesis, show my high expertise in the systematics of the genus *Macrobiotus*. My studies on the *M. hufelandi* complex that I conducted during this period were financed under the SONATA BIS project. In this project, I acted as an investigator/scholarship holder and one of the research models used in it was the aforementioned group of tardigrades. My doctoral thesis was defended with honors, but earlier my research on the *M. hufelandi* complex was also appreciated by experts. That is, I obtained funding for the ETIUDA 7 grant (NCN), which is in the

form of a scholarship for doctoral students with already formally opened dissertations and is intended to help applicants in finalizing their doctoral thesis.

About halfway through my PhD studies, I also began to extend my taxonomic research interests to the entire superfamily Macrobiotoidea. Therefore, I applied for funding for my additional research in the NCN call, PRELUDIUM 16. My project on the integrative revision of the family Macrobiotidae received funding, and the research itself was continued for more than a year after defending my doctoral thesis. As part of these studies, I published several original scientific papers, of which I consider articles 1, 3, 9, 14, 63, 64, and 68 to be the most important.

In the period before obtaining the doctoral degree, I published 61 original scientific papers in total. The vast majority of them were related to the taxonomy of tardigrades, but the knowledge, skills, and analytical tools that I gained during this time allowed me to participate in some interesting research on other groups of invertebrates. The studies on slugs and shell snails (articles 8 and 10) and antlions (article 11) should be mentioned here. In addition, I was able to turn my hobbyist interest in beekeeping into scientific work. Participation in research projects on the honey bee resulted in several interesting papers on the behavior of this important insect (e.g. articles 4-6, 22).

In the period before obtaining the doctoral degree, I also actively participated in international and national scientific conferences, as well as several foreign internships/research trips, which are described in detail in the relevant sections.

C. PERIOD AFTER THE DOCTORAL DEGREE

After obtaining a doctoral degree, I started working as an assistant professor at the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences (ISEA PAS). In this unit, I intensified and still conduct research on tardigrades. It was during this period of more than a year that I published a significant part of the research that became part of my habilitation achievement presented here. To date, my scientific achievements include 14 original scientific papers published after obtaining a doctoral degree (Articles 62-75), and two more publications are being now in review in recognized scientific journals indexed on the JCR list. The works published by me are to a large extent a continuation of previously crystallized research trends concerning the integrative taxonomy of tardigrades, but also their biology and ecology.

During the first year of work at the ISEA PAS, I also crystallized research topics that I would like to work on more thoroughly in the coming years. They concern the biodiversity and ecology of tardigrades. Using my specialist knowledge of the taxonomy and identification of Tardigrada, I want to study more closely the patterns of their distribution as well as the factors that may influence these patterns. Therefore, this year I prepared a project application as part of the SONATINA 6 call of the National Science Centre, which received funding and has just started. My research project uses an altitudinal gradient, which gives a unique opportunity to study the causes of tardigrade species richness and their distribution. The key point of the project will be the identification of tardigrades using integrative taxonomy and DNA metabarcoding methods. The project itself is highly integrative, as it combines the analysis of many different types of data such as morphological, morphometric, ecological, functional, and genetic data.

In terms of extending my scientific activity to include the ecological aspect, I started a collaboration with a PhD student BARTŁOMIEJ SURMACZ and my colleague Dr. MATTEO VECCHI, who under my supervision wrote projects under the PRELUDIUM 21 and POLONEZ BIS 2 programs, which received funding. In addition, I have actively participated in the editorial work in journals in which I served as associate editor and reviewed many articles for various international and well-recognized journals.

D. FUTURE PLANS

In the near future, I plan to continue research on all of the topics mentioned above appearing in my research work so far. As part of the SONATINA 6 NCN grant awarded to me, I will study the distribution patterns of tardigrades with the altitudinal gradient of the Italian Alps. Together with BARTŁOMIEJ SURMACZ, MSc (funding under the PRELUDIUM 21 grant from the National Science Centre), I will also study the distribution patterns of tardigrades in the gradient of the intensity of human impact in forest environments in Poland. In turn, together with Dr. MATTEO VECCHI (funding under the POLONEZ BIS 2 NCN grant), I will study the ecology of tardigrade communities in ephemeral environments such as rock pools in the Karkonosze Mountains and the Italian Apennines. In all these projects, in addition to advanced statistical methods and modern DNA sequencing techniques, the crucial part will be the preparation of the morpho-genetic reference databases based on the

accurate identification of tardigrades using integrative taxonomy methods. Therefore, I do not plan to abandon my studies on the taxonomy, phylogeny, and evolution of Tardigrada, but rather I plan to develop it further based on the material analyzed within the already received and ongoing but also future scientific projects.

6. Presentation of significant scientific activity carried out at more than one university, scientific institution, especially at foreign institutions.

As I mentioned above, I obtained my doctoral degree at the Jagiellonian University, and to this day I continue my scientific activity at the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences (ISEA PAS).

Before obtaining a doctoral degree, I completed three research trips abroad. Two of them were held as part of the European Commission's SYNTHESYS+ grants, where I conducted research at the Natural History Museum at the University of Copenhagen under the supervision of Professor REINHARDT KRISTENSEN and Professor MARTIN SØRENSEN. These studies focused on the taxonomy of tardigrades and the most important publications that resulted from these trips include articles: 14, 15, 16, 36, 44, 47, 67, 68. Furthermore, I completed a four-month scientific internship at the Water Research Institute (CNR), in Italy, as part of the ETIUDA 7 grant, where my internship supervisor was Dr. DIEGO FONTANETO. Its results include, above all, active participation in the scientific life of the host team and establishing close collaboration with this team, learning statistical methods and programming in the R environment, and developing my interests in ecology and evolution of meiofauna. The aftermath of this trip is a scientific publication on the diversification rate in three large groups of tardigrades (article 66) and close scientific cooperation under the currently implemented SONATINA 6 NCN grant.

During my employment at the ISEA PAS, I have completed one research trip so far, a month-long study visit to Spain, as part of the START 2021 scholarship of the Foundation for Polish Science. I completed the visit at the University of Barcelona. During the trip, I got acquainted with the research team of Professor MIQUEL ARNEDO. I had the opportunity to interact with all its members and learn about the details of their research and the analytical tools they use. I was familiarized with the concept and protocols of genetic research used in biodiversity studies, such as metabarcoding and low-coverage genome sequencing, which are implemented in the visited laboratory.

Through interaction with team members, I was also introduced to research on functional ecology as well as the evolution of functional traits using the concept of hypervolumes as well as advanced analytical techniques of morphometric data. Under the supervision of Professor ARNEDO, during the short period of my study trip, I conducted phylogenetic analyses of spiders in order to date individual speciation events of specific clades and to study the rate of diversification of these organisms. Thus, the study trip allowed me to actually establish collaboration with the unit to which I went, which will continue through possible future joint publications and grant applications that were discussed in detail with my supervisor and his team. As mentioned above, I would like to focus on the distribution and differentiation of meiofauna as part of my future research. Therefore, establishing cooperation with a research group with extensive experience in this field is extremely valuable to me and will contribute to increasing my chances when applying for further research funding.

In addition, I have already planned two more trips abroad. The first will take place in early 2023 as part of the European Commission's SYNTHESYS+ grant, where I will conduct research on the taxonomic revision of the enigmatic family Eohypsybioidea at the Natural History Museum at the University of Copenhagen. The second trip is a six-month internship stay in the unit with which I collaborate as part of the SONATINA 6 project, Water Research Institute (CNR), in Italy. During this internship, I will process the data collected in the first two years of the project and prepare the final versions of scientific publications, drawing as much as possible from the skills and knowledge of the host team, which includes world-renowned scientists dealing with ecology, biodiversity, and the evolution of meiofauna.

7. Presentation of teaching and organizational achievements as well as achievements in popularization of science.

Due to the fact that I have been employed as a researcher since obtaining my doctoral degree, my teaching experience is very limited. During my doctoral studies at the Jagiellonian University, I conducted courses for biology undergraduate students. These were the courses "Zoology - field activities - invertebrates" (in the academic year 2015/2016, 2016/2017, 2017/2018, 2018/2019) and the course "Fauna and Flora of Poland" (in the academic year 2016/2017, 2017/2018 , 2019/2020). In total, it was 381 teaching hours. I also participated in events popularizing science several times, such as

the Małopolska Researchers' Night. In my achievements from the period before obtaining my doctoral degree, I also have a popular science article.

After obtaining a doctoral degree, for the first year of work at the ISEA PAS, I did not conduct didactic classes. However, it should be mentioned here that from 2023 I will act as a supervisor and mentor of the principal investigators of the two previously mentioned projects (PRELUDIUM 21, POLONEZ BIS 2), which will be implemented at ISEA PAS. So far, my role in these projects has been focused on cooperation with their PIs on the application, which concerned the concept, planning, and methodology of the proposed research. In addition, from the beginning of 2023, I will take over the function of a tutor for two students who will carry out year-long internships at the ISEA PAS, also working in the ongoing grants. In addition, as part of popularization activities after obtaining a doctoral degree, I will co-present an interactive show "Secrets of the bee family" during Biologists' Night in January 2023. There, participants will learn the basics of honey bee biology and learn what beekeeping is all about. Participants will also learn about the elements of a hive and will see what it consists of and how it happens that we receive bee products such as honey or pollen. Regarding my organizational activities as part of my work at ISEA PAS, one should mention here the function of the chairman of the committee for the implementation of the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers at ISEA PAS, and the representative of young researchers in the Scientific Council of the Institute in the 2023-2026 term.

Popular articles:

1. **Stec, D.** (2014) Co gryzie nasze pszczoły? *Wszechświat*, 115(4-6): 95-99.

8. Other information.

My research from the period before obtaining the doctoral degree was appreciated several times and I was awarded several scholarships, including the annual scholarship of the Minister of Science and Higher Education for outstanding students (in 2014), three times the annual scholarship of the Minister of Science and Higher Education for outstanding doctoral students (in 2016, 2017 and 2018), a stipend under the ETIUDA 7 grant (2019-2020) and a START stipend funded by the Foundation for Polish Science (awarded in 2021 and fully implemented after obtaining a PhD degree at the ISEA PAS).

In turn, in the transition period between obtaining a doctoral degree and after, my scientific achievements were awarded a three-year scholarship for outstanding young scientists financed by the Minister of Science and Higher Education (2020-2023). An extremely important distinction for me is also the Award of the Prime Minister for an outstanding doctoral dissertation, which I received in the first half of December this year.

Tardigrades have won the hearts of many people around the world, as evidenced by frequent mentions of them in popular science articles appearing on the Web. This also applies to research conducted by me or research in which I participated. Here are some examples:

- https://tech.wp.pl/odkryto-nowy-gatunek-niesporczaka-z-zadziwiajacymi-zdolnosciami,6841262768687648a
- https://dzienniknaukowy.pl/planeta/odkryto-nowy-gatunek-niesporczaka-z-zadziwiajacymi-zdolnosciami
- https://www.rnz.co.nz/news/national/476420/new-species-of-microscopic-creatures-found-living-in-new-zealand-s-glaciers
- https://www.sci.news/biology/glacier-tardigrades-11270.html
- https://www.sciencealert.com/scientists-have-literally-unearthed-a-whole-new-species-of-tardigrade
- https://www.sciencealert.com/brand-new-species-tardigrade-discovered-rock-japan-carpark-macrobiotus-shonaicus-hufelandi
- https://www.sci.news/biology/new-species-tardigrade-japan-05784.html
- https://blog.pensoft.net/2021/05/31/guest-blog-post-new-tardigrade-species-honours-eurovision-song-contest-winner/
- https://www.ibtimes.com/new-tardigrade-species-named-after-eurovision-winner-3213632

In all recently awarded research projects of the National Science Centre, which I mentioned above, actions are also planned to popularize the results of our research, and as such knowledge about tardigrades (workshops, lectures, popular science articles, etc.). Therefore, I am convinced that the press report presented on the research I am conducting or in which I participate is not the last.

Signed by /
Podpisano przez:

Daniel Piotr Stec

Date / Data:
2022-12-20
14:20