SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

Marcin Górniak, PhD

Gdansk, 2023

1. Name

Marcin Górniak

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

Master's degree in Biology - Faculty of Biology, Geography, and Oceanology, University of Gdańsk, 2001, Master's thesis title: "Construction of a Molecular Key for Species Identification in the Genera Cetraria Ach., Platismatia W. Culb., and C. Culb. and Tuckermannopsis Gyel.," supervised by Prof. dr. hab. Grzegorz Węgrzyn.

Doctoral degree in Biological Sciences in the field of Biology - Institute of Biology, Faculty of Biology, Geography, and Oceanology, University of Gdańsk, 2007, Doctoral dissertation title: "Classification of the Order Orchidales Based on Analysis of Selected DNA Fragments," supervised by Prof. dr hab. Dariusz L. Szlachetko.

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

2005 – 2006: Department of Plant Taxonomy and Nature Conservation, Faculty of Biology, University of Gdańsk - Senior Technician.

2007 – 2008: Department of Plant Taxonomy and Nature Conservation, Faculty of Biology, University of Gdańsk - Specialist.

2008 – 2013: Department of Plant Taxonomy and Nature Conservation, Faculty of Biology, University of Gdańsk - Assistant Professor.

2013 – 2021: Department of Molecular Evolution, Faculty of Biology, University of Gdańsk - Assistant Professor.

2021 – Present: Department of Evolutionary Genetics and Biosystematics, University of Gdańsk - Assistant Professor.

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

4A. Title of scientific achievement

The use of low-copy nuclear molecular markers in phylogenetic analyses at various taxonomic levels within the Orchidaceae family

4B. A series of published scientific articles forming the basis of the postdoctoral achievement

1. **Górniak, M***., Paun, O., Chase, M.W. Phylogenetic relationships within Orchidaceae based on a low-copy nuclear coding gene, *Xdh*: Congruence with organellar and nuclear ribosomal DNA results. *Mol. Phylogenet. Evol.* 2010, *56*, 784–795. https://doi.org/10.1016/j.ympev.2010.03.003
   IF_{2010}: **3.889**, quartile (JIF): **Q1**; MNiSW[1]: 32
   citations: **94** (WoS); **98** (Scopus)

   My contribution to the creation of the publication included: developing the concept of the work, participating in the design of new primers for the amplification of the *Xdh* gene for representatives of the Orchidaceae family, isolating, amplifying, and sequencing DNA, depositing the sequences in the NCBI database, preparing the matrix for analysis, conducting phylogenetic analysis, literature review, manuscript writing, creating figures, incorporating changes in the text based on reviewers' suggestions, and responding to reviews. I estimate my contribution at 80%.

2. Szlachetko, D.L., Kolanowska, M., Muller, F., Vannini, J., Rojek, J., **Górniak, M***. First Guatemalan record of natural hybridisation between Neotropical species of the Lady's Slipper orchid (Orchidaceae, Cypripedioideae).*PeerJ* 2017, *5*, e4162. https://doi.org/10.7717/peerj.4162
   IF_{2017}: **2.118**, quartile (JIF): **Q2**; punktacja MNiSW[1]: **35**
   citations: **11** (WoS), **12** (Scopus)

   My contribution to the creation of the publication included: developing the concept of the work, isolating, amplifying, and sequencing DNA, preparing the matrix for analysis, conducting phylogenetic analysis, literature review, writing the discussion section of the manuscript, creating some of the figures, incorporating changes in the text based on reviewers' suggestions, and responding to reviews. I estimate my contribution at 50%.

3. Szlachetko, D. L., **Górniak, M***., Kowalkowska, A. K., Kolanowska, M., Jurczak-Kurek, A., and Morales, F. A. The natural history of the genus *Cypripedium* (Orchidaceae). *Plant Biosyst. Int. J. Deal. Aspects Plant Biol*. 2020, 155, 772–796.

IF$_{2021}$: **1,781**, quartile (JIF): **Q3**; punktacja MEiN^{2}: **40**
citations: **8** (WoS), **7** (Scopus)

My contribution to the creation of the publication included: developing the concept of the work, isolating, amplifying, and sequencing DNA, depositing the sequences in the NCBI database, preparing matrices for analysis, conducting phylogenetic analyses, capturing micro-morphological images, including scanning electron microscopy (SEM) images, conducting literature review, writing the manuscript, creating figures 1-6, incorporating changes in the text based on reviewers' suggestions, and responding to reviews. I estimate my contribution at 60%.

4. **Górniak, M***., Szlachetko, D.L., Kowalkowska, A.K., Bohdanowicz, J., Canh, C.X. Taxonomic placement of *Paphiopedilum canhii* (Cypripedioideae; Orchidaceae) based on cytological, molecular and micromorphological evidence. *Mol. Phylogenet. Evol.* 2014, *70*, 429–441**.** https://doi.org/10.1016/j.ympev.2013.08.016
IF$_{2014}$: **3,916,** quartile (JIF): **Q1**; MNiSW^{1}: **30**
citations: **10** (WoS), **11** (Scopus)

My contribution to the creation of the publication included: developing the concept of the work, designing new primers for the amplification of the *Xdh* gene for representatives of the Cypripedioideae subfamily, isolating, amplifying, and sequencing DNA, depositing the sequences in the NCBI database, preparing matrices for analysis, conducting phylogenetic analyses, capturing micro-morphological images, including scanning electron microscopy (SEM) images, conducting literature review, writing the manuscript, creating some of the figures, incorporating changes in the text based on reviewers' suggestions, and responding to reviews. I estimate my contribution at 80%.

5. **Górniak, M***., Szlachetko, D.L., Olędrzyńska, N., Naczk, A.M., Mieszkowska, A., Boss, L., Ziętara, M.S. Species phylogeny versus gene trees: A case study of an incongruent data matrix based on *Paphiopedilum* Pfitz. (Orchidaceae). *Int. J. Mol. Sci.* 2021, *22*, 11393. https://doi.org/10.3390/ijms222111393
IF$_{2021}$: **6,208** , quartile (JIF): **Q1**; MEiN^{2}: **140**
citations: **2** (WoS); **3** (Scopus)

My contribution to the creation of the publication included: developing the concept of the work, designing new primers for the amplification of the *PhyC* gene for representatives of the subfamily Cypripedioideae, isolating, amplifying, and sequencing DNA, depositing the sequences in the NCBI database, preparing matrices for analysis, phylogenetic analysis, literature review, manuscript writing, creating figures, incorporating changes in the text based on reviewers' suggestions, and responding to reviews. I estimate my contribution at 85%.

* the role of the corresponding author in articles published with other authors.

Total Impact Factor 1 year **IF** in the year of publication of the scientific achievement is **17,912**

Total ministerial points of scientific achievement: **277** points, including **97** points (scoring based on the list of the Ministry of Science and Higher Education dated January 25, 2017) and **180** points (scoring based on the list of the Ministry of Education and Science dated December 21, 2021).

4C. Description of the series of publications constituting the scientific.

Despite numerous studies on the phylogeny of individual taxa within Orchidaceae Juss., the classification of many systematic units continues to be a source of controversy and discussion. Previous classification systems were based on various features of both vegetative, generative, and genotypic characteristics. The abundance of publications on this topic indicates that the challenge of systematizing this diverse taxon, abundant in species and genera, remains open.

Traditional taxonomic systems rely on morphological and anatomical features. Due to convergent evolution influenced by pollinator pressures, certain morphological characteristics of flowers have independently evolved in different species during the course of evolution. The selection of these features for analysis can lead to the creation of polyphyletic taxa. To address these issues, rapidly advancing molecular biology techniques such as PCR and DNA sequencing, widely used in taxonomic research, have been employed. The application of molecular techniques has allowed the establishment of phylogenetic relationships between studied plant groups. Taxonomic studies of Orchidaceae using molecular features have been conducted at both lower taxonomic levels (genera, tribes) and at the family level.

Due to the variability of molecular markers at the lower taxonomic level, noncoding chloroplast markers and the ITS1-5.8S rDNA-ITS2 fragment from the nuclear genome (nrITS) have been used. At the family level, the focus has been on genes encoding proteins from the plastid genome, such as *rbcL* (Cameron et al. 1999, Chase et al. 1994), *matK* (Freudenstein et al. 2004, **Górniak 2007)**, *psaB* (Cameron 2004), and *ycf1* (Neubig et al. 2009). Additionally, studies involving the nuclear coding sequence of the 18S rDNA fragment (Cameron and Chase

2000) and the mitochondrial intron *nad1 b-c* (Freudenstein and Chase 2001) have been conducted. However, due to the limited variability of both mentioned markers, phylogenetic analyses using them did not resolve the relationships within individual subfamilies.

Another challenge in determining relationships within the Orchidaceae family is widespread intergeneric and interspecific hybridization. Due to the maternal inheritance of chloroplast markers, commonly used in molecular systematics, they only reflect the phylogeny of one of the parental species, a phenomenon known as chloroplast capture. This complicates understanding the evolutionary history of the studied group. Similar issues may arise when using repetitive units of rDNA, including nrITS, as they undergo evolutionary processes leading to the homogenization of rDNA units (concerted evolution). Verification of results obtained from analyses of sequences derived from uniparentally inherited genomes or rDNA can be carried out using low-copy nuclear markers encoding proteins.

In **Publication 1**, I utilized a low-copy *Xdh* gene fragment to address taxonomic issues at the family level in Orchidaceae. Considering limitations of the nrITS marker for phylogeny at lower taxonomic levels (Cox et al. 1997, Chochai et al. 2012), I sought to determine whether low-copy nuclear markers could also be used to address taxonomic issues at the genus level. I chose orchids characterized by the presence of two fertile stamens and a unique lip structure resembling a slipper (Cypripedioideae) as a model. This subfamily includes five genera: *Selenipedium* Rchb.f., *Cypripedium* L., *Phragmipedium* Rolfe, *Mexipedium* V.A.Albert & M.W.Chase, and *Paphiopedilum* Pfitzer. I incorporated information from molecular, cytological, and morphological (macro- and micromorphological) studies in my research on this orchid group (**Publications 2-5**). In these publications, I present phylogenetic relationships based on DNA sequence analysis and use a molecular clock to determine the divergence times of analyzed species in the context of geological changes during the Eocene and Miocene.

I focused on two of the most species-rich genera, *Cypripedium* (**Publication 2 and 3**) and *Paphiopedilum* (**Publication 4 and 5**). Previous publications highlight many unresolved issues regarding the phylogeny and intrageneric systematics of these taxa (Cox et al. 1997, Chochai et al. 2012, **publikacja 4,** Guo et al. 2015). Molecular studies based solely on the nuclear nrITS sequence, in many cases, do not confirm classifications based on morphological

features. In the case of the *Paphiopedilum* genus, analyses of low-copy nuclear markers also revealed unresolved or conflicting topologies (Guo et al. 2015). The use of a single gene for creating a classification system is often prone to error, as the analysis of selected DNA sequences is used as a source of phylogenetic information for the entire organism. Analyzing two different genes may reveal different evolutionary lineages (Guo et al. 2015). It is crucial to include as many molecular markers as possible in the analysis, considering that the observed sequence variability may result from homoplasy, recombination, incomplete lineage sorting, or even the sequencing of pseudogenes.

In my work, I critically analyze phylogenies based on molecular features, comparing them with morphological concepts. I also propose hypotheses to explain probable causes of discrepancies. My research aligns with one of the main currents in contemporary biology. The publications included in my habilitation achievement bridge the gap between the nrITS era and next-generation sequencing (NGS). NGS has provided the opportunity to explore a significant portion of the genetic material through RADseq (restriction site-associated DNA sequencing) and, in many cases, has reinstated morphological concepts that were displaced by nrITS phylogenetics (Brandrud et al. 2018). Despite the many advantages of NGS, I argue that using several nuclear markers along with morphological feature analysis allows reconstructing the evolutionary history of studied taxa without the need for high-budget projects.

Publication 1: **Phylogenetic relationships within Orchidaceae based on a low-copy nuclear coding gene, *Xdh*: Congruence with organellar and nuclear ribosomal DNA results**

The first study pertains to determining phylogenetic relationships within the Orchidaceae family based on a low-copy nuclear *Xdh* gene fragment. Attempts to classify the Orchidaceae family date back to the 18th century (Juss). Since then, numerous classification systems have been developed based on the analysis of morphological characteristics (Burns-Balogh and Funk 1986, Dressler 1981, 1993, Rasmussen 1985, Szlachetko 1995). Due to the utilization of various morphological features or the emphasis placed on specific characteristics, contemporary systems are in conflict with one another. The use of molecular

features, such as DNA sequences, has allowed the verification of existing systems based on inherited traits. Chase et al. (2003) synthesized molecular classification based on dozens of publications, proposing five subfamilies, which I present below in Newick format: (Apostasioideae, (**Vanilloideae**, (Cypripedioideae, (**Epidendroideae**, **Orchidoideae**)))). The distinction of these subfamilies was in line with some morphological concepts. However, the phylogenetic relationships between them were controversial, as they suggested that the characteristic of a single fertile stamen (subfamilies possessing this trait are indicated in bold) evolved twice in the course of Orchidaceae evolution. Morphological-based systems indicated the monophyly of one-stamen orchids: (Apostasioideae, (Cypripedioideae, (**Vanilloideae**, (**Epidendroideae**, **Orchidoideae**)))).

In this study, I employed the parsimony method and Bayesian analysis to estimate phylogenetic relationships within the Orchidaceae family, focusing on subfamilies and tribes. I obtained DNA sequences of the *Xdh* gene fragment for 154 taxa, including 126 genera of Orchidaceae and taxa from an outgroup. Until the publication of this article, for which I am the first author, molecular systematics of Orchidaceae were based solely on chloroplast DNA fragments, non-coding mitochondrial genome fragments, and nuclear repetitive rDNA. The choice of the *Xdh* marker proved to be very appropriate but required extensive work in the laboratory. Nevertheless, the results were highly spectacular. The level of variability of the studied marker contributed to the determination of phylogenetic relationships within the Orchidaceae family. The general topology of the *Xdh* trees is consistent with previously published phylogenetic trees. The five recognized subfamilies are monophyletic and well-supported. My results indicate that the monandrous state evolved independently in the ancestors of Vanilloideae and Epidendroideae/Orchidoideae.

My analysis also clarified relationships between tribes in the subfamily Epidendroideae, such as Vandeae *sensu lato* to Collabieae and Epidendreae to Calypsoeae and Malaxideae to Dendrobieae. The phylogenetic position of "problematic" taxa like *Bromheadia* Lind., *Imerinaea* Schltr. and *Sirhookera* Kuntze, whose phylogenetic position was unclear, was also resolved.

Phylogenetic relationships of leafless (lacking active plastid genes) species from the

genera *Corallorhiza* Châtel., *Gastrodia* R.Br., *Limodorum* L., *Neottia* Guett., and *Wullschlaegelia* Rchb. f. were analyzed on such a broad scale for the first time in molecular phylogenetics. Subsequent analyses based on NGS sequencing results from 292 low-copy nuclear genes (Pérez-Escobar et al. 2021) confirmed my results and conclusions, reaffirming my assumption that the proper analysis of individual markers can lead to the same results. The high citation rate of this article is a direct testament to the scientific interest in my findings. I anticipate that at the time of assessing my scientific achievements, it will have received 100 citations in the Scopus database.

Publication 2: **First Guatemalan record of natural hybridisation between Neotropical species of the Lady's Slipper orchid (Orchidaceae, Cypripedioideae)**

In 2008, the first author of this article received a set of colorful photographs of multi-flowered species from the genus *Cypripedium L*., taken by Fred Muller, an orchid enthusiast from Guatemala. Mr. Muller suspected that he had discovered a naturally occurring hybrid in a sympatric population of *C. irapeanum* Lex. and *C. dickinsonianum* Hágsater. Previous reports indicated that no hybridization occurred between these species (Hagsater 1984, Cribb 1997). Both mentioned species, as well as *C. molle* Lindl., belong to the Irapeana section. Since the discovery of the putative hybrid, both *C. irapeanum* and *C. dickinsonianum* have maintained stable populations at this particular location. Many individuals of the putative hybrid have also bloomed every year since their discovery. Observations conducted by Fred Muller revealed that both species are pollinated by small species of the *Trigona* genus (Jurine, 1807), as well as other small bees. A high percentage of fruit set was also observed in this population. Upon establishing collaboration with Mr. Muller, we had the opportunity to examine the morphology of the mixed population, conduct analyses of molecular marker variability, and perform embryological studies on the putative new Cypripedium taxon.

To detect hybridization, I used the nrITS fragment, the low-copy Xdh gene, and the plastid gene *matK*. Evaluating the variability of these markers for individuals from the sympatric population and additionally for *C. molle*, which is closely related to *C. irapeanum*, allowed me to detect hybridization. Basing conclusions solely on the highly copied nrITS marker can be insufficient. Based on the analysis of variability in both nuclear markers, I

determined the heterogeneity of sequences, demonstrating the presence of the ITS allele and the *Xdh* allele of both *C. irapeanum* and *C. dickinsonianum* in the genome of the potential hybrid. Meanwhile, the identical chloroplast fragment between the hybrid and *C. irapeanum* allowed me to identify *C. irapeanum* as the seed parent.

Another intriguing aspect for me was to examine whether the studied hybrids were capable of reproducing. Hybrid populations, especially the F1 generation, often suffer from reduced fertility, resulting in both weak seed viability and the production of unbalanced gametes (Rieseberg 1997). To investigate this, I invited Dr. Joanna Rojek, a plant embryologist, who demonstrated undisturbed development of ovules and megagametophytes, suggesting that the *C. fred-mulleri* hybrid is likely fertile.

Based on the evidence of gene flow between *C. dickinsonianum* and *C. irapeanum*, as well as certain mixed morphological characteristics in the studied population discovered by Mr. Muller in Guatemala, we decided to describe it as the first natural hybrid in the Irapeana section, under the name *Cypripedium fred-mulleri* Szlach., Kolan. & Górniak, hybr. nov.

Through my work, I have emphasized the need to use single-copy nuclear markers to detect hybridization as a complement to alpha taxonomy because morphological traits do not always strictly confirm the hybrid origin of the studied mixed specimens. *C. fred-mulleri* is more similar to *C. irapeanum* than *to C. dickinsonianum* in terms of flower morphology. This phenomenon, indicating the similarity of hybrid flowers to the maternal lineage, is common within the Orchidaceae family (Bateman & Farrington 1987, Bateman & Hollingsworth 2004, Bateman, Smith & Fay 2008, R. Bateman pers. comm. 2017).

Publication 3: **The natural history of the genus *Cypripedium* (Orchidaceae)**

One of the most taxonomically and biogeographically interesting genera of orchids is undoubtedly the genus *Cypripedium* L.. While representatives of *Cypripedium* are easily distinguishable from other lady's slipper orchids by their plicated leaves and single-chambered ovary, some species have been considered members of smaller genera: *Arietinum* L.C. Beck, *Calceolus* Mill., *Criosanthes* Raf., Fissipes Small, *Hypodema* Rchb., or *Sacodon* Raf. However, none of these proposals are currently accepted. Recent intensive research on the

intra-generic classification of *Cypripedium* has led to a significant increase in the number of sections. In Cribb's (1997) concept, *Cypripedium* species were included in 11 different sections. An additional section - *Sinopedilum* - was recently proposed by Perner (2008). The division of *Cypripedium* into two subgenera and 13 sections was accepted by Eccarius (2009).

Although Cypripedium is considered a monophyletic genus (Cox et al. 1997, Cribb 1997, Eccarius 2009), relationships between sections representing Asian and North American species remain unclear. Recent molecular studies have indicated a monophyletic grouping of eight sections (*Subtropica, Obtusipetala, Trigonopedia, Sinopedilum, Bifolia, Flabelinervia*, *Arietinum*, and *Cypripedium*), as well as a paraphyletic grouping of sections *Irapeana* and *Retinervia*, and subsections *Macrantha* and *Cypripedium* from section *Cypripedium* (Li et al. 2011).

Based on molecular phylogenetic analysis using nrITS and chloroplast DNA fragments (Li et al. 2011), Liu et al. (2012), Chen and Liu (in Chen et al. 2013) suggested the creation of additional monotypic sections (*Californica, Palangshanensia, Wardiana*). The total number of sections in the *Cypripedium* genus reached 15. Based on the same study (Li et al. 2011), Frosch and Cribb (2012) divided the genus into 13 sections. The main difference was the inclusion of *C. palangshanense* in the *Enantiopedilum* section and *C. wardii* in the *Subtropica* section. Although both systems are based on the same results (Li et al. 2011), they also differ in the species composition of the *Macrantha* subsection and *Cypripedium* subsection within the *Cypripedium* section. Only in Chen and Liu's classification (in Chen et al. 2013) is the *Macrantha* subsection monophyletic. However, in both systems, the *Cypripedium* subsection is polyphyletic. The authors maintained the grouping of the *Cypripedium* subsection based on morphological traits, leaving Asian species: *C. calceolus*, *C. shanxiense*, *C. henryi*, and *C. segawae*, along with North American species.

In my research, I aimed to resolve the described conflict by additionally using a low-copy *Aco* gene fragment from the nuclear genome. The analysis of this marker is consistent with the results obtained based on the analysis of the nrITS fragment (Li et al. 2011, publication 3). Based on this, I decided to combine both matrices to increase the phylogenetic signal. The results obtained from the nrITS/Aco matrix (with a noticeable increase in bootstrap support) suggest the polyphyletic nature of the *Cypripedium* subsection. Additionally, in this

study, I performed a reanalysis (created a new alignment) of chloroplast DNA markers from Li et al.'s work (2011), obtaining in my analysis a clade of the *Cypripedium* subsection supported by a bootstrap test at the level of 91. The result I obtained can be explained by an alternative DNA matrix arrangement compared to Li et al. (2011).

I have thus identified a probable conflict between chloroplast and nuclear regions, suggesting a possible hybridization process in this group. To ultimately resolve this conflict, a significantly larger number of nuclear molecular markers are required, as the genomes of potential hybrids may be mosaic. For this reason, we suggest maintaining the approach of Li et al. (2011) regarding the *Cypripedium* subsection based on morphological characteristics and chloroplast DNA analysis.

In this study, I also estimated the divergence times of individual evolutionary lineages within the *Cypripedium* genus. I used the nrITS sequence and concatenated chloroplast DNA fragments for molecular clock analysis. According to the analyses, the most recent common ancestor of *Cypripedium* appeared in the early Eocene. The *Irapeana* section clade is the oldest phylogenetic lineage within the *Cypripedium* genus. Analyses indicate that three major evolutionary lineages diverged after the climatic optimum of the middle Eocene (approximately 41 million years ago). Other significant evolutionary processes occurred in the late Oligocene and early Miocene. During this period, numerous divergences occurred within groups of species characterized by transcontinental disjunction.

Additionally, this work features micro-morphological photos obtained under a light microscope and a scanning electron microscope (SEM). The photos were taken in collaboration with Dr. Agnieszka Kowalkowska (currently dr. hab. Agnieszka Kowalkowska, Prof. UG), who also described the mentioned photos as part of this study. Numerous photos of selected *Cypripedium* species and *Selenipedium aequinoctiale* can serve as source material for comparative analysis once a phylogenetic tree of the Cypripedioideae subfamily based on multiple molecular markers is obtained.

Publication 4: Taxonomic placement of *Paphiopedilum* canhii (Cypripedioideae; Orchidaceae) based on cytological, molecular and micromorphological evidence

In 2009, on rocky limestone in northern Vietnam, a new lithophytic species from the genus *Paphiopedilum* Pfitzer was discovered. *Paphiopedilum canhii* Aver. & O. Gruss was described in 2010 by Averyanov and Gruss based on specimens from the orchid enthusiast Chu Xuan Canh's collection (Vietnam, Hanoi). Morphological characteristics such as marbled leaves, single-flowered inflorescence, and the petal/sepal ratio indicated a close relationship of this species with the Barbata section (Kraenzl.) V.A. Albert & Börge Pett. However, Averyanov et al. (2010) noticed certain leaf features, staminodium, lip, and inner whorl petal characteristics that suggested an intermediate position for *P. canhii* between the Barbata and Parvisepalum Aver. & Crbb. sections. Consequently, they proposed describing a new section for *P. canhii*. Later, Braem and Gruss (2011) suggested a new subgenus, *Megastaminodium* Braem & O. Gruss, for this species. Subsequently, Averyanov (Averyanov et al. 2011) placed *P. canhii* in a new monotypic section, *Pygmaea Aver*., within the subgenus Paphiopedilum. Until my publication, nucleotide sequences for phylogenetic analysis to verify the systematic position of this newly described species had not been obtained.

During a scientific expedition to Vietnam in 2011, I obtained material from the collection of C. X. Canh, which I used to isolate and sequence DNA. I was the first person in the world to obtain DNA sequences of this newly described species.

In this work, using parsimony and Bayesian analysis, I estimated the phylogenetic relationships within the Paphiopedilum genus, also indicating the systematic position of the problematic taxon. Additionally, I determined the chromosome number (2n=26) in collaboration with dr. hab. Jerzy Bohdanowicz, Prof. UG, and took photos of flower structures using a light microscope and SEM (in collaboration with dr. Agnieszka Kowalkowska). Determining the chromosome number and micro-morphological features allowed me to exclude close relationship between *P. canhii* and the *Barbata* section (2n=28-46).

In the phylogenetic analysis, I selected species representing all intra-generic taxa of Paphiopedilum and species forming the outgroup. I used three chloroplast markers, nrITS,

and a low-copy nuclear *Xdh* gene fragment. This was the first study that used a low-copy marker for phylogenetic analysis of the *Paphiopedilum* genus on such a broad scale. Until the publication of my work, the molecular systematics of this genus relied solely on nrITS (Cox et al. 1997, Chochai et al. 2012) and chloroplast DNA fragments (Chochai et al. 2012).

My analyses, based on five molecular markers, are consistent with the results published by Chochai et al. (2012) regarding relationships between subgenera. All three subgenera distinguished by Cribba (1998), namely *Parvisepalum*, *Brachypetalum*, and *Paphiopedilum*, are well-supported in chloroplast DNA and combined analyses. On the other hand, none of the nuclear markers (nrITS and *Xdh*) supported the monophyly of the widely recognized subgenus *Paphiopedilum sensu* Cribb 1998. The lack of this support was a puzzle since morphological traits (Cribb 1998) and chloroplast DNA analysis (Chochai et al. 2012, Górniak et al. 2014) unequivocally supported this taxon. Another study (Guo et al. 2015), which presented phylogenetic trees of four nuclear genes, did not resolve this issue, revealing conflicts between them. I presented a hypothesis regarding the cause of the conflict in the topology of nuclear phylogenetic trees and the lack of support for the monophyly of the subgenus *Paphiopedilum* in **publication 5**.

In summary, both molecular and morphological features indicated that *P. canhii* should be placed in a separate taxon. The isolated position of *P. canhii* in nuclear phylogenetic trees presented in this article, combined with a unique set of morphological characteristics, confirms the status of the distinct subgenus *Megastaminodium* within the *Paphiopedilum* genus. Therefore, to maintain the monophyly of the subgenera, I suggested elevating sections *sensu* Cribb (1998) from the subgenus *Paphiopedilum* to subgenus rank.

My work highlighted the need to use additional molecular markers outside the chloroplast and nrITS genome regions to fully understand the evolutionary history of the studied taxa. The choice of the low-copy *Xdh* gene revealed conflicts in the topology of chloroplast and nuclear trees, suggesting probable hybridization within the subgenus *Paphiopedilum* and the hybrid origin of *P. canhii*. Several years later, Guo et al. (2021) demonstrated the close relationship of the chloroplast genome of *Paphiopedilum canhii* to the multiflower sections (*Coryopedilum, Pardalopetalum, and Cochlopetalum*) within the subgenus *Paphiopedilum*, corroborating my hypothesis about the hybrid origin of *P. canhii*.

Publication 5: Species phylogeny versus gene trees: A case study of an incongruent data matrix based on *Paphiopedilum* Pfitz. (Orchidaceae)

The intrageneric classification of *Paphiopedilum* Pfitzer primarily relies on flower morphology and leaf types (Brieger et al. 1971, Karasawa & Saito 1982, Cribb 1998, Bream et al. 1998, Hennessy et al. 2000, Bream & Chiron 2003). Analyses of chloroplast DNA (Chochai et al. 2012, **publication 4**) strongly support this classification, which distinguishes three subgenera: *Parvisepalum, Brachypetalum*, and *Paphiopedilum*. The relationships between sections within the subgenus Paphiopedilum were also confirmed by the analysis of plastid DNA (Chochai et al. 2012, publication 4), revealing the existence of two evolutionary lineages: one containing single-flowered species (sections *Barbata* and *Paphiopedilum*) and the other containing multi-flowered species (i.e., sections *Coryopedilum* - *Pardalopetalum* and *Cochlopetalum*). Within the latter lineage, *P. canhii* is also found, which was further supported by the analysis of chloroplast genome (Guo et al. 2021).

However, it is worth noting that the phylogenetic position of the analyzed taxa reconstructed based on chloroplast genomes is not supported by low-copy nuclear genes *Xdh* (Górniak et al. 2014), *Aco, Def4, Rad51, Lfy* (Guo et al. 2015), and nrITS (Chochai et al. 2012, Górniak et al. 2014) analysed until the publication of the present study.

In this study, I designed new primers for the low-copy nuclear gene *PhyC*, the phylogenetic analysis of which confirmed the division of the subgenus Paphiopedilum into the two evolutionary lineages mentioned above (*P. canhii* was not analyzed). Despite obtaining congruence in the topology of the *PhyC* gene tree with the plastid tree, I wondered about the reasons for the incongruence in the tree topologies of the remaining nuclear markers.

In this work, I conducted a reanalysis of all the aforementioned markers from Guo et al. (2015) and Górniak et al. (2014) (**publication 4**), confirming conflicts in topology. Phylogenetic analyses for all markers were conducted using the Bayesian method based on the molecular clock theory, applying secondary calibration points. I noticed a certain pattern of incongruence/lack of resolution in phylogenetic relationships between sections and the divergence time (tMRCA) within the subgenus Paphiopedilum.

The topology of the Aco and Def4 gene trees showed unresolved relationships (polytomy) between sections, but strongly supported the subgenus Paphiopedilum, just like the combined analysis of plastid markers. Additionally, the divergence time of the subgenus Paphiopedilum was consistent with the plastid tree.

On the other hand, the topologies of the remaining nuclear gene trees displayed various relationships between sections, but these were inconsistent with morphological concepts. The conflict concerned the positions of sections *Barbata* (Xdh), *Cochlopetalum* (Rad51), and *Barbata* and *Cochlopetalum* (Lfy). It is worth noting that both of these sections have a higher chromosome number than the ancestral (pleiomorphic) one. Previous studies have shown that the increased chromosome number was due to their divergence (Karasawa & Saito 1982).

Additionally, a common feature of these phylogenetic trees was the lack of support for the subgenus Paphiopedilum. I assumed that the lack of support for the subgenus Paphiopedilum in the analysis of *Xdh, Lfy, Rad51, PhyC* markers might be due to weak phylogenetic signals. Therefore, I decided to perform a phylogenetic analysis by combining these matrices to obtain support for the subgenus Paphiopedilum.

A very interesting result of this analysis (analysis of the combined matrix *Xdh, Lfy, Rad51, PhyC*) was also that the topology of the obtained tree concerning relationships between sections was identical to the topology of the *Lfy* gene tree. This result confirms that combining matrices with conflicting phylogenetic signals is inappropriate and can lead to the distortion of the evolutionary history of the studied taxa. While combining these matrices, I was aware of this error, but I aimed to strengthen the signal that was not in conflict (the clade of subgenus *Paphiopedilum*).

What also drew my attention in these analyses was a significant difference in estimating the divergence time of the subgenus *Paphiopedilum* (tMRCA) based on the combined markers *Xdh, Lfy, Rad51, PhyC* compared to the combined analysis of *Aco/Def4* markers and the chloroplast tree. This was the moment when I saw all the puzzle pieces fall into place.

Based on the analysis of all conflicting phylogenetic tree topologies, I hypothesized that these conflicts were caused by ancestral hybridization, which occurred in the ancestor of the subgenus Paphiopedilum and preceded the differentiation into sections within this subgenus. This hypothesis also explained the conflict between sections in nuclear genes and the agreement between the analysis of maternally inherited chloroplast DNA and morphological features. I supported the conflict in nuclear genes based on processes directly resulting from hybridization (Stebbins 1957, Grant 1958, Buerkle et al. 2000, Folk et al. 2018).

The nodes on the phylogenetic trees for the subgenus *Paphiopedilum* in the analysed studies concerned proto-Paphiopedilum (before hybridization - trees Xdh, Lfy, Rad51, PhyC) and *Paphiopedilum* (after hybridization - trees *Aco, Def4*, and chloroplast tree).

To test my hypothesis, I also used the Phylonet program (Than et al. 2008), which, based on the conflicting topologies of phylogenetic trees (not matrices) obtained in this study, confirmed ancestral hybridization as the most likely cause of incongruence in the topologies of phylogenetic trees.

Summary

The reconstruction of the phylogeny of Orchidaceae based on low-copy nuclear genes has resolved many taxonomic issues at each of the studied levels. I have demonstrated that the variability of the analysed markers, when the appropriate analysis accounting for possible evolutionary pathways is applied, is suitable for analysis at both the family and genus levels. In particular, I have highlighted the potential use of these markers for detecting both hybridization in young hybrids and ancestral hybridization. I have also indicated a way to analyse molecular data that exhibit conflicting phylogenetic signals. Despite conflicts in the topologies of phylogenetic trees when considering morphological traits and analyses of maternally inherited markers, I have described the evolutionary history of the studied taxon.

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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

Below is an overview of my scientific activities. The complete list of my scientific achievements is presented in Attachment 4, titled " List of scientific or artistic achievements which present major contribution to the development of a specific discipline”

5A. Prior to obtaining a doctoral degree

I conducted my master's thesis as part of an interdisciplinary molecular-taxonomic team, which was the result of collaboration between the Department of Plant Taxonomy and Nature Conservation, led by Prof. dr. hab.. Dariusz L. Szlachetko, and the Department of Molecular Biology, led by Prof. dr. hab. Grzegorz Węgrzyn. After completing my studies in 2001, I enrolled in the Environmental Doctoral Studies at the University of Gdańsk. My supervisor was Prof. Dr. hab. Dariusz L. Szlachetko, who entrusted me with the task of establishing the Molecular Taxonomy Laboratory in his home department. My responsibility was to introduce molecular biology techniques, including PCR and DNA sequencing, and apply them in taxonomic research. To fulfill this task, I undertook a short-term internship at the Department of Plant Systematics and Geography at the University of Warsaw, where I familiarized myself with the procedures for obtaining ITS1-5.8S-ITS2 ribosomal rDNA sequences using the Sanger method. Throughout my doctoral studies, I implemented these techniques and also supervised master's and doctoral students at the KTRiOP.

In 2003, I embarked on an internship at the University of Copenhagen (Denmark), funded by the Copenhagen Biosystematics Centre (COBICE) as part of the European scholarship program "Improving Human Potential." This internship focused on molecular techniques and sequence data analysis. During my stay, I participated in scientific seminars and informal meetings, which prepared me for the challenges I

would encounter in laboratory work and sequence data analysis. This internship opened up the possibility of utilizing plastid sequences in plant systematics and drew my attention to the potential use of the matK gene as an alternative or verification method for the ITS1-5.8S-ITS2 region in plant systematics. I used the DNA sequences obtained during the internship in publications, including Górniak, M., Mytnik-Ejsmont, J., Rutkowski, P., Tukałło, P., Minasiewicz, J., & Szlachetko, D. L. (2006). "Phylogenetic relationships within the subtribe Spiranthinae sl (Orchidaceae) inferred from the nuclear ITS region" and Kułak, M., Górniak, M., & Romowicz, A. (2006). "Tribal and subtribal relationship of Epidendroideae Lindl. (Orchidaceae) with emphasis on Epidendreae Humb., Bonpl. & Kunth based on matK gene."

Another significant milestone in my scientific career was presenting a lecture titled "Molecular Taxonomy versus Orchid Classification" at the Dresdner Orchideen-Welt conference in Dresden in 2004. The lecture addressed the discrepancies between molecular systematics based on the ITS1-5.8S-ITS2 fragment and morphological characteristics in the genus Paphiopedilum. At that time, I was unaware that I would eventually resolve this conflict in the distant future (**Publication 5**).

Recognizing that the epicenter of molecular systematics in the Orchidaceae family resided in the Jodrell Laboratory at Kew Gardens in London, I established contact with prof. M. W. Chase, who was the head of the molecular laboratory at the time. Through the European BioMoBil program, I completed three scientific internships at the Jodrell Laboratory between 2005 and 2006. During my first visit, my focus was on learning DNA isolation techniques and the long-term storage of DNA material for research purposes, known as the "DNA bank." The second visit centered on utilizing DNA cloning techniques to obtain homogeneous products for DNA sequencing. The third visit involved familiarizing myself with the stages of work following sequence data acquisition, including sequence assembly (contig creation), DNA sequencing, determination of DNA evolution models, and methods for constructing phylogenetic trees. My stays at the Jodrell Laboratory provided me with the expertise of a molecular taxonomist, which I applied during data analysis and the

writing of my doctoral thesis. They also allowed me to become acquainted with the scientific community collaborating with the Jodrell Laboratory at Kew Gardens.

5B. After obtaining a doctoral degree

Encouraged to continue our collaboration, I asked Prof. M. W. Chase to become my academic supervisor as part of the Synthesys program. After receiving funding (Grant No. GB-TAF-4470), I completed another internship at the Jodrell Laboratory. The scientific outcome was the publication of my first work on the phylogeny of the Orchidaceae family based on low-copy *Xdh* gene sequences (**Publication 1**) in 2010.

In 2011, as part of a research project funded by the Ministry of Science and Higher Education (MNiSW), I organized a scientific expedition to Vietnam. The objective was to search for rare/new species of the genus Paphiopedilum. During the expedition, I also initiated collaboration with orchid collector Chu Xuan Canh. Mr. Canh possessed one of the most valuable species for scientific study, and a species was named in his honor in 2010 (*P. canhii*). The scientific outcome of my expedition is reflected in Publication 2.

In 2011, upon the request of the Dean of the Faculty of Biology, prof. dr. hab. Dariusz L. Szlachetko, I joined the scientific team in the newly established Department of Molecular Evolution. Prof. dr. hab. Marek S. Ziętara became the head of this department. Due to new organizational and educational responsibilities, I limited my scientific activity to my home unit. Currently, following another change in the department in 2022, I am part of the Molecular Evolution and Bioinformatics Laboratory in the Department of Evolutionary Genetics and Biosystematics.

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

Teaching classes taught

I have been actively involved in teaching since I started working as an assistant professor. I have conducted various forms of classes, including exercises, lectures, workshops, and seminars, across several undergraduate and graduate programs at the Faculty of Biology, as well as at other faculties such as the Faculty of Mathematics and Physics and the Faculty of Law and Administration. I have developed original programs for exercises in Introduction to Bioinformatics, Tree of Life, as well as materials for online classes and exercise instructions. A significant part of my teaching involves mentoring students and supervising their diploma theses, acting as an auxiliary supervisor. Here is a list of selected teaching achievements:

Preparation and Conducting of Classes:

1. Scientific Methods of Crime Scene Investigation Using Biological Techniques – Lecture and exercises for second-year students at the Faculty of Law and Administration (Criminology program).

2. Crime Scene Evidence Discovery – Lecture segment for first-year students at the Faculty of Law and Administration (Criminology program).

3. Biodiversity and Taxonomy – Exercises for first-year students at the Faculty of Mathematics and Physics (Bioinformatics program).

4. Molecular Taxonomy and Phylogenetics – Exercises for third-year students at the Faculty of Mathematics and Physics (Bioinformatics program).

5. Tree of Life – Exercises for third-year students at the Faculty of Mathematics and Physics (Bioinformatics program).

6. Basics of Biology – Lecture and exercises for first-year students at the Faculty of Biology (Medical Biology program).

7. Introduction to Bioinformatics – Exercises for third-year students at the Faculty of Biology (Medical Biology, Genetics, and Experimental Biology programs).

8. Bioinformatics in Diagnostics – Exercises for third-year students at the Faculty of Biology (Genetics and Experimental Biology programs).

9. Bioinformatics for Biologists – Exercises for third-year students at the Faculty of Biology (Biology program).

10. Molecular Methods in Species Identification – Lecture segment for second-year students at the Faculty of Biology (Nature Resource Conservation program).

Research assistance in the preparing the bachelor and master theses

Academic Supervision of Students:

Bachelor's Theses Supervisor (2015–2020), University of Gdańsk, various study programs including Biology, Medical Biology, and Bioinformatics:

1. Damian Brodzik - Phylogenetic Relationships within the Orchidaceae Family, with a Focus on the Epidendroideae Subfamily, Based on Nuclear and Plastid Markers, in Relation to Molecular Clock Concept.

2. Sebastian Zielonka - Taxonomic Position of Paphiopedilum rungsuriyanum O. Gruss, Rungruang, Chaisur & Dionisio sp. nov.

3. Marta Wirkus - Influence of External Group on the Subfamily Cypripedioideae Phylogeny.

4. Bogna Szczepaniak - Molecular Evolution of the MatK Gene in Orchidaceae.

5. Izabela Gos - Attracting Long Branches and Morphological Adaptation to Pollinators in Lankesterella Ames and Eurystyles Wawra.

6. Aleksandra Chomik - Phylogenetic Relationships within the Genus Paphiopedilum (Pfitzer) Based on Nuclear and Plastid Markers Using Molecular Clock.

7. Marcin Bianek - Molecular Evolution of Plastid tRNA Genes in the Orchidaceae Family.

8. Karolina Kubić - Molecular Evolution and Analysis of Variability of HIV-1 env Gene under the Influence of DNA/rAd5 Vaccine.

9. Agnieszka Bilak - Analysis of Variability and Molecular Evolution of the HIV-1 env Gene under the Influence of the DNA/rAd5 Vaccine.

10. Marlena Rozwadowska - Analysis of Mitochondrial Genome Rearrangements in Selected Nematoda Species.

11. Aleksandra Zalewska - Phylogenetic Position and Molecular Evolution of the SARS-CoV-2 Virus Based on the Spike Gene Sequences.

Master's Theses Supervisor (2009–2022), University of Gdańsk, various study programs including Biology, Medical Biology:

1. Piotr Krajewski - Phylogenetic Analysis of the Oncidium Genus in the Context of the Oncidinae Subtribe (Orchidaceae).

2. Joanna Kuźniarska - Phylogenetic Analysis of the Bulbophyllum Genus in the Context of the Bulbophyllinae Subtribe (Orchidaceae).

3. Andrzej Recław - Phylogenetic Relationships within the Paphiopedilum Genus (Orchidaceae) Based on Mitochondrial nad1 Intron Sequences.

4. Maciej Leszczyński - Phylogenetic Relationships within the Paphiopedilum Genus (Orchidaceae) Based on Nuclear Xdh Gene Sequences.

5. Katarzyna Lange, Adrianna Lubińska - Phylogenetic Relationships within the Paphiopedilum Genus (Pfitzer) Based on Nuclear ITS and Chloroplast matK Gene Sequences.

6. Tomasz Koliński - Systematics of the Vargasiella C. Schweinf. Genus (Orchidaceae) Based on DNA Sequences.

7. Katarzyna Mystkowska - DNA Barcoding of Protected Vascular Plants in the Pomeranian Voivodeship.

8. Barbara Urasińska - Application of Chloroplast matK Gene as a DNA Barcode for Protected Vascular Plants in the Gdańsk Pomerania Region.

9. Natalia Olędrzyńska - Phylogenetic Relationships within the Paphiopedilum Genus (Pfitzer) in the Context of Reticulate Evolution.

10. Damian Brodzik - Mitochondrial Genome Sequence Analysis of the Gyrodactylus salaris Malmberg, 1957 Hybrid (Monogenea, Gyrodactylidae).

11. Sebastian Zielonka - Taxonomic Position of Paphiopedilum fairrieanum (Lindley) Stein (Orchidaceae, Cypripedioideae).

12. Joanna Smętek - Assessment of DNA Methylation Changes in In Vitro Culture Depending on the Method of Propagation.

13. Karolina Kubić - Analysis of Variability and Molecular Evolution of HIV-1 Subtype B Based on the env Gene.

14. Aleksandra Zalewska - Comparative Genomic Analysis and Lytic Properties of Two Bacteriophages Infecting Pseudomonas aeruginosa: vB_Pae575P-3 and vB_Pae1369P-5.

Auxiliary Supervisor in Doctoral Programs (ongoing):

1. mgr Iwona Skorowska - Phylogeny and Taxonomy of Oncidiinae (Orchidaceae) in the Guyana Upland.

2. mgr Aleksandra Zalewska - Recombination in Bacteriophages and Its Significance in Pseudomonas aeruginosa-Infecting Viruses – Microbiological, Molecular, and Phylogenetic Studies.

Other activity related to teaching activities

28.03.2014 – I participated in the 2nd Didactic Conference "Academic didactics: tradition and modernity" organized by the Educational Quality Team of the Faculty of Biology of the University of Gdansk.

Science popularization activities

I have also been actively involved in the implementation of popular science and educational programs of the Faculty of Biology aimed at promoting biological knowledge: "Invite a Scientist to School" and "Discover the Work of a Biologist." I carried out this activity by conducting original workshops titled "From Leaf to Phylogenetic Tree."

As part of presenting the research topics conducted at the Department of Plant Taxonomy and Nature Conservation and the Department of Molecular Evolution, I prepared meetings with young people and adults during the Baltic Festival of Science, "Night of Biologists," and open days at the Faculty of Biology of the University of Gdańsk.

Organizational activities

I utilized my organizational skills as a co-organizer of the Baltic Festival of Science at the Faculty of Biology (9th, 10th, 11th editions) and the Summer School of Taxonomy at the Faculty of Biology, University of Gdańsk (September 18-20, 2013). I consider the preparation and co-creation of the Molecular Taxonomy Laboratory in the Department of Plant Taxonomy and Nature Conservation at the Faculty of Biology as a personal success. As a representative of assistant professors, I am also involved in the work of committees organizing the activities of the Faculty of Biology (member of the Faculty of Biology Council - 2021 to the present) and in activities related to the organization of education at the Faculty of Biology, University of Gdańsk (member of the Biomedical Biology program council - 2016-2020, 2021-present, member/vice-chair of the Genetics and Experimental Biology program council - 2023-present).

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.

7.1.1 Contribution to the Systematics of the Orchidaceae Family and Molecular Plant Identification

Due to my ability to work in the research team of prof. dr. hab. Dariusz L. Szlachetko, I had access to numerous rare species from the Orchidaceae family. Both during my doctoral studies and after obtaining my doctoral degree, my role in Professor's team was focused on laboratory work to obtain sequence data and their phylogenetic analysis. The result of this collaboration is 10 scientific articles and two monographs in which I am a co-author. These works concern the systematic position/phylogeny of various species/systematic groups within the Orchidaceae family (see Appendix 4). Additionally, I was involved in work related to alpha taxonomy. I am a co-author of a monograph on African orchids (Szlachetko, D. L., Mytnik-Ejsmont, J., Kras, M., Rutkowski, P., Baranow, P., & **Górniak, M**. (2010). *Orchidaceae of West-Central Africa*. Gdańsk University Press.) and six new genera for science: *Ceratopetalorchis* Szlach., Górniak & Tukałło, Richardiana 3(4): 158 (2003), *Andinorchis* Szlach., Mytnik & Górniak, Polish Bot. J. 51(1): 31 (-32) (2006), *Brassiopsis* Szlach. & Górniak, Biodivers. Res. Conservation 1-2: 12 (2006), *Christensonella* Szlach., Mytnik, Górniak & Śmiszek, Polish Bot. J. 51(1): 57 (-58) (2006*), Irenea* Szlach., Mytnik, Górniak & Romowicz, Biodivers. Res. Conservation 1-2: 5 (2006), *Siederella* Szlach., Mytnik, Górniak & Romowicz, Biodivers. Res. Conservation 1-2: 4 (-5) (2006), encompassing over 60 new species combinations in total.

I was also involved in research related to conservation genetics, focusing on species-level variability analysis. I collaborated with dr. Aleksandra Naczk and dr. Julita Minasiewicz, publishing in renowned journals such as the Botanical Journal of the Linnean Society and Conservation Genetics. The experience gained in working with microsatellite DNA was utilized in the molecular identification of pure *Boechera stricta* lines, collaborating with dr. Joanna Rojek (Rojek, J., Kapusta, M., Kozieradzka-

Kiszkurno, M., Majcher, D., **Górniak, M**., Sliwinska, E., Sharbel, T. F., & Bohdanowicz, J. (2018). *Establishing the cell biology of apomictic reproduction in diploid Boechera stricta (Brassicaceae).* Annals of Botany, 122(4), 513–539).

In addition to internal unit collaboration, I also work on research projects where I am invited as an expert. For example, I initiated cooperation with Dr. Hab. Anna Jakubska-Busse (currently head of the Botany Department at the University of Wrocław), resulting in publications such as Jakubska-Busse, A., Proćków, J., Górniak, M., & Gola, E. M. (2012). *Is Epipactis pseudopurpurata distinct from E. purpurata (Orchidaceae)? Evidence from morphology, anatomy, DNA and pollination biology.* Botanical Journal of the Linnean Society, 170(2), 243–256; Jakubska-Busse, A., Żołubak, E., Górniak, M., Łobas, Z., Tsiftsis, S., & Steiu, C. (2020). *A Revision of the Taxonomy and Identification of Epipactis greuteri (Orchidaceae, Neottieae).* Plants, 9(6), 783; Górniak, M., Jakubska-Busse, A., & Ziętara, M. S. (2021). Genetic history of the remnant population of the rare orchid Cypripedium calceolus based on plastid and nuclear rDNA. Genes, 12(6), 940. In 2022, I was invited to collaborate with Dr. Yan-Yan Guo from Henan Agricultural University (China) in analyzing the results of chloroplast and mitochondrial genome sequencing of a *Selenipedium* species, one of the five genera in the subfamily Cypripedioideae with an unresolved systematic position based on sequence data.

Another significant stage in my scientific work was the establishment of a taxonomic-molecular team for a project related to molecular plant identification (DNA Barcoding). The project's executors involved in plant collection and identification were dr. Magdalena Lazarus, dr. Marta Kolanowska, and MSc. Aleksandra Naczk. Students from the "Zioło" Science Circle, affiliated with the Department of Plant Taxonomy and Nature Conservation at the University of Gdańsk, were also involved in this project. The project titled "DNA Barcode (DNA Barcoding) and DNA Bank of Protected Vascular Plants in the Pomeranian Voivodeship" was carried out as part of a competition organized by the WFOŚ in Gdańsk and co-financed by the University of Gdańsk. The project collected over 180 (437 specimens) protected vascular plant species. In addition to supervising the project, my role was to obtain *rbcL* and *matK* gene

fragments for the identified specimens and deposit them in the BARCODE OF LIFE DATA SYSTEM (Bold Systems v4) for the purpose of plant identification by other users. I selected the *rbcL* gene as the main marker for identification, with the *matK* gene as a supplementary marker. In the Bold database, I deposited sequences for 122 species (117 *rbcL*; 73 *matK*) covering 305 specimens for the *rbcL* gene and 157 specimens for the *matK* gene, totaling 462 sequences. One of the advantages of the BOLD database is the inclusion of sequence chromatograms available to all users in .ab1 format, allowing for sequencing quality assessment, which is crucial for identification. In addition to the BOLD database, I deposited (as an author/co-author) over 1200 sequence records of Orchidaceae family representatives as part of my scientific work. These records are available in the NCBI database.

I was also invited by prof. dr. hab. Maria Łuczkiewicz from the Department of Pharmacognosy at the Gdańsk Medical University to assist in the molecular identification of *Salvia apiana* (Lamiaceae) - Krol, A., Kokotkiewicz, A., **Gorniak, M**. et al*. Evaluation of the yield, chemical composition and biological properties of essential oil from bioreactor-grown cultures of Salvia apiana microshoots.* Sci Rep 13, 7141 (2023).

7.1.2 Analysis and Interpretation of Data from Large-Scale DNA Sequencing (NGS)

In the era of next-generation sequencing (NGS), obtaining a large number of sequence data is not a problem. Financial resources can be a barrier, but the real challenge lies in data analysis. Through collaboration with students/graduates of the Bioinformatics program (B.Sc. Maciej Pietkiewicz) from the Faculty of Mathematics and Physics and participation in bioinformatics training, I acquired skills in the analysis of genomic data. I applied these skills in collaboration with the team of prof. dr. hab. Grzegorz Węgrzyn, analyzing the genomes of newly discovered bacteriophages. The result of this collaboration is three publications in the International Journal of Molecular Sciences, published in 2020. I also initiated cooperation with Dr. Lidia Boss, an expert in studying toxin-antitoxin (TA) systems in *Dickeya dadantii* bacteria. As part of this collaboration, I conducted a phylogenetic analysis of complete genomes of bacteria from the Pectobacteriaceae family and provided data on the occurrence of

these systems to determine if there is a correlation between relatedness and the presence/loss of these systems (Boss, L., Górniak, M., Lewańczyk, A., Morcinek-Orłowska, J., Barańska, S., & Szalewska-Pałasz, A. (2021*). Identification of three type II toxin-antitoxin systems in model bacterial plant pathogen Dickeya dadantii 3937.* International Journal of Molecular Sciences, 22(11), 5932).

Aware that the evolutionary history of genomes is mosaic, I am critical of drawing conclusions about the phylogeny of organisms based on complete genomes, which has become a standard in the era of NGS. Using information from a large part of the genome can obscure the signal of hybridization, which is often a driver of speciation. What interests me is breaking down the signal from before hybridization and indicating which part of the ancestors' genome remained after the selection process in recombinants. The experience I gained in detecting hybridization in plants is now being used in the analysis of bacteriophage genomes. In addition to the aforementioned analyses for prof. dr. hab. Grzegorz Węgrzyn's team, I established close cooperation with dr Jurczak-Kurek, an expert in isolating bacteriophages from aquatic environments. Together, we are building a research team (both of us are employed in the Laboratory of Molecular Evolution and Bioinformatics) focusing on describing the impact of hybridization on expanding the host range of bacteriophages and their use in phage therapy. My role in this is a broad analysis of bioinformatic data from NGS sequencing. The result of our collaboration is a publication on the impact of recombination on bacteriophage genomes: **Górniak, M.**, Zalewska, A., & Jurczak-Kurek, A. (2022). *Recombination events in putative tail fibre gene in Litunavirus phages infecting Pseudomonas aeruginosa and their phylogenetic consequences*. Viruses, 14(12), 2669. MSc. Aleksandra Zalewska is also part of our team, for whom I serve as a co-supervisor in her PhD project.

Górniak

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(Applicant's signature)