e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 72 (2024), No 4
https://doi.org/10.3409/fb_72-4.13

Going down the rabbit hole: insight into the future of *Paramecium* (Ciliophora, Protista) biodiversity surveys

Sebastian Tarcz

Accepted November 25, 2024 *Published online December 11, 2024* *Issue online December 31, 2024*

Review article

Tarcz S. 2024. Going down the rabbit hole: insight into the future of *Paramecium* (Ciliophora, Protista) biodiversity surveys. Folia Biologica (Kraków) **72**: 130-142.

Paramecium, a research subject in many areas of life sciences, appeared to be a ciliate genus with a well-known biodiversity structure. However, the understanding of its biological diversity has been evolving rapidly in recent years, driven by the discovery of new taxa and an expanded knowledge of the distribution of known species. Most future insights into *Paramecium* biodiversity are expected to come from molecular data, particularly through eDNA sampling. As one of the most recognisable microeukaryotes, commonly found in freshwater ecosystems, and with over a century of biodiversity research – including extensive reference data from GenBank records and living culture collections – *Paramecium* holds significant potential to become a model ciliate for studies in biodiversity and biogeography. This review addresses the challenges of species identification within the *Paramecium* genus, the current state of knowledge on its biodiversity and other factors that may shape future research. Despite some existing bottlenecks, new approaches to data acquisition and analysis will enable researchers to integrate diverse lines of evidence, allowing for exceptional explorations of *Paramecium* species and populations.

Key words: ciliates, DNA barcoding, environmental DNA, metabarcoding, microeukaryotes, model organism, species identification.

Sebastian Tarcz✉, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland.
E-mail: tarcz@isez.pan.krakow.pl

Although ciliates, like many other single-celled microbial eukaryotes, are key components of trophic food webs in various habitats (Lynn 2008, 2012), they are still severely underestimated in terms of their biodiversity (Medinger *et al.* 2010; Weisse 2014). It was previously supposed that 83-89% of the ciliate diversity remains undescribed (Foissner *et al.* 2008). This problem is caused by the species' complex structure (Caron 2013; Nanney & McCoy 1976), the under-sampling of many habitats (Foissner *et al.* 2008; Fokin 2010/2011) and the proper selection of a DNA marker, especially when the systematic identification relies solely on molecular analyses (Zhan *et al.* 2019). Most ciliates are free-living in various environments, including ponds, lakes, estuaries, salt marshes and oceans (Lynn 2016). Their distribution is an intensely-debated issue with two hypotheses: the 'ubiquity model" (UM) (Fenchel & Finlay 2004; Finlay *et al.* 2006) (also called "everything everywhere, but the environment selects") and the "moderate endemicity model" (MEM) (Foissner 2006; Foissner *et al.* 2008).

One of the most studied ciliate genera is *Paramecium* (Beale & Preer 2008; Sonneborn 1975; Wichterman 1986), the species of which are model organisms in many fields of biological and medical surveys (Long

et al. 2023; Van Houten 2023). *Paramecium* was probably observed for the first time under the microscope by Antony van Leeuwenhoek (Van Houten 2019), then named in 1752 by John Hill (Woodruff 1921), and is considered, together with *Tetrahymena*, *Stentor* and *Vorticella*, to be one of the flagship ciliate genera (Lynn 2016).

Paramecia are visible to the naked eye due to their size (50-300 µm in length, depending on the species) (Fokin 2010/2011). They are free-living, predatory ciliates, which inhabit mainly freshwater and less frequently brackish water reservoirs (Brette 2021; Fokin 2010/2011), but so far have not been found in marine ecosystems (Fokin 2023). It is believed that the phylogenetic history of *Paramecium* dates back hundreds of millions of years (De Souza *et al.* 2020), with a fossil being discovered in a 200 million-year-old piece of amber (Schönborn *et al.* 1999). As with all other ciliates (Verdonck *et al.* 2022), Paramecia have two distinctly functioning, differentiated nuclei in one cytoplasm: a germline micronucleus; and a somatic, transcriptionally active macronucleus (Long *et al.* 2023). The *Paramecium* genus contains over twenty morphological species divided into six subgenera: *Paramecium*, *Cypriostomum*, *Helianter*, *Chloroparamecium*, *Viridoparamecium* and *Neobursaridium* (Serra *et al.* 2022). Within some of the morpho-species, the existence of cryptic species has been reported (Greczek-Stachura *et al.* 2021; Melekhin *et al.* 2022, 2024; Potekhin A. & Mayén-Estrada 2020; Przyboś & Tarcz 2016; Sonneborn 1975). While some of these species appear to have a worldwide distribution (Long *et al.* 2023; Melekhin *et al.* 2022; Tarcz *et al.* 2018), other *Paramecium* species are less extensively spread and may even be endemic (Krenek *et al.* 2015; Potekhin & Mayén-Estrada 2020; Przyboś *et al.* 2014). The state of knowledge of *Paramecium* biodiversity has been changing dynamically in recent years, both through the description of new taxa (Krenek *et al.* 2015; Potekhin & Mayén-Estrada 2020; Serra *et al.* 2022) and new knowledge on the ranges of known species (Przyboś & Tarcz 2018; Tarcz *et al.* 2023).

In recent years, the application of molecular approaches, particularly the availability of nucleotide sequences (for example, from the GenBank database), has allowed for a more accurate appraisal of the complex structure of ciliates and other microeukaryote species (Bass & Bell 2016). Although it is thought that to assess biodiversity the collected DNA sequence information should be applied to the background of the genetic, morphological, physiological and ecological data (Caron 2013; Dunthorn *et al.* 2014; Stoeck *et al.* 2014), it appears that in future most of the knowledge of *Paramecium*, as well as the biodiversity of other organisms, will be based on molecular data (Hoban *et al.* 2022; Porter & Hajibabaei 2018). Thus, the application of suitable molecular markers to facilitate species identification has been in the recent past and is still crucial to properly assess the biodiversity of *Paramecium* and other microbial eukaryotes.

DNA markers for Paramecium biodiversity assessments

Initially, *Paramecium* species were determined morphologically based on the shape and size of their cells, characteristics of the nuclear apparatus, contractile vacuoles and the presence or absence of endosymbionts (Fokin 2010/2011). The identification of cryptic species or syngens was based on the results of mating reactions (Chen 1956; Sonneborn 1970). However, the strain crosses technique, which has been used for years, requires *Paramecium* cultures in an appropriate stage of sexual maturity, and complementary mating types of standard strains. These two issues greatly complicate the determination of cryptic species.

The introduction of molecular techniques, such as isozyme patterns (Allen *et al.* 1973), RAPD (Przyboś *et al.* 2006), RFLP (Maciejewska 2006), ARDRA (Przyboś *et al.* 2007a) and PFGE (Rautian & Potekhin 2002) has facilitated studies on genetic polymorphism within the genus *Paramecium*. The main disadvantages of the above analysis techniques were relatively low reproducibility, limited resolution, ambiguity and incomparability of the results obtained by different research teams (Laimeheriwa *et al.* 2018; Matsumoto *et al.* 2022). The application of DNA sequencing techniques and the presence of sequenced DNA fragments in public databases (e.g. GenBank) has allowed for the above problems to be solved.

Although the first phylogenetic analyses based on studying DNA fragments from ciliates of the genus *Paramecium* date back to the early 1980s (Kumazaki *et al.* 1982), their use became more widespread only about 20 years later (Barth *et al.* 2006; Coleman 2005; Fokin *et al.* 2004; Hori *et al.* 2006; Maciejewska 2007; Przyboś *et al.* 2007b; Strüder-Kypke *et al.* 2000).

Nevertheless, a weak point of the above analyses was their selectivity (studies have focused on a small number of *Paramecium* strains) and a lack of consistency (every study concerned a different genome fragment). It is worth noting that the identification of a universal DNA marker is fundamental when it

is the only tool used to delineate boundaries between species of eukaryotic microorganisms (Caron 2013), especially when the objects of a study are morphologically indistinguishable, and without the possibility of performing strain crosses.

The response to the above challenge was the concept of DNA barcoding, which first appeared in the scholarly literature about two decades ago (Hebert *et al*. 2003). It was the first widely-accepted attempt to improve taxonomic research based on molecular data. However, it was instantly evident that there was no single DNA barcode for all living organisms (Moritz & Cicero 2004). Similarly, various markers for ciliates have been suggested as the best tools for DNA barcoding (Pawlowski *et al.* 2012; Stoeck *et al.* 2014; Strüder-Kypke & Lynn 2010).

For the genus *Paramecium*, despite the application of numerous ribosomal, mitochondrial and nuclear DNA fragments (Barth *et al.* 2006; Coleman 2005; Hori *et al*. 2006; Maciejewska 2007; Przyboś *et al.* 2011; Stoeck *et al.* 2014; Strüder-Kypke *et al.* 2000), two genetic markers are commonly used: various nuclear rDNA fragments; and the mitochondrial COI gene fragment (Barth *et al.* 2006; Greczek-Stachura *et al.* 2021; Krenek *et al.* 2015; Melekhin *et al.* 2022). However, it has been established that in some circumstances, highly conserved rDNA segments might produce confusing results in taxonomic studies (Przyboś & Tarcz 2019). With regards to the COI mtDNA fragment, it is thought that mitochondrial genes evolve 5 to 10 times faster than nuclear genes (Brown *et al.* 1979), making them better molecular markers for closely-related taxa, as has been demonstrated by previous surveys on the genus *Paramecium* (Krenek *et al.* 2015; Przyboś & Tarcz 2019).

The reliability of DNA barcoding, regardless of the marker that is used, primarily depends on the quantity and quality of the reference data that links the obtained sequences to taxonomic designations (Hleap *et al.* 2021; Keck *et al.* 2023). Based on molecular data collected over the last 20 years or so, the genus *Paramecium* has a good reference base for rDNA and COI mtDNA fragments. Therefore, a suitable DNA marker, when used in future metabarcoding studies, could be one of the 'cures for the bottleneck problem' in understanding the biodiversity of *Paramecium* and other microeukaryotes.

'Bottlenecks effects' in Paramecium biodiversity surveys

Exploring microeukaryote biodiversity is crucial to better understand their important roles and functions in ecosystems. However, it seems that with the above issue, problems are encountered with what one might refer to as the 'bottleneck effect'. Generally, the term 'bottleneck effect' corresponds to a kind of restriction – or in the case of a population, with a decrease in its genetic diversity (Nei *et al.* 1975). In turn, restrictions in sampling, processing, and data analysis methodologies mostly cause the 'bottleneck effect' in protistan or ciliate biodiversity surveys.

It appears that the biodiversity assessment problems concerning *Paramecium* are just as relevant for ciliates as for the other microeukaryotes (small eukaryotic, mostly unicellular organisms: protists, algae or fungi). They can be classified as follows:

1. Sampling and detection biases (including the abovementioned undersampling).

The biodiversity of microeukaryotes can be difficult to capture in surveys due to differences in population sizes, environmental circumstances and spatial-temporal fluctuations. According to Lehtiniemi *et al.* (2022), there is a need for optimising sampling frequencies, since the present approaches may miss out on diverse kinds of microeukaryotes. For example, in the case of *Paramecium*, a significant amount of its biodiversity data (especially its tropical biodiversity) is acquired through incidental sampling (Przyboś *et al.* 2013; Przyboś & Tarcz 2018; Tarcz *et al.* 2023) rather than through planned surveys (Melekhin *et al.* 2024; Potekhin & Mayén-Estrada 2020; Tarcz *et al.* 2018). Therefore, most areas remain unexplored (e.g. Afrotropical, Nearctic, Indomalayan and Australasian realms), and what is interesting is that this issue concerns *Paramecium* – one of the most recognisable microeukaryotes. So what about the biodiversity knowledge of the other, less-known representatives of Protista?

2. Technical bottlenecks in the sequencing and reusing of molecular data.

Molecular approaches such as metabarcoding provided breakthroughs in many microeukaryote surveys; however, the huge amount of sequencing data may overwhelm computing analysis workflows (Forster *et al.* 2019). In contrast, another problem is the renewed and easy access to DNA barcodes or raw sequencing data, which can be used repeatedly in other biodiversity studies (Paupério *et al.* 2023). Currently, in terms of *Paramecium,* most of the molecular data is deposited in the GenBank database as separate records connected with particular species, cryptic species or populations. However, the minority data – which may quickly become the majority – is obtained from surveys not directly related

to *Paramecium,* and may somehow be lost due to its presence in the 'flood' of HTS output (Abraham *et al.* 2024; da Silva & Fernandes 2023, 2024).

3. Taxonomic gaps.

Incomplete reference databases are a significant bottleneck for identifying protists (Gelis *et al.* 2024), particularly ciliates (Boscaro *et al.* 2017). Although the amount of reference data for the genus *Paramecium* seems sufficient for metabarcoding (Long *et al.* 2023), discoveries in recent years have shown that we still know too little about its biodiversity (Krenek *et al.* 2015; Melekhin *et al.* 2022, 2024; Serra *et al.* 2022). Furthermore, some *Paramecium* species (e.g. *P. africanum*, *P. jankowskii*, *P. ugandae* and *P. wihtermanii*) have been described only based on morphological characteristics; therefore, their proper affiliation cannot be established due to a lack of living strains (cf Krenek *et al.* 2015) – a source of the reference molecular data for these species.

4. Time-consuming procedures for species designation.

Despite frequent occurrences in aquatic ecosystems, ciliates are often disregarded due to their time-consuming and costly morphological identification (Hering *et al.* 2018). For example, in the case of *Paramecium,* the key traits used to distinguish morphospecies are the cell size and shape, type and number of micronuclei, structure of contractile vacuoles and the occurrence of endosymbionts (Fokin 2010/2011), or mating tests (strain crosses) in the case of cryptic species designation (Sonneborn 1970). Moreover, many other ciliates are fragile and fast-moving, and they frequently require challenging preservation and staining methods for their proper identification (Dopheide *et al.* 2009).

These problems with species designations are compounded by a crisis in taxonomy, due to its underfunding and the decreasing number of taxonomists, including protozoologists (Britz *et al.* 2020; Löbl *et al.* 2023; Orr *et al.* 2020).

5. Limited understanding of ecological dynamics and distribution.

Complex factors such as founder effects, population bottlenecks and genetic drift make it difficult to adequately assess the environmental and evolutionary dynamics of ciliates (Ganser *et al.* 2021) including *Paramecium* (Przyboś *et al.* 2011; Tarcz *et al.* 2018). The reasons for this may be the description of a new species from one or at most two or three locations – the so-called Wallacean shortfall (Lomolino 2004) or poor knowledge of the distribution of particular species, which mainly boils down to the enigmatic statement the 'everything is everywhere, but, the environment selects' (De Wit & Bouvier 2006). In the case of *Paramecium*, some species are also known from one or a small number of sites (Krenek *et al.* 2015; Potekhin & Mayén-Estrada 2020; Serra *et al.* 2022), which in practice limits the understanding of their ecological dynamics and distribution.

6. 'Shelf life' of new species.

Discoveries of new species often depend on one or a few specimens, leading to delays as researchers wait for additional context, sometimes for decades (SOSA 2024). This phenomenon has been referred to as the 'shelf life' – a period from the first specimen sampling to the formal species description, which on average may last around 21 years (Fontaine *et al.* 2012). In the case of *Paramecium buetschlii*, for example, which was sampled in Norway in 2005, its 'shelf life' lasted 10 years (Krenek *et al.* 2015).

I realise that these are not all the issues researchers face in studying the biodiversity of microeukaryotic organisms such as *Paramecium*. Nevertheless, it can be assumed that they will significantly impact the future understanding of protistan biodiversity, as they are more comprehensive and also apply to other organisms (Twyford *et al.* 2024).

Towards eDNA metabarcoding of *Paramecium* – a potential model organism for freshwater microeukaryote biodiversity surveys

Environmental DNA, or eDNA, refers to the genetic material found in environmental samples such as sand, water and air, which includes entire cells, extracellular DNA and perhaps whole animals (Taberlet *et al.* 2012; Ruppert *et al.* 2019); for example, ciliates (Kulaš *et al.* 2021). Similarly, in the past decade, environmental RNA (eRNA) metabarcoding studies, with molecular techniques used to identify and analyse the diversity of organisms in an environmental sample by sequencing specific, standardised genetic markers, have been extensively performed to examine microeukaryotes (Cook *et al.* 2024). Generally, microeukaryotic eDNA research is altering how scientists interpret and monitor ecosystems. These techniques offer non-invasive, cost-effective and compassionate approaches for investigating biodiversity, ecological relationships and environmental changes. These investigations have implications for conservation biology, public health, environmental monitoring, and evolutionary studies, while providing critical insights into the roles that microeukaryotes play in sustaining healthy ecosystems (Blattner

et al. 2021; Metz *et al.* 2023; Pawlowski *et al.* 2016; Rishan *et al.* 2024). The use of eDNA metabarcoding has resulted in a lot of new data, especially in relation to the biodiversity of ecosystem assessments, allowing for the detecting of new taxa including cryptic species and assessing the distribution of individual microeukaryote species (Abraham *et al.* 2024). It provides an opportunity for studying not only the soil, freshwater and marine habitats (Huang *et al.* 2024; Schenekar 2023; Zimmermann *et al.* 2024), but also enables sampling for sedimentary DNA (Nguyen *et al.* 2023). The latter makes it possible to assess changes in a microeukaryote species community over time.

Although to date, there have been no studies dedicated to the genus *Paramecium* based on the metabarcoding of environmental DNA, it seems that it is only a matter of time before such studies will be performed. The application of eDNA for species detection and the biogeography assessment in *Paramecium* will potentially provide a large amount of new data, which may dramatically change our view of its biodiversity.

The frequent presence of *Paramecium* in freshwater ecosystems suggests its great potential as a model organism for studying microeukaryote biodiversity (Lynn 2016; Anand & Paul 2022). Recently published metabarcoding studies support this hypothesis. Moreover, they can also be applied to tropical areas, which remain poorly understood in the case of *Paramecium* biodiversity (Abraham *et al.* 2024; da Silva & Fernandes 2023, 2024; Fernandes *et al.* 2021; Lansac-Tôha *et al.* 2022). The second aspect, which is directly connected with the previous one, is the estimated number of freshwater bodies on Earth. It is supposed that the global extent of natural lakes is 304 million, which occupy 4.2 million km^2 in area, and are dominated in size by millions of water bodies smaller than 1 km^2 (Downing *et al.* 2006). This, in turn, indicates a huge number of isolated freshwater habitats in which *Paramecium* species are very likely to occur. The third issue concerns the reference data for metabarcoding eDNA surveys. In the case of *Paramecium*, there are a large number of rDNA and COI mtDNA sequences that have been deposited in GenBank for more than 20 years. In addition, collections of living ciliates of the genus *Paramecium* can be a reservoir of reference materials for molecular and morphological studies. For example, the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences (Krakow, Poland) houses a collection of more than 1,500 live strains of various *Paramecium* species that were established in the early 1960s. Finally, *Paramecium* is a genus of ciliates that has been well-examined through systematic studies (Fokin 2010/2011; Krenek *et al.* 2015; Long *et al.* 2023; Serra *et al.* 2022), which is important for identifying and matching the molecular data obtained during environmental DNA analyses.

Going down the rabbit hole. Whether the faster we run, the further we drift away from the goal of understanding and recognising *Paramecium* biodiversity?

Recognising the biodiversity of microbial eukaryotes is essential for sustaining the Earth's ecosystems and supporting human activities that rely on balanced, healthy environments. Their roles in nutrient cycling, climate regulation and disease prevention highlight the need for a deeper scientific understanding and conservation efforts (Cavalier-Smith 2004; Corliss 2002; Falkowski *et al.* 2008; Sogin *et al.* 2006). Similarly, ciliates from *Paramecium* genus are important due to their role in maintaining the ecological balance, their use as model organisms in research and their potential applications environmental monitoring (Lynn 2008).

Although *Paramecium* was reported in many freshwater ecosystems in the abovementioned eDNA metabarcoding surveys, its identification was limited to the genus (da Silva & Fernandes 2023, 2024; Fernandes *et al.* 2021; Lansac-Tôha *et al.* 2022) or species level only (Abraham *et al.* 2024), with no data on the occurrence of cryptic species. Understandably, the objective of these studies was a biodiversity assessment across a broad spectrum of eukaryotic microorganisms, rather than focusing solely on specific groups such as ciliates, or even the *Paramecium* genus. Although for this purpose the variable V4 region of the small subunit rDNA was employed, it is too conservative to accurately assess biodiversity among closely-related taxa (Zhan *et al.* 2019). A similar issue has been observed with cryptic species in *Paramecium* (Przyboś & Tarcz 2019). Therefore, the results of the survey performed by Abraham *et al.* (2024) showing that *Paramecium tetraurelia* has been identified in studied water samples may be imprecise, because this taxon has an identical V4 sequence variant with eleven other cryptic species of the *P. aurelia* complex (Przyboś & Tarcz 2019). Over a decade ago, the CBOL Protist Working Group (Pawlowski *et al.* 2012) proposed a two-step DNA barcoding approach. The first step involved the application of a universal eukaryotic pre-barcode – for example, the V4 domain of the 18S rDNA gene – followed by group-specific barcodes; for instance, COI for ciliates (Strüder-Kypke & Lynn 2010) or amoebozoa (Kosakyan *et al.* 2015).

Another solution may be the application of multi-marker eDNA metabarcoding, which allows for a simultaneous analysis of several DNA fragments, and therefore results in increased accuracy, broader taxonomic coverage and resolutions at different taxonomic levels (Cordier *et al.* 2019; Topstad *et al.* 2021). In conclusion, problems in relation to methodological adjustments have been and remain one of the main challenges regarding DNA metabarcoding (Cristescu 2014; Diniz-Filho *et al.* 2024; Iwaszkiewicz-Eggebrecht *et al.* 2024).

The second problematic area is connected with the data, its collection and its subsequent analysis, especially its integration (Lapatas *et al.* 2015) and management (Wandelt *et al.* 2012). There is a great potential for sampling site numbers (Downing *et al.* 2006) associated with problems caused by natural factors, geopolitical crises or financial obstacles, making it almost impossible to screen such a large number of water bodies for sampling a particular microeukaryote genus such as *Paramecium*. An opportunity to support *Paramecium* biodiversity assessments could be citizen science, which has become a powerful tool in protistology and planktonic research, enabling volunteers and non-professionals to conduct broad-scale monitoring and data collection that would be difficult for research institutions alone (Buonanno *et al.* 2020; Fry *et al.* 2024; Simoniello *et al.* 2019). For example, projects such as 'Plankton Planet' (de Vargas *et al.* 2022) and 'PlanktoScope' (Pollina *et al.* 2022) highlight the role of citizen science in large-scale surveys of plankton populations and coastal ecosystems. Due to such initiatives, which engage citizen scientists, researchers may gain access to valuable real-time data across vast geographic areas. Another option may be to teach field biologists how to collect an environmental sample, isolate the DNA and send it to a lab (Rieder *et al.* 2024).

A second idea that soon could aid in the acquisition of samples for microeukaryote eDNA metabarcoding is the application of autonomic samplers, which are tools used for the automated and remote collection of water samples from aquatic environments, without the need for a human presence (Govindarajan *et al.* 2022; Preston *et al.* 2024). However, autonomous sampling for eDNA metabarcoding is used more frequently in marine environments due to its logistical advantages in covering large, remote areas, the need for continuous monitoring in stable waters and the cost-benefit considerations that make it worthwhile for such expansive ecosystems. In turn, in freshwater environments, manual sampling is often more feasible, efficient and cost-effective, due to the smaller scale and higher accessibility of these ecosystems (Bernos *et al.* 2023).

eDNA metabarcoding is widely recognised for generating extensive datasets containing multispecies information from environmental samples such as water, soil and sediment (Altermatt *et al.* 2023). These datasets, while they are initially collected for specific research purposes, offer significant potential for reanalyses to address a range of scientific questions, including those related to biodiversity patterns and species distribution changes (Dickie *et al.* 2018). This capability reduces the need for repetitive field sampling, as well as offering considerable time and resource savings. A key challenge, however, lies in enhancing the accessibility of this data (Berry *et al.* 2021). The application of the FAIR principles – Findability, Accessibility, Interoperability, and Reusability (Wilkinson *et al.* 2016) – is therefore essential for effective data management and reuse, ensuring the datasets are accessible across different platforms and are usable by diverse research communities (Abarenkov *et al.* 2023). Importantly, eDNA repositories could be regarded as modern counterparts to traditional natural history collections (Monfils *et al.* 2017), providing a complementary resource for biodiversity and taxonomic research. While they do not supplant physical collections, eDNA repositories – for example, PR2 or EUKARYOME (Guillou *et al.* 2013; Tedersoo *et al.* 2024) – offer valuable genetic snapshots that contribute to our understanding of past and present ecosystems, particularly at the molecular level. Similarly, to traditional collections that house physical specimens such as bones, skins, preserved plants, and living or frozen microeukaryotes, eDNA repositories archive molecular data representing the biological material from organisms present in the environment at the time of sampling (Parducci *et al.* 2017; Thomsen & Willerslev 2015). While eDNA repositories will offer exciting new ways to document and study biodiversity, they will complement rather than replace traditional natural history collections or surveys based on morphological features (Chen *et al.* 2024; Westgaard *et al.* 2024). However, eDNA can provide critical genetic data that may be otherwise lost, especially in cases where physical sampling is difficult or impossible (e.g. for extinct or cryptic species).

Currently, Artificial Intelligence (AI) is playing an increasingly prominent role in various fields, including taxonomic research (Karbstein *et al.* 2024). In this area, taxonomists, as well as protozoologists and ciliatologists, are confronted with two major challenges: the need to analyse vast volumes of data ranging from images and morphometric measurements to

environmental and molecular datasets; and the proliferation of concepts, methods and definitions, etc. Such a situation requires urgent coordination and the integration of data and specialists from different fields (Clamp & Lynn 2017). A striking example is the 'well-studied' genus *Paramecium*, where our current efforts suggest that we may be closer to the beginning than the conclusion of unravelling its true biodiversity. It is considered that combining integrative taxonomy with artificial intelligence (AI) may help delimit species in a less subjective and more integrative and rapid way (Karbstein *et al.* 2024), in a manner similar to Deep Learning (DL) which is 'on track to become an integral part of the future biologist's toolkit' (Borowiec *et al.* 2022) and may therefore bring about improvements in various aspects of ciliate biodiversity research.

Concluding remarks

Paramecium, one of the most well-known protists, holds a distinguished place in the history of microbiology. First observed by van Leeuwenhoek in the 17th century, has since been a staple in biology education (Becz *et al.* 2024; Clarke *et al.* 2002; Elwess *et al.* 2017). *Paramecium*, as one of the most recognisable microeukaryotes, is fairly well understood in terms of its taxonomy and distribution, and may have great potential to be a model genus in biodiversity and the biogeography studies of ciliates. As an abundant key player in freshwater ecosystems, *Paramecium* has a profound impact on microbial communities, carbon cycling and nutrient dynamics, processes crucial for maintaining the balance of an ecosystem. By investigating its species diversity and distribution, we can explore how *Paramecium* populations respond to changing environmental conditions, including the effects of climate change on freshwater ecosystems.

Taking into account the issues mentioned in the above review of previous research, which indicate new opportunities but also emerging problems, one may venture to conclude that for the *Paramecium* genus as well as for other microeukaryotes, a 'new dawn for the study of its biodiversity and biogeography' is approaching (Pinseel *et al.* 2024).

Conflict of Interest

The author declares no conflict of interest.

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