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Morphological Classification, Species Diversity, and Ecological Functions of Ciliate Communities

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Abstract Ciliate communities are crucial in ecosystems, and studying their diversity and ecological functions is essential for understanding microbial community structures and functions, as well as for environmental monitoring and ecological conservation. This study reviews the morphological classification, species diversity, and ecological functions of ciliate communities. By analyzing ciliate morphological characteristics, classification standards, and methods, this study explores species richness and distribution patterns, using genetic and molecular biology techniques to identify ciliate species and investigate their biogeographical patterns. Ciliates play significant ecological roles in nutrient cycling, energy flow, and interactions with other microorganisms and higher trophic levels. Case studies of ciliate communities in freshwater, marine, and soil environments illustrate their specific manifestations and provide recommendations for future research. This study aims to offer a systematic review of ciliate diversity and ecological functions, providing scientific basis for ecosystem management and conservation.

Keywords Ciliate communities; Morphological classification; Species diversity; Ecological functions; Molecular tools

1 Introduction

Ciliates are a diverse group of single-celled eukaryotes that play crucial roles in aquatic ecosystems. They are key components of the microbial loop, acting as predators of bacteria and protozoa, and providing nutrition for higher trophic levels (Kulaš et al., 2021a). Ciliates are found in various aquatic environments, including freshwater, brackish, and marine ecosystems, where they contribute to nutrient cycling and energy flow (Weisse et al., 2017; Fernandes et al., 2020). Traditional morphological methods have been used to study ciliate communities, but recent advancements in molecular techniques have revealed a much higher diversity than previously recognized (Rossi et al., 2016).

Understanding the diversity and ecological functions of ciliate communities is essential. Ciliates serve as bioindicators of environmental conditions, making them valuable for monitoring ecosystem health (Kulaš et al., 2021a; 2021b). Their sensitivity to changes in water quality and environmental pressures allows researchers to assess the impact of human activities on aquatic ecosystems (Kulaš et al., 2021b). Additionally, ciliates play a significant role in the food web, influencing the population dynamics of bacteria, algae, and other protists, and serving as prey for zooplankton (Weisse et al., 2017). This makes them integral to the stability and functioning of aquatic ecosystems. Studying ciliate diversity also provides insights into the evolutionary processes and biogeographic patterns of microorganisms, contributing to our understanding of biodiversity at the microbial level.

This study aims to provide a comprehensive overview of the morphological classification, species diversity, and ecological functions of ciliate communities. We will summarize the current knowledge on the morphological and molecular diversity of ciliates in various aquatic environments, discuss the ecological roles and functional diversity of ciliates, highlighting their contributions to nutrient cycling, energy flow, and ecosystem stability, and evaluate the use of ciliates as bioindicators for environmental monitoring, assessing the effectiveness of different methodological approaches in studying ciliate communities . Furthermore, we will identify gaps in current research and suggest future directions for studying ciliate diversity and functions, emphasizing the integration of morphological and molecular techniques. By addressing these objectives, this study aims to enhance our understanding of ciliate communities and their importance in aquatic ecosystems, providing a foundation for future research and conservation efforts.

2 Morphological Classification of Ciliates

2.1 Overview of ciliate morphology

Ciliates are a diverse group of unicellular eukaryotes characterized by the presence of cilia, which are hair-like organelles used for movement and feeding. The cellular structure of ciliates is among the most complex in eukaryotes, featuring a dual nuclear apparatus that includes a macronucleus and one or more micronuclei, along with highly specialized ciliary patterns (Abraham et al., 2019). Luo et al. (2019) conducted morphological and molecular biological analyses, particularly of the 18S rRNA gene sequences, on species from two genera of the family Psilotrichidae (Figure 1). Their work confirmed the taxonomic status of these species and expanded our understanding of the biodiversity and geographical distribution of this group of ciliates. Ciliate morphology includes various structures such as oral apparatus, body cilia arrangement, and caudal cilia, which are crucial for their classification and ecological functions (Chen et al., 2015; Qu et al., 2020).

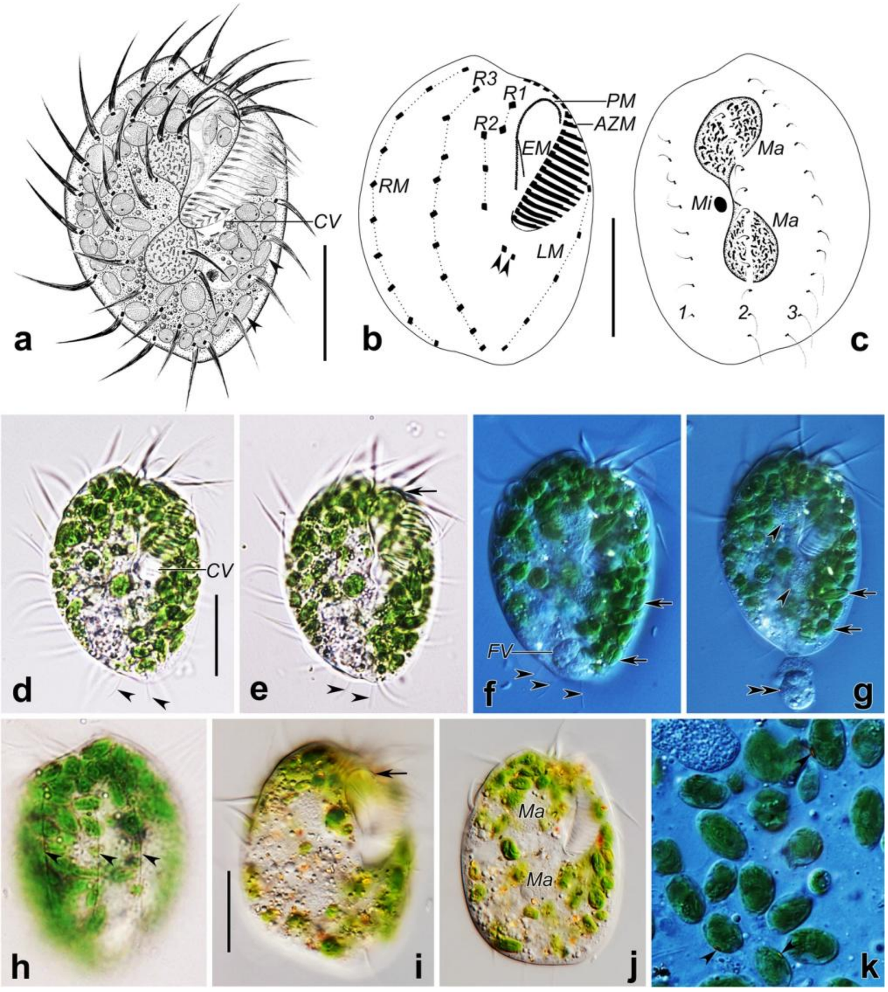


Figure 1 Hemiholosticha kahli nov. spec. in vivo (a, d–k) and after protargol impregnation (b, c) (Adopted from Luo et al., 2019)
Image caption: a Ventral view of a representative individual, arrowheads show the algae. b, c Ventral (b) and dorsal (c) views of a representative specimen. d–g Ventral views of representative individuals, showing the contractile vacuole, arrowheads in (d–f) indicate the long posterior dorsal bristles, arrowheads in (g) show the macronuclear nodules, arrow in (e) shows the distinctly curved paroral membrane, arrows in (f, g) show the green algae, double arrowheads show the food discharged from the food vacuole in (f). h Dorsal view, showing the dorsal ribs (arrowheads). i, j Ventral views of a slightly squeezed specimen, showing the distinctly curved paroral membrane (arrow), macronuclear nodules and the granules. k Details of the green algae, arrowheads indicate the red eyespots (Adapted from Luo et al., 2019)

Figure 1 from Luo et al.'s study shows the morphological characteristics of the newly discovered ciliate species *Hemiholosticha kahli*. The images, taken from in vivo observations and protargol impregnation, detail the morphological features of the species. The figure displays the species' wide oval shape, three sharp ribs on the dorsal side, and a large number of green algae inside the body. These algae give the ciliate a green appearance, enhancing its unique identification. Figure 1 also shows detailed cellular structures, such as the nucleus, cilia arrangement, and body surface texture. These morphological characteristics provide important references for the classification and study of ciliates, highlighting the importance of morphology in ciliate research.

2.2 Classification methods and criteria

The classification of ciliates has traditionally relied on detailed morphological and ultrastructural analyses, including the study of ciliary structures and internal cytoskeletal derivatives (ciliary structures). The study by Chi et al. (2020) provides detailed methods and examples of ciliate classification and species diversity, demonstrating how molecular and morphological analyses can be combined to improve taxonomic diagnosis and understand phylogenetic relationships among species (Figure 2). Modern approaches combine molecular data, such as 18S rRNA gene sequences, with classical morphological methods to resolve taxonomic uncertainties and improve species identification (Zhao et al., 2016; Abraham et al., 2019). Techniques such as live cell observation, staining methods, and molecular markers (e.g., cox1 gene, ITS region) are used to delineate species and understand their phylogenetic relationships (Zhao et al., 2016; Abraham et al., 2019; Wang et al., 2020).

Figure 2 from Chi et al. (2020) shows a maximum likelihood (ML) phylogenetic tree based on 18S rDNA sequences, including 91 species of heterotrich ciliates and 5 species of karyorelictean ciliates. This figure indicates that *Gruberia foissneri* forms a highly supported independent clade with other Gruberia species (100% ML, 1.00 BI), representing the family Gruberiidae. Additionally, *Linostomella vorticella* and two other Linostomella sequences form a sister group with the Condylostomides clade. These phylogenetic relationships emphasize the evolutionary differentiation among various ciliate groups, providing significant insights into their classification and evolutionary history.

2.3 Key morphological traits used in classification

Key morphological traits used in the classification of ciliates include the structure and arrangement of the oral apparatus, the pattern of somatic kineties, and the presence of specific ciliary structures such as caudal cilia and undulating membranes (Chen et al., 2015; Qu et al., 2020). For instance, the novel ciliate *Platynematum rossellomorai* is distinguished by its large anterior oral area, two caudal cilia, and a small number of somatic kineties, which are critical for its classification within the genus *Platynematum* (Qu et al, 2020). Similarly, the novel species *Sterkiella subtropica* is identified by its unique ciliature and nuclear apparatus, as well as its ontogenetic events, which include the formation of specific cirri patterns (Chen et al., 2015). These morphological characteristics, combined with molecular data, provide a comprehensive framework for the classification and understanding of ciliate diversity and evolution (Abraham et al., 2019; Luo et al., 2019).

3 Species Diversity of Ciliate Communities

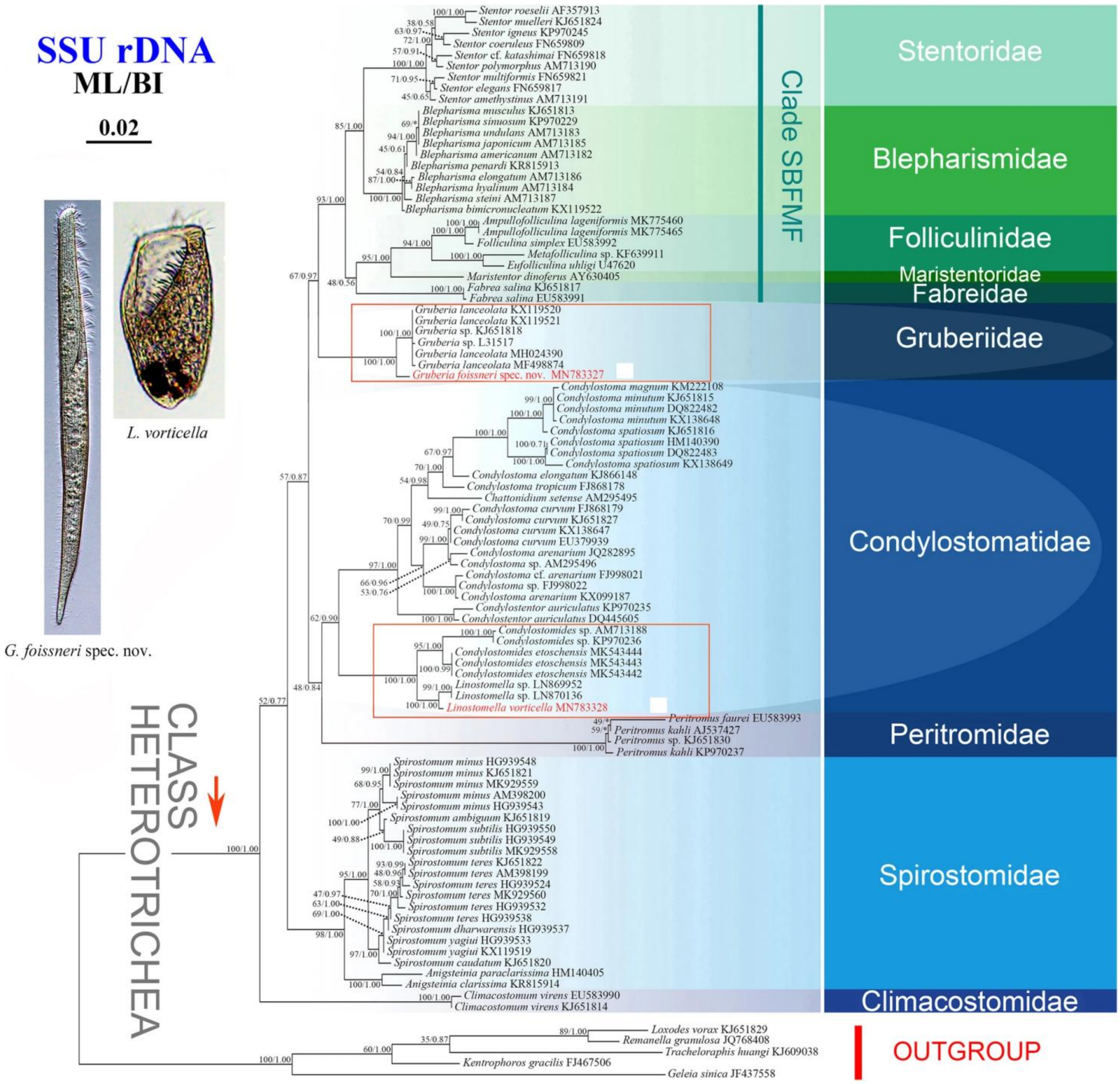
3.1 Species richness and abundance

Ciliate communities exhibit significant species richness and abundance across various aquatic environments. For instance, a study conducted in the Brazilian Atlantic Forest identified 409 ciliate taxonomic units (OTUs), with a notable diversity in freshwater compared to brackish environments (Fernandes et al., 2020). Similarly, research in the Pistoia province of Italy highlighted the presence of hidden biodiversity, including rare species and resistance forms, which are often missed by traditional morphological methods but detected through molecular techniques (Rossi et al., 2016). These findings underscore the importance of employing both morphological and molecular approaches to capture the full extent of ciliate diversity.

3.2 Genetic and molecular approaches to identifying species

Advancements in genetic and molecular techniques have revolutionized the identification and classification of ciliate species. The use of high-throughput sequencing and metabarcoding has provided deeper insights into ciliate diversity and taxonomy. For example, the mitochondrial *cox1* gene, nuclear ITS1 and ITS2, and the hypervariable

D2 region of LSU rDNA have been identified as promising markers for species delineation in the genus *Frontonia* (Zhao et al., 2016). Additionally, the hypervariable V4 region of the small subunit rDNA and the D1-D2 region of the large subunit rDNA have been effective in species delimitation for the genus *Euplotes* (Zhao et al., 2018). These molecular markers offer high resolution and accuracy, facilitating the identification of cryptic species and enhancing our understanding of ciliate taxonomy.



Fiture 2 Maximum likelihood (ML) phylogenetic tree inferred from 18S rDNA sequences (91 heterotrichean and 5 karyorelictean taxa) (Adopted from Chi et al., 2020)

Image caption: The posterior probabilities from the Bayesian inference (BI) were mapped onto the ML tree. Asterisks indicate a mismatch in branching pattern between the ML and BI trees. The newly sequenced species in this study are shown in red font. The scale bar corresponds to 2 substitutions per 100 nucleotide positions (Adopted from Chi et al., 2020)

3.3 Biogeographical patterns of ciliate diversity

Biogeographical patterns of ciliate diversity reveal significant spatial and environmental influences on community composition. Studies in floodplain lakes in Brazil demonstrated that molecular data could detect broad-scale spatial patterns, while morphological data reflected local environmental controls (Lansac-Tôha et al., 2022). This dual approach highlighted the importance of both spatial factors and environmental variables in shaping ciliate assemblages. Furthermore, research in the Krka River, Croatia, using environmental DNA (eDNA) metabarcoding, showed significant differences in ciliate community structure across different sampling locations, influenced by hydrological parameters and saprobiological classification (Kulaš et al., 2021). These findings emphasize the need to consider both biogeographical and environmental factors in studies of ciliate diversity.

The integration of morphological and molecular approaches is crucial for a comprehensive understanding of ciliate species diversity. Genetic and molecular techniques provide high-resolution tools for species identification, while biogeographical studies reveal the complex interplay between spatial and environmental factors in shaping ciliate communities. This holistic approach enhances our ability to monitor and conserve ciliate biodiversity in various aquatic ecosystems.

4 Ecological Functions of Ciliate Communities

4.1 Role in nutrient cycling

Ciliate communities play a crucial role in nutrient cycling within aquatic ecosystems. They contribute significantly to the decomposition of organic matter, thereby facilitating the recycling of essential nutrients such as nitrogen and phosphorus. For instance, studies have shown that ciliate communities in the Northern Beibu Gulf are influenced by the concentrations of phosphorus and nitrogen, which in turn affect their biomass and diversity (Wang et al., 2014). This indicates that ciliates are integral to nutrient dynamics, particularly in eutrophic conditions where nutrient availability is high. Additionally, ciliates are involved in the microbial loop, where they consume bacteria and other microorganisms, thus recycling nutrients back into the ecosystem (Kulaš et al., 2021a; Zhao and Langlois, 2022).

4.2 Contribution to energy flow in ecosystems

Ciliates are pivotal in the transfer of energy through aquatic food webs. They occupy intermediate trophic levels, acting as both predators and prey. By preying on bacteria and other protozoa, ciliates help control microbial populations and facilitate the flow of energy to higher trophic levels, such as zooplankton and small fish (Vilas-Boas et al., 2020). In riverine floodplains, for example, ciliates contribute to the overall ecosystem functioning by maintaining high abundances during stable hydrological conditions, which supports the energy flow within these dynamic environments (Vlaičević et al., 2022). Moreover, heterotrichous ciliates, such as those studied in the Pistoia province, play significant roles in material transfer and energy flow in aquatic food webs (Chi et al., 2020).

4.3 Interactions with other microorganisms and higher trophic levels

Ciliates interact extensively with other microorganisms and higher trophic levels, influencing community structure and ecosystem health. They serve as bioindicators of environmental conditions due to their sensitivity to pollutants and changes in water quality (Jiang et al., 2013; Kulaš et al., 2021a). For example, ciliate communities in the Krka River have been shown to respond to hydrological parameters and saprobiological classifications, indicating their potential as bioindicators for environmental monitoring (Kulaš et al., 2021a). Additionally, ciliates provide nutrition for higher trophic levels, such as zooplankton and small fish, thereby linking microbial processes to larger food web dynamics (Vilas-Boas et al., 2020). The presence of specific ciliate taxa can also indicate the health of aquatic ecosystems, as seen in the functional groups of marine ciliates that respond to varying levels of pollution and eutrophication (Jiang et al., 2013).

5 Case Studies of Ciliate Communities in Different Environments

5.1 Freshwater ecosystems

Ciliate communities in freshwater ecosystems exhibit significant diversity and play crucial roles in the microbial loop, contributing to nutrient cycling and energy flow. Studies have shown that freshwater ciliates are highly diverse, with distinct community structures influenced by environmental parameters such as light exposure and hydrological conditions. For instance, research conducted on the Krka River in Croatia revealed that ciliate communities in biofilm samples from different river sections showed significant differences in community structure, with hydrological parameters being the main structuring factors (Kulaš et al., 2021a; 2021b). Additionally, the diversity of ciliates in freshwater systems of the Brazilian Atlantic Forest was found to be higher than in brackish environments, with a considerable fraction of the detected diversity not represented in current molecular databases (Fernandes et al., 2020). These findings highlight the importance of freshwater ciliates in ecosystem functioning and their potential as bioindicators for environmental monitoring.

5.2 Marine ecosystems

Marine ciliate communities are equally diverse and play essential roles in marine food webs, particularly in nutrient cycling and primary production. Studies in coastal waters of China have documented over 100 new species of marine ciliates, emphasizing the large, undiscovered diversity in these habitatsn (Liu et al., 2017). The application of taxonomic relatedness indices, such as taxonomic distinctness (Δ*) and average taxonomic distinctness (Δ+), has proven effective in assessing the impact of environmental stressors like eutrophication on marine ciliate communities. These indices showed significant correlations with changes in nutrient levels, demonstrating their robustness as indicators for marine environmental assessment (Xu et al., 2011). Furthermore, the functional diversity of marine ciliates, including their roles as predators and mixotrophs, underscores their ecological significance in maintaining the balance of marine ecosystems (Weisse et al., 2017).

5.3 Soil and terrestrial habitats

Soil and terrestrial habitats host a remarkable diversity of ciliates, with many species yet to be discovered. Soil ciliates contribute to ecosystem functioning by participating in nutrient cycling and responding to environmental changes. Studies have shown that soil ciliate communities are influenced by factors such as soil type and vegetation cover. For example, grasslands and hardwood forests are characterized by a higher abundance of K-strategist ciliates, while more stressed ecosystems like arable lands and deserts have a higher proportion of r-strategist ciliates. Recent research in South Korea has identified 18 newly recorded ciliate species from soil and inland waters, further highlighting the high ciliate diversity in these habitats. The hidden biodiversity of soil ciliates, often missed by traditional morphological methods, can be better understood through molecular techniques, which reveal a more comprehensive picture of their community structure and ecological roles (Rossi et al., 2016).

6 Methods for Studying Ciliate Communities

6.1 Sampling techniques and protocols

Sampling techniques for studying ciliate communities are crucial for obtaining representative and reliable data. In freshwater biotopes, prolonged observations of differentially treated sample aliquots are often employed to capture the hidden biodiversity, including rare species and resistance forms. This approach combines morphological identification with molecular techniques such as Sanger sequencing to provide comprehensive insights into ciliate fauna (Rossi et al., 2016). In stream biofilms, samples are collected from various streams with different levels of human impact, and both microscopy and terminal restriction fragment length polymorphism (T-RFLP) analysis of 18S rRNA sequences are used to assess ciliate diversity. Additionally, periphyton samples from light- and dark-exposed lithified tufa/stones in karstic rivers are collected to investigate ciliate community structure, with environmental DNA (eDNA) metabarcoding being employed alongside traditional microscopy analyses (Kulaš et al., 2021).

6.2 Microscopic and imaging technologies

Microscopy remains a fundamental tool for studying ciliate communities, allowing for the direct observation and identification of ciliates based on their morphological characteristics. Traditional microscopy analyses are used to investigate ciliate community structures in various aquatic environments, such as biofilms and periphyton samples (Kulaš et al., 2021). However, microscopy alone may not capture the full extent of ciliate diversity, as it can miss rare species and those with subtle morphological differences (Rossi et al., 2016). To address this limitation, advanced imaging technologies and error-correcting algorithms for pyrosequences are employed to enhance the accuracy of taxon identification and reduce discrepancies in richness estimates (Santoferrara et al., 2014). These technologies provide a more detailed and accurate representation of ciliate communities, complementing traditional microscopy methods.

6.3 Molecular and genetic tools

Molecular and genetic tools have revolutionized the study of ciliate communities by detecting genetic diversity that is not apparent in morphological examinations. Techniques such as Sanger sequencing, pyrosequencing, and Illumina sequencing are used to assess ciliate diversity and community structure (Santoferrara et al., 2014; Rossi et al., 2016; Kulaš et al., 2021). DNA barcoding (metabarcoding) effectively enhances the accuracy and efficiency of biodiversity surveys of ciliates (Figure 3). This method improves understanding of ciliate community assembly and their ecological functions (Zhao et al., 2022). Mitochondrial *cox1* gene, nuclear ITS1 and ITS2, and the highly variable D2 region of LSU rDNA are promising candidate genes for species delimitation, providing high-resolution analysis below the species level (Zhao et al., 2016). Combining molecular techniques with morphological analysis offers a robust framework for resolving conflicts in species identification and understanding the ecological functions of ciliate communities (Zhao et al., 2016).

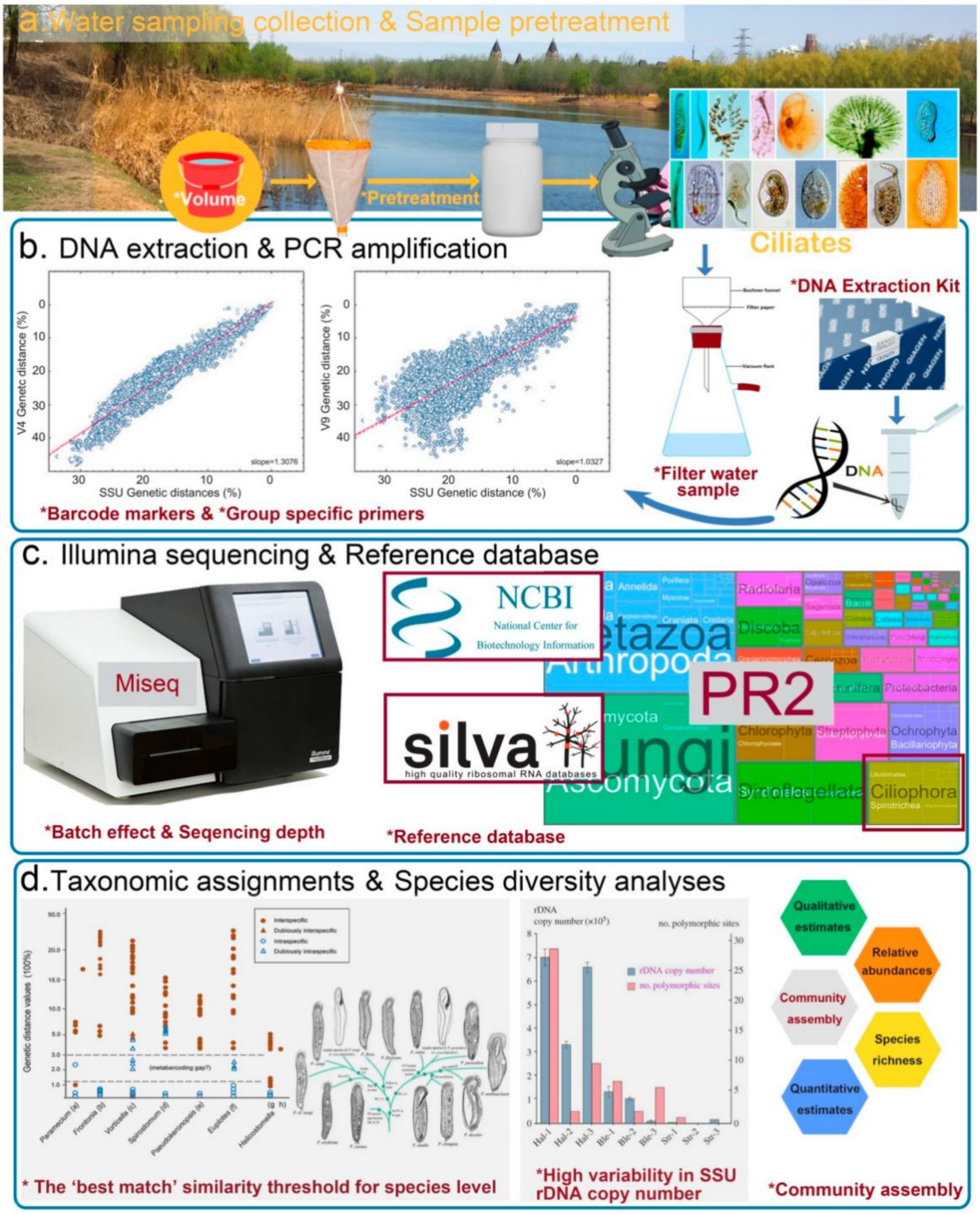


Figure 3 The flow chart of the metabarcoding processing steps (water sampling collection, sample pretreatment, DNA extraction, and PCR amplification, NGS-Illumina sequencing for example, reference database selection, and taxonomic assignment) of ciliate biodiversity (Adopted from Zhao et al., 2022)

Figure 3 from Zhao et al. (2022) shows a flow chart of the barcoding processing steps used to assess ciliate biodiversity. It includes water sample collection, sample pretreatment, DNA extraction, PCR amplification, NGS-Illumina sequencing, reference database selection, and taxonomic assignment. The flow chart highlights potential factors that can affect biodiversity assessments, such as sampling volume, filter materials, DNA extraction methods, and PCR amplification fidelity. The figure emphasizes the importance of standardizing each step to produce reliable and reproducible results in barcoding studies, which is crucial for effective biodiversity surveys and ecological research.

7 Challenges and Future Directions

7.1 Technical and methodological challenges

The study of ciliate communities faces several technical and methodological challenges. Traditional morphological methods, while valuable, often miss a significant portion of "hidden biodiversity," including rare species and resistance forms, which are more readily detected by molecular techniques (Rossi et al., 2016; Weisse, et al., 2017). The discrepancies between morphological and molecular approaches can lead to identification errors and incomplete biodiversity assessments (Rossi et al., 2016). Additionally, the reliance on single molecular markers in many studies can result in an incomplete understanding of ciliate diversity and biogeography. The integration of high-throughput DNA sequencing with traditional methods has shown promise but requires further refinement to ensure comprehensive and accurate biodiversity assessments (Fernandes et al., 2020; Kulaš et al., 2021a).

7.2 Knowledge gaps and research needs

Despite significant advancements, there remain substantial knowledge gaps in our understanding of ciliate diversity and ecological functions. For instance, the ecological roles of many ciliate species, particularly those that are rare or dormant, are not well understood (Weisse et al., 2017). There is also a need for more detailed taxonomic revisions and the exploration of new habitats to uncover the full extent of ciliate diversity. Furthermore, the functional diversity of ciliates and their contributions to ecosystem processes, such as primary production and nutrient cycling, require more experimental research to link ecophysiological performance to functional genes (Weisse et al., 2017). The geographic distribution of ciliates and the factors influencing their biogeography also remain areas needing further investigation.

7.3 Emerging trends and innovations

Emerging trends and innovations in the study of ciliate communities include the use of high-throughput DNA sequencing and environmental DNA (eDNA) metabarcoding, which have significantly enhanced our ability to detect and classify ciliate species (Fernandes et al., 2020; Kulaš et al., 2021a). These molecular techniques have revealed a higher number of ciliate taxa than traditional methods and have provided new insights into the community structure and ecological roles of ciliates in various environments (Kulaš et al., 2021a). Additionally, the integration of molecular data with traditional morphological approaches offers a more robust analytical framework for studying ciliate biodiversity and bioindicator potential (Kulaš et al., 2021a). Future research should focus on developing more comprehensive and integrative methodologies that combine morphological, molecular, and functional analyses to advance our understanding of ciliate communities and their ecological functions (Rossi et al., 2016; Weisse et al., 2017; Fernandes et al., 2020; Kulaš et al., 2021a).

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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