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Integrative Taxonomy in Algae: Combining Morphological, Molecular, and Ecological Data for Species Delimitation

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Abstract Integrative taxonomy has emerged as a robust framework for species delimitation by combining morphological, molecular, and ecological data. This study focuses on the application of integrative taxonomy to algae, aiming to enhance species delimitation accuracy. Traditional morphological methods often face challenges due to high levels of morphological plasticity and convergence in algae. By incorporating molecular data, such as DNA barcoding, and ecological information, we can achieve a more comprehensive understanding of species boundaries. This approach not only aids in the accurate identification of species but also helps in uncovering cryptic diversity and understanding evolutionary relationships. Our findings demonstrate that integrative taxonomy, through the use of multiple data sources, provides a more reliable and nuanced method for species delimitation in algae, thereby contributing significantly to biodiversity studies and conservation efforts.

Keywords Integrative Taxonomy; Algae; Species Delimitation; Morphological Data; Molecular Data

1 Introduction

Algal taxonomy has evolved significantly over the past two and a half centuries, driven by technological advancements such as improved microscopy, culture techniques, and digital access to collections (Leliaert, 2021). Traditionally, species delimitation in algae relied heavily on morphological characteristics. However, the high levels of morphological plasticity and convergence in algae often complicate species identification using morphology alone (Darienko et al., 2015). The advent of molecular techniques, particularly DNA sequencing, has revolutionized algal taxonomy by providing more accurate and objective methods for species delimitation. Molecular data have uncovered numerous cryptic species and reshaped our understanding of algal diversity and distribution (Leliaert, 2021).

Integrative taxonomy, which combines morphological, molecular, ecological, and other data, has emerged as a robust approach for species delimitation. This approach addresses the limitations of using a single type of data, such as the potential misidentifications due to phenotypic plasticity in morphological methods or the issues of introgression and incomplete lineage sorting in molecular methods. By integrating multiple lines of evidence, integrative taxonomy provides a more comprehensive and accurate framework for identifying species (Heethoff et al., 2011; Boisselier-Dubayle et al., 2012). This is particularly important in groups with high morphological plasticity or cryptic diversity, where traditional methods may fail to delineate species boundaries accurately (Darienko et al., 2015; Jesus et al., 2019; Leliaert, 2021).

This study is to explore the application of integrative taxonomy in the study of algae, focusing on how the combination of morphological, molecular, and ecological data can enhance species delimitation. This review aims to highlight the challenges and limitations of traditional algal taxonomy. Discuss the advancements and benefits of integrative taxonomy in algae. Provide case studies demonstrating the successful application of integrative taxonomy in algal research. Offer insights into future directions and potential improvements in the field of algal taxonomy. By synthesizing findings from multiple studies, this review seeks to underscore the importance of a holistic approach to taxonomy and its implications for biodiversity research and conservation.

2 Morphological Data in Algal Taxonomy

2.1 Traditional morphological methods

Traditional morphological methods have long been the cornerstone of algal taxonomy. These methods primarily involve the examination of physical characteristics such as cell shape, size, structure, and reproductive features. Historically, taxonomists have relied on these visible traits to classify and differentiate algal species. The use of light microscopy has been fundamental in these studies, allowing researchers to observe and document the intricate details of algal morphology. However, the reliance on morphological characteristics alone has often led to challenges, particularly due to the high degree of morphological plasticity and convergence observed in many algal species (Darienko et al., 2015; Leliaert, 2021).

2.2 Microscopic techniques

Advancements in microscopic techniques have significantly enhanced the resolution and accuracy of morphological studies in algal taxonomy. Improved light microscopy, coupled with electron microscopy, has allowed for more detailed observations of cellular and subcellular structures. These techniques have enabled taxonomists to identify subtle morphological differences that were previously undetectable, thus refining species boundaries. For instance, the use of scanning electron microscopy (SEM) has been instrumental in examining the surface structures of algal cells, providing critical insights into species differentiation. Additionally, digital imaging and analysis have facilitated the documentation and comparison of morphological traits, further aiding in the accurate classification of algae (Leliaert, 2021).

2.3 Limitations of morphological approaches

Despite the advancements in microscopic techniques, morphological approaches in algal taxonomy are not without limitations. One of the primary challenges is the phenotypic plasticity exhibited by many algal species, where environmental factors can induce significant morphological variations within the same species. This plasticity can lead to misidentifications and taxonomic ambiguities. Moreover, morphological convergence, where unrelated species evolve similar physical traits, complicates the accurate delimitation of species based solely on morphology. These limitations underscore the need for integrative approaches that combine morphological data with molecular and ecological information to achieve more robust and reliable species delimitation (Darienko et al., 2015; Papakostas et al., 2016; Leliaert, 2021).

3 Molecular Data in Algal Taxonomy

3.1 DNA barcoding

Molecular data have revolutionized the field of algal taxonomy, providing more precise and reliable methods for species delimitation compared to traditional morphological approaches. The integration of molecular techniques has allowed for the identification of cryptic species and has reshaped our understanding of algal diversity and distribution (Leliaert, 2021). DNA barcoding involves the use of short, standardized gene regions to identify species. This method has been particularly useful in algae, where morphological plasticity and convergence often complicate species identification. Commonly used barcode markers in algae include the V4 and V9 regions of the nuclear ribosomal operon and the ITS-2 region (Darienko et al., 2015). These markers have been effective in distinguishing species within genera such as *Coccomyxa*, revealing multiple phylogenetic lineages that correspond to distinct species (Darienko et al., 2015). However, the choice of barcode markers is crucial, as some markers may provide better resolution than others. For instance, COI and 28S markers have shown higher resolution in species delimitation compared to ITS2 in other taxa (Nolasco et al., 2022).

3.2 Phylogenetic analysis

Phylogenetic analysis uses DNA sequence data to reconstruct evolutionary relationships among species. This approach has been instrumental in redefining species boundaries in algae, often revealing cryptic diversity that is not apparent from morphological data alone (Leliaert, 2021). Multi-locus phylogenetic methods, which combine data from multiple unlinked loci, have proven particularly effective in accounting for confounding factors such as incomplete lineage sorting and hybridization (Leliaert et al., 2014). For example, studies on the genus Aphonopelma have demonstrated the utility of combining mitochondrial and nuclear markers to achieve consistent and reliable species delimitation (Hamilton et al., 2014). Similarly, the use of coalescent-based methods has provided statistical support for species boundaries, enhancing the robustness of phylogenetic inferences (Leliaert et al., 2014).

3.3 Genomic approaches

Genomic approaches involve the use of high-throughput sequencing technologies to analyze large portions of the genome. These methods offer a comprehensive view of genetic diversity and have the potential to uncover fine-scale population structure and evolutionary history (Leliaert, 2021). For instance, the integration of genomic data with traditional morphological and ecological data has led to the identification of distinct evolutionary units within species complexes, even in the presence of mitonuclear discordance (Papakostas et al., 2016). Genomic approaches also facilitate the identification of chemophenetic markers, which can provide additional insights into species delimitation. The use of untargeted metabolomics, combined with genomic data, has been shown to distinguish taxa based on chemophenetic markers at various levels of complexity, offering a more nuanced understanding of species boundaries (Figure 1) (Peters et al., 2023). The integration of molecular data through DNA barcoding, phylogenetic analysis, and genomic approaches has significantly advanced the field of algal taxonomy. These methods provide a more accurate and comprehensive framework for species delimitation, addressing the limitations of traditional morphological approaches and uncovering hidden biodiversity (Hamilton et al., 2014; Leliaert et al., 2014; Darienko et al., 2015; Papakostas et al., 2016; Leliaert, 2021; Nolasco et al., 2022).

Figure 1 Overview of images showing main phenotypic characters of the investigated species (Adopted from Peters et al., 2023)

This figure presents a comparative overview of phenotypic characteristics of four species, including *Riccia glauca*, *R. sorocarpa*, *R. warnstorfii*, and *Lunularia cruciata* (used as an outgroup). The images are organized into several rows, each showing different aspects of the plant's morphology (Adopted from Peters et al., 2023)

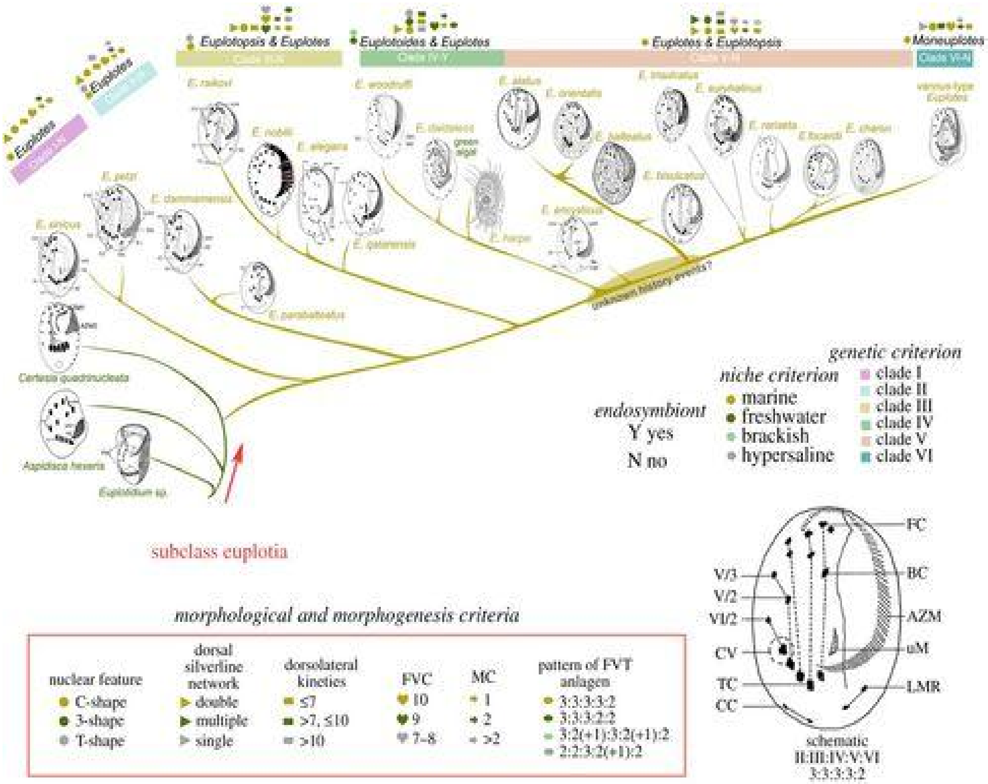

Figure 2 Possible evolutionary trajectories of different Euplotes lineages (Adopted from Nolasco et al., 2022)

Image caption: This figure presents the possible evolutionary trajectories of different Euplotes lineages, highlighting their morphological, ecological, and genetic characteristics (Adopted from Nolasco et al., 2022)

4 Ecological Data in Algal Taxonomy

4.1 Habitat characteristics

Ecological data play a crucial role in the taxonomy of algae, providing insights into the environmental contexts in which different species thrive. This section delves into the various ecological aspects that are integral to the classification and understanding of algal species. The habitat characteristics of algae are diverse and can significantly influence their taxonomy. Algae inhabit a wide range of environments, from freshwater to marine ecosystems, and even extreme habitats such as those polluted by heavy metals. For instance, the genus *Coccomyxa* is known for its cosmopolitan distribution and ecological versatility, thriving in both terrestrial and aquatic habitats (Darienko et al., 2015; Malavasi et al., 2016). The ability of *Coccomyxa* to colonize such varied environments underscores the importance of considering habitat characteristics in species delimitation. Similarly, the study of *Euplotes* has shown that ecological speciation plays a significant role in the diversification of this genus, with different clades adapting to specific environmental conditions (Zhao et al., 2018).

4.2 Environmental interactions

Environmental interactions, including symbiotic relationships and responses to abiotic factors, are critical for understanding algal taxonomy. Algae often engage in symbiotic relationships, which can influence their morphological and genetic characteristics. For example, *Coccomyxa* species are known to form symbioses, which can affect their ecological and physiological traits (Darienko et al., 2015). Additionally, environmental variables such as nutrient availability, light intensity, and pollution levels can drive speciation and influence species boundaries. The case study on *Paragorgia* octocorals demonstrated that cryptic species boundaries are often associated with specific environmental variables, highlighting the need for integrative approaches that consider these interactions (Figure 3) (Darienko et al., 2015; Herrera and Shank, 2015).

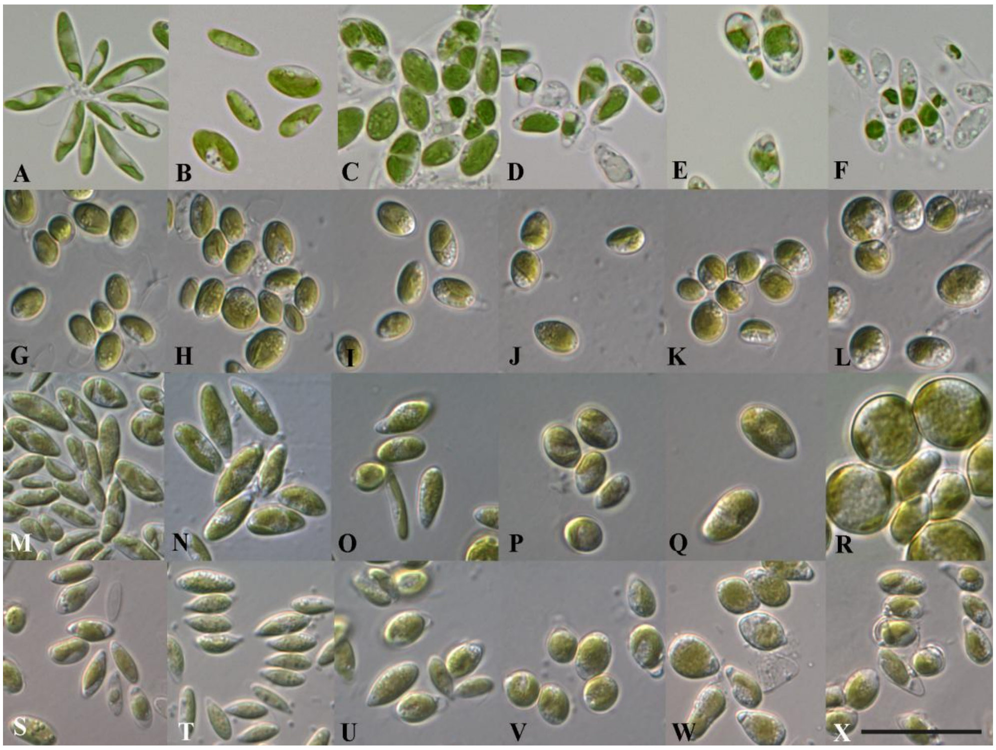

Figure 3 Phenotypic plasticity of various Coccomyxa strains under different salinities (Adopted from Darienko et al., 2015)
Image caption: This figure is likely used to demonstrate the capacity of Coccomyxa species to adapt to different saline environments (Adopted from Darienko et al., 2015)

4.3 Biogeographical patterns

Biogeographical patterns provide valuable information about the distribution and evolutionary history of algal species. Molecular data have revealed that widely distributed taxa may actually comprise different species with more restricted ranges, challenging traditional morphological classifications (Leliaert, 2021). For instance, the genus *Coccomyxa* has been shown to form a monophyletic group with distinct phylogenetic lineages that correspond to different biogeographical regions (Darienko et al., 2015). This biogeographical differentiation is essential for understanding the evolutionary processes that shape algal diversity. Moreover, the use of DNA-based taxonomy has uncovered numerous cryptic species, further emphasizing the importance of biogeographical data in species delimitation (Malavasi et al., 2016; Leliaert, 2021). Ecological data, encompassing habitat characteristics, environmental interactions, and biogeographical patterns, are indispensable for the accurate taxonomy of algae. Integrative approaches that combine these ecological aspects with molecular and morphological data provide a comprehensive framework for species delimitation and enhance our understanding of algal diversity and evolution.

5 Integrative Approaches for Species Delimitation

5.1 Combining morphological and molecular data

Integrative taxonomy has emerged as a robust framework for species delimitation by combining multiple lines of evidence, including morphological, molecular, and ecological data. This approach addresses the limitations of traditional taxonomy, which often relies on a single type of data, and provides a more comprehensive understanding of species boundaries. Combining morphological and molecular data is a cornerstone of integrative taxonomy. Morphological data, which include physical characteristics and structures, have traditionally been used for species identification. However, morphological convergence and plasticity can complicate species delimitation. Molecular data, particularly DNA barcoding, offer a complementary approach by providing genetic markers that can distinguish between species even when morphological differences are subtle or absent.

For instance, the study on green microalgae (*Coccomyxa*) demonstrated the effectiveness of combining phenotypic and genetic parameters for species characterization. The use of DNA barcode markers (V4, V9, and ITS-2 regions) alongside morphological and physiological analyses revealed new lineages and putative new species, highlighting the importance of a polyphasic approach in microalgae taxonomy (Darienko et al., 2015). Similarly, the application of convolutional neural networks to integrate morphological and molecular data has shown high accuracy in species identification across various taxa, including beetles, butterflies, fishes, and moths (Yang et al., 2021).

5.2 Integrating ecological information

Ecological data provide additional layers of information that can be crucial for species delimitation. These data include habitat preferences, ecological interactions, and geographical distribution, which can help distinguish species that are morphologically and genetically similar but occupy different ecological niches. In the case of the *Pnigalio soemius* complex, integrating ecological data such as host-plant associations and geographical separation, along with molecular and morphological analyses, helped resolve species boundaries and identify cryptic species. This integrative approach also suggested that endosymbiont infections could play a role in reproductive isolation and genetic diversification (Gebiola et al., 2012). Another example is the study on photosynthetic sea slugs, where ecological data were used to evaluate candidate species identified through DNA barcoding and morphological analysis, revealing significant cryptic diversity (Krug et al., 2013).

Another example is the study by Zheng et al. (2024) that illustrates how environmental pressure drives the evolution of specific genes that are essential for survival in diverse habitats. The identification of PSGs related to detoxification, ubiquitin proteolysis system, and energy metabolism highlights the complex interaction between genetic evolution and environmental adaptation in *P. lima* and *P. arenarium*. These findings help us understand how species evolve to thrive in challenging environments (Figure 4).

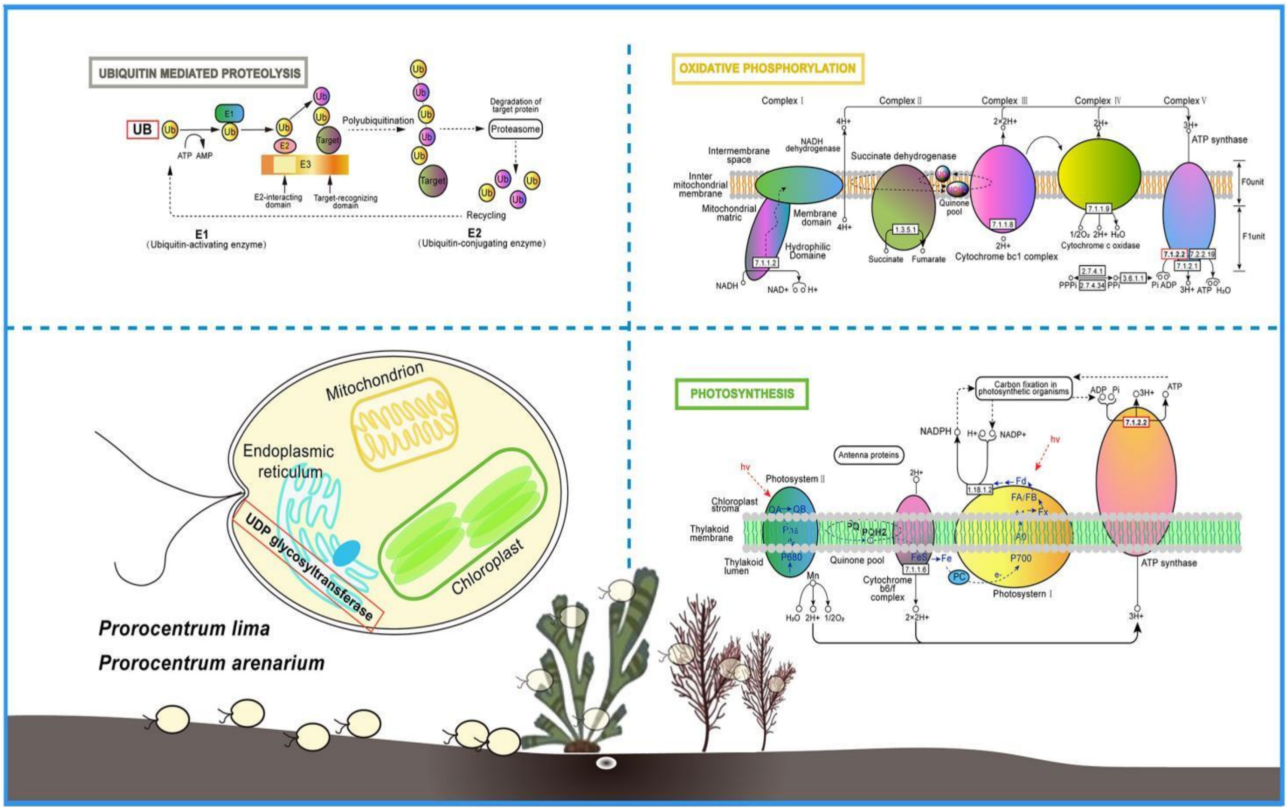

Figure 4 Schematic of adaptation in the evolution of *P. lima* and *P. arenarium* (Adopted from Zheng et al., 2024)

Image caption: This figure presents a schematic representation of the adaptive evolution processes in *Prorocentrum lima* and *Prorocentrum arenarium*. The diagram is divided into four sections, each illustrating key biological pathways and systems that have undergone adaptive evolution in these species (Adopted from Zheng et al., 2024)

5.3 Case studies of integrative taxonomy

The study on *Limnadopsis* species employed both mitochondrial (COI) and nuclear (EF1α) markers along with morphological characters to delineate species. This integrative approach allowed for the identification of 11 species, including three previously undescribed ones. The congruence between molecular and morphological data under various species concepts underscored the robustness of this method (Schwentner et al., 2011).

In the *Brachionus calyciflorus* complex, widespread mitonuclear discordance posed a challenge for species delimitation. By integrating molecular data (ITS1 and 28S nuclear DNA markers) with ecological information, researchers were able to resolve phylogenetic conflicts and infer species boundaries. This approach demonstrated the potential of integrative taxonomy to address complex introgression scenarios and provided insights into the competitive abilities of different species under various growth conditions (Papakostas et al., 2016).

The integrative taxonomic approach applied to *Homoscleromorph* sponges combined multiple datasets, including external morphology, anatomy, cytology, spicule shapes, geography, reproduction, genetic sequences, and metabolomics. This comprehensive approach not only facilitated the description of new species but also elucidated their phylogenetic relationships within the phylum Porifera. The study highlighted the importance of integrating diverse data types for both species delimitation and classification (Boury-Esnault et al., 2013).

6 Challenges and Limitations

6.1 Data integration challenges

Integrative taxonomy aims to combine multiple data sources, such as morphological, molecular, and ecological data, to achieve more accurate species delimitation. However, integrating these diverse data types presents significant challenges. One major issue is the lack of standardized methods for combining different data types, which can lead to inconsistencies and difficulties in interpretation (Schwentner et al., 2015). For instance, while molecular data can provide high-resolution insights into genetic differences, morphological data may be subject to phenotypic plasticity, complicating the integration process (Darienko et al., 2015). Additionally, the computational tools required for such integration, like convolutional neural networks or machine learning algorithms, are still in developmental stages and may not be universally applicable across all taxa (Yang et al., 2021; Pyron, 2023).

6.2 Inconsistencies in data sets

Inconsistencies between different data sets are another significant limitation in integrative taxonomy. Molecular data, for example, can sometimes show discordance with morphological or ecological data due to phenomena like introgression, incomplete lineage sorting, or horizontal gene transfer (Papakostas et al., 2016; Yang et al., 2021). This discordance can lead to conflicting species boundaries, making it difficult to draw definitive conclusions (Gebiola et al., 2012; Papakostas et al., 2016). Moreover, the choice of molecular markers can also influence the results, as different markers may provide varying levels of resolution and may not always correlate with morphological or ecological traits (Darienko et al., 2015; Heethoff et al., 2011). These inconsistencies necessitate a cautious and iterative approach to species delimitation, often requiring multiple rounds of data collection and analysis to resolve conflicts (Yeates et al., 2011; Gebiola et al., 2012).

6.3 Technical and practical limitations

Technical and practical limitations also pose significant challenges to integrative taxonomy. High-quality data collection is resource-intensive, requiring specialized equipment and expertise in multiple disciplines, including molecular biology, ecology, and computational science (Pante et al., 2015; Papakostas et al., 2016). Additionally, the need for large datasets to train machine learning models or to perform robust statistical analyses can be a limiting factor, especially for rare or poorly studied taxa (Yang et al., 2021). Practical issues such as the availability of specimens, the preservation of samples, and the accessibility of remote or understudied habitats further complicate the process (Heethoff et al., 2011; Pante et al., 2015). These limitations highlight the need for collaborative efforts and the development of more efficient and accessible methodologies to advance the field of integrative taxonomy (Dayrat, 2005; Heethoff et al., 2011).

7 Advances and Future Directions

7.1 Technological innovations

Recent technological advancements have significantly propelled the field of integrative taxonomy, particularly in algae research. High-throughput sequencing and metabarcoding technologies have revolutionized our understanding of microbial eukaryotes, including algae, by providing detailed insights into their diversity and ecological roles (Zhao et al., 2018). The development of convolutional neural networks, such as the morphology-molecule network (MMNet), has further enhanced species identification by integrating morphological and molecular data, achieving high accuracy across various taxa (Yang et al., 2015). These innovations underscore the importance of combining multiple data sources to improve species delimitation and identification.

7.2 Improving data integration methods

The integration of diverse data types - morphological, molecular, ecological, and more—remains a cornerstone of modern taxonomy. However, challenges persist in effectively combining these data. Recent studies have highlighted the need for a more structured approach to data integration, proposing flexible procedures and stopping rules to resolve disagreements among disciplines (Padial et al., 2010). Additionally, the iterative nature of integrative taxonomy, which treats species boundaries as hypotheses to be tested with new evidence, has been suggested as a robust framework for refining species delimitation (Schlick-Steiner et al., 2021). This iterative approach allows for continuous improvement and validation of species hypotheses, ensuring that taxonomic decisions are well-supported by comprehensive evidence.

7.3 Future research priorities

Future research in integrative taxonomy should focus on several key areas to further advance the field. First, there is a need to develop and standardize quantitative methods for data integration, moving beyond the current qualitative and comparative approaches (Schlick-Steiner et al., 2021). Second, expanding the use of integrative taxonomy to a broader range of taxa, including those with high morphological plasticity and genetic diversity, will help address the taxonomic impediment and improve species discovery and description (Pante et al., 2015; Darienko et al., 2021). Finally, fostering collaboration among taxonomists, molecular biologists, ecologists, and other specialists will be crucial for the continued evolution of integrative taxonomy, ensuring that it remains a dynamic and interdisciplinary field (Dayrat, 2005; Yang et al., 2022).

8 Concluding Remarks

Integrative taxonomy, which combines morphological, molecular, and ecological data, has proven to be a robust framework for species delimitation across various taxa. Studies have demonstrated that this approach can effectively uncover cryptic species and resolve taxonomic ambiguities that traditional methods alone might miss. For instance, the application of integrative taxonomy in photosynthetic sea slugs revealed twenty candidate species within three nominal taxa, highlighting the method’s ability to identify unrecognized diversit. Similarly, in the rotifer *Brachionus calyciflorus* complex, integrative taxonomy successfully addressed mitonuclear discordance and provided a clearer understanding of species boundaries. The approach has also been effective in delineating species in microalgae, where morphological convergence and plasticity often complicate traditional classification. Furthermore, integrative taxonomy has been applied to various other organisms, including insects, mites, and salamanders, consistently demonstrating its utility in resolving complex taxonomic issues.

The continued use of integrative taxonomy is crucial for advancing our understanding of biodiversity. By incorporating multiple lines of evidence, this approach provides a more comprehensive and accurate picture of species diversity and evolutionary relationships. It helps bridge the gap between different disciplines, fostering collaboration and enhancing the robustness of taxonomic conclusions. Moreover, integrative taxonomy is essential for addressing the “taxonomy crisis”, where many species remain undescribed or misidentified, potentially leading to their extinction before they are even recognized. The approach also has practical implications for conservation, drug discovery, and ecological studies, as accurately identified species are fundamental to these fields.

Future research should focus on expanding the application of integrative taxonomy to a broader range of taxa and ecosystems. This includes developing and standardizing molecular markers for groups where suitable markers are still under debate, such as microalgae and protists (Darienko et al., 2015). Additionally, there is a need for more comprehensive datasets that integrate morphological, molecular, and ecological data, as well as advanced analytical methods to handle these complex datasets. Researchers should also prioritize the formal description and naming of newly delimited species to ensure effective communication and data sharing within the scientific community and beyond. Finally, interdisciplinary collaboration and the development of new technologies, such as machine learning for species delimitation, should be encouraged to further enhance the accuracy and efficiency of integrative taxonomy.

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Conflict of Interest Disclosure

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