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Review Article

Culture practices and characterization of diatoms using morphological and molecular based methods: A review

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Abstract

From the early culturing research of Louis Pasteur (1857-1864) the current use of microbial cultures in scientific research for genetics, virology, bacteriology, mycology and the characterization of algae is extensively documented. Further, cultures have been invaluable in studying the genetic production of amino acids, and general metabolic processing for basic cellular functions and pharmaceuticals. In diatom research, cultures have been instrumental in studying systematics, cell movement, contaminant impacts and resistance, silica valve formation, lipid (biofuel) production and advancements in nanotechnology. The continued documentation of morphological variations in diatom species supported by molecular data, using cultures, is scientifically essential in determining diatom evolutionary relationships and the functional realities within their cellular ecosystem services. This article reviews selected procedures and methods of isolation and culturing specifically related to phycology, including small- and large-scale open culture systems, and closed systems using photobioreactors.

Keywords: Diatom, Isolation, Cultivation, Characterization, Photobioreactor

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Introduction

Diatoms are unique unicellular, photosynthetic eukaryotes that first appeared over 200 million years ago. They are highly versatile ecologically, play a fundamental role as primary producers in aquatic ecosystems, and are of great value in biotechnology and materials science (Risjani, 2025; Rabiee et al., 2021). The production of silica frustules and lipids for energy storage are key features that set diatoms apart from other photosynthetic protists. This combination of defensive silica frustule protection and large vacuoles for lipids is widely cited as primary reasons for survival and dominance in aquatic ecosystems (Behrenfeld et al., 2021). The autecological functionality of diatoms can be linked to 10 classes based on functional valve morphogensis: raphid pennates (Bacillariophyceae), araphid pennates (Fragilariophyceae), and centric diatoms (8 classes, Corethrophyceae - Plagiogrammophyceae) (Kociolek et al., 2026). The evolutionary trajectory in their autecology's exists as single cells or form distinct colonial structures, such as zigzags (*Tabellaria*), bands (*Fragilaria*), fans (*Meridion*), and stellate forms (*Asterionella*) (Mishra et al., 2017). Centric species typically populate lotic and nearshore planktonic zones, while pennate forms dominate sediment and biofilm communities. Biologically, these organisms divide rapidly undergoing generational diminution cycles in frustule size due to rapid reproduction, until reaching a minimum threshold (Tong and Derek, 2023).

Due to their biological and physiological ability to grow rapidly, these unicellular organisms have promising industrial potential for multifaceted applications (Abdelhay et al., 2025; Bhat et al., 2025; Bhattacharjya et al., 2021; Biswas et al., 2025; Bouazzara and Chaibi, 2025; Risjani 2025). They are rich sources of carotenoids, lipids (including free fatty acids) (Vella et al., 2019), and other pigments, such as fucoxanthin (Xia et al., 2013). Diatoms also produce biosilica, a natural material useful for biomedical engineering (Yoo et al., 2025; Reid et al., 2021; Saxena and Tiwari, 2023). The biosilica in frustules further acts as a pH buffer, aiding the intracellular conversion of bicarbonate into metabolizable dissolved CO_2 (Sharma et al., 2021). Diatoms have evolved to thrive in diverse and often harsh environments, ranging from extreme acidity or salinity to high pressure and radiation. This resilience is the basis for their importance in providing ecosystem services (Jin and Agustí, 2018). Furthermore, their rapid life cycles and environmental sensitivity make them excellent bioindicators for assessing water quality (Beranda et al., 2020).

In addition to their benefit for various applications, diatoms can be isolated from various substrates in aquatic environments and incubated in laboratory cultures for characterization. Many taxonomic studies have used cultures for morphological analysis to characterize diatoms (e.g. Kryk et al., 2021; Rybak et al., 2021; Riaux-Gobin et al., 2022; Luthfi et al., 2024a, b). Other studies have used cultures in both morphological and molecular approaches (e.g. Prasetiya et al., 2019; Kulikovskiy et al., 2020a, b; Tseplik et al., 2021a, b; Pane et al., 2024). At present, about 12,000 of the estimated 100,000 to 200,000 species worldwide have been identified (Wang et al., 2022). The diversity of tropical diatoms in the world is still significantly underestimated. Despite the widespread publication of morphological and molecular tools, taxonomic misidentifications are common, and cryptic species are widespread. This article presents a literature review of methods for isolating, culturing, and characterizing diatoms using both morphological and molecular approaches. The paper provides comparison between various methods and their limitations. Through this review, we hope to present a more comprehensive understanding in the development of research in the field of diatom taxonomy and provide direction for further global research.

Isolation of single species

Diatoms originate from coastal and lake sediments, coral, stones, and living materials, such as seaweed and seagrass, which can be isolated in a laboratory. There are several isolation methods, such as isolation with a micropipette, an agar medium, or a capillary pipette.

Isolation with micropipette

The micropipette method is the most widely used technique for isolating monoclonal diatom cultures. It allows for the precise separation of single cells under a microscope, resulting in relatively contamination-free cultures. Although studies have demonstrated the effectiveness of this method for most species, the success rate is greatly influenced by culture conditions and cell resistance.

Examining some examples of isolation will highlight the variability and success of the process. Several

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studies have applied variations to the technique to improve its effectiveness. For example, Prasetiya et al. (2019) used the micropipette technique to isolate *Haslea nusantara* and maintain it in medium-term culture under controlled conditions; however, only one of the initial six cells survived. Tseplik et al. (2021b) obtained similar results when they obtained non-axenic monoclonal cultures from the *Achnanthidium gladius* strain. This confirms that the method is reliable, even though the cultures are not completely free of contamination. Chen et al. (2013) successfully isolated *Synedra sp.* using the same method and maintained it in AF/2 medium. A comparison of these three studies demonstrates the broad applicability of micropipette isolation across marine and freshwater environments, yielding consistent results in forming single cultures. However, success rates are highly influenced by autecology, cell resistance, media conditions, and environmental factors such as temperature and lighting. Micropipette isolation is generally faster and more reliable than other methods, especially for taxonomic, physiological, and biotechnological applications. However, it is limited by the need for high technical skill and low success rates when environmental conditions are suboptimal.

Isolation with agar medium

The agar medium isolation method is a widely used approach to obtain pure diatom cultures. Solid media allow cells to grow separately, so single cells can form colonies that can be easily observed and isolated. This method's flexibility is an advantage, as it can be tailored to the species' needs and the study's environmental conditions. Several studies have applied variations to the technique to improve its effectiveness. For example, Kimura and Tomaru (2013) demonstrated that altering the agar surface with nylon mesh influences the growth pattern of *Chaetoceros tenuissimus* and enhances the likelihood of sterile media preparation. Proper autoclaving and compaction are essential for successful diatom culture on solid media. Sanniyasi et al. (2022) combined liquid and solid culture approaches to isolate *Nitzschia palea*, demonstrating that transitioning between the two media types increases isolation efficiency while improving long-term culture stability. Renta and Chen (2022) optimized the agar concentration in f/2 medium for the growth of Amphora sp. using the streak plate technique, demonstrating that 0.4% agar yielded the highest number of colonies and maintained culture viability for up to 8 weeks without contamination. These three studies demonstrate that agar methods can be adapted to various strategies according to the needs of research, whether emphasizing technical modifications, sterilization procedures, or media combinations. Overall, these studies show that the successful isolation of diatoms and the stability of the culture in solid media depend largely on technical adjustments, such as manipulating the surface structure of the medium, the use of an optimal agar concentration (0.4%) to promote long-term viability, and the inclusion of a transition phase between liquid and solid media to improve the efficiency of culture purification. Thus, although this method is not as precise as micropipettes, it remains a relevant alternative for diatom isolation, especially when large sample sizes are involved or stable cultures are required for long-term use.

Isolation with capillary pipette

The isolation method using a capillary pipette is a classic approach that is widely used in diatom and microalgae research. The process involves selecting a single cell under a microscope and aspirating it with a thin Pasteur pipette. Though relatively simple, this technique can separate individual cells from complex samples with high accuracy and does not require sophisticated equipment. However, the operator's technical skills are important because small errors can lead to isolation failure.

Several studies have examined the application of this method to different groups of algae, yielding mixed results. Single-cell DNA analysis is possible using this technique (Hamilton et al., 2015). Tsuchikane et al. (2018) emphasized the importance of repeated washing to ensure pure cells and subsequent cultures, as this procedure reduces the risk of contaminants being carried over from the natural environment. Iba (2014) demonstrated that selecting appropriate media, such as f/8-Si for dinoflagellates or f/2 with silicate supplementation for diatoms, significantly impacts the survival of isolated cells. Meanwhile, Susilaningsih (2014) demonstrated that using supporting devices, such as 24-well plates, increases the accuracy of single-cell transfer while minimizing the risk of cell loss. A more modern approach is demonstrated by Won et al. (2023), who optimized the capillary method using micro-scale 96-well plates to achieve higher cell maintenance efficiency. Additionally, An et al. (2023) added antibiotics to the culture medium to suppress bacterial growth, a common issue in capillary-based isolation.

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These various approaches demonstrate that the capillary method is highly flexible. Variations in media, support devices, and antibiotic supplementation make it suitable for use with various species and environmental conditions. Though simpler than micropipette isolation, the capillary method remains relevant because it can produce stable monoclonal cultures for diatom physiology, ecology, and biotechnology applications. Table 1 compares the three isolation methods, including their advantages and limitations.

Table-1. Comparison of diatom isolation methods.

Method	Principle	Advantages	Limitations	Reference
Micropipette	Single cell separation using a micropipette under an inverted microscope.	High accuracy, capable of producing pure monoclonal cultures, suitable for morphological and molecular analysis.	Requires high technical skill; longer process; success depends on the species.	Prasetiya et al. (2019) ; Tseplik et al. (2021b); Chen et al. (2013)
Capillary	Single-cell selection using a thin glass capillary pipette (Pasteur).	Relatively simple, flexible, can be modified (e.g., 24- or 96-well plates, antibiotic supplementation).	Success rate is influenced by skill; risk of contamination if washing is suboptimal.	Tsuchikane et al. (2018) ; Iba (2014) ; Susilaningsih, 2014) ; Won et al. (2023); An et al. (2023)
Agar	Cells are grown on solid agar medium until single colonies form.	Practical for large sample sizes; easy to observe; flexible (can be combined with liquid culture).	Risk of cross-contamination; not as precise as micropipettes; slower growth.	Kimura and Tomaru (2013) ; Sanniyasi et al. (2022)

Cultivation of diatoms

A culture is defined as an artificial environment in which diatoms grow. Mass diatom cultures are achieved under controlled laboratory conditions and in the field. The first step in the laboratory is small-scale culture, followed by mass culture. There are two main approaches to mass microalgae cultivation: open pond systems (Matsumoto et al., 2017) and closed photobioreactors (Tam et al., 2021).

Open ponds are cheaper and more energy efficient but susceptible to contamination, which reduces productivity. Conversely, closed photobioreactors reduce contamination and increase biomass yield; however, they have higher installation and operational costs (Admirasari et al., 2025).

Small laboratory scale

Small-scale diatom culture involves cultivating species in small volumes of nutrient-enriched water under laboratory-controlled conditions. Culture systems can use adaptive laboratory approaches (Saxena et al., 2022a; LaPanse et al., 2023). For small-scale cultures, it is essential to use thoroughly sterilized or cleaned vessels. These vessels are filled with filtered seawater that maintains a salinity of 28–34%. To promote growth, the water is supplemented with specific nutrient formulations such as TMRL, Walne, or Guillard and Ryther's F media. Pure diatom strains, previously isolated and grown in the laboratory, are introduced into these containers. Although borosilicate glass brands like Corning or Pyrex are standard for these setups, the scale of the culture system can vary significantly, ranging from test tubes to large concrete tanks depending on the required biomass volume (Pachiappan et al., 2015). Mass cultivation of Diatom using local salts, for example rock salt and table salt can substitute the use

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of standard seawater. It exhibits growth and stupendous productivity. This culture method is costly effective, because it can reduce the cost than standard Guillard's f/2-Si composition (Saxena et al., 2022b).

Open cultivation

Open cultivation is the most widely used method for the large-scale production of microalgae and diatoms because it is simple and inexpensive. This system typically consists of an open pond or series of ponds that use sunlight as the main energy source, resulting in low energy costs. Proper pond design, strain selection, and management of external factors such as temperature, pH, and light intensity are crucial for successful cultivation. Several studies have highlighted the potential and limitations of this method. For example, Matsumoto et al. (2017) used a large-scale pond system to cultivate different diatom strains according to the season. They cultivated *Fistulifera solaris* at warm temperatures and *Mayamaea sp.* at low temperatures. This approach-maintained biomass and lipid production throughout the year. This approach confirms that selecting temperature-adaptive strains is key to successful open cultivation. Meanwhile, Min et al. (2021) combined raceway ponds with wastewater effluent as the culture medium and added a lighting system to maintain stable growth even as natural light intensity decreased. Their use of artificial lighting technology demonstrates that, despite the simplicity of the open system, design innovations can significantly increase biomass productivity. Thus, these studies demonstrate that the success of open cultivation depends on both natural factors and technical strategies that support system efficiency. This open-water aquaculture system holds great promise for large-scale production. However, because the system is directly exposed to the environment, there is a risk of contamination by microorganisms and predators (Narala et al., 2016). Productivity often varies significantly due to daily and seasonal climate changes that influence temperature, light, and nutrient availability (Smachetti et al., 2018). Furthermore, the rate of water evaporation in open ponds can change the ionic concentrations of the medium, ultimately impacting the stability of cell growth.

Close cultivation with photobioreactors

Closed cultivation with photobioreactors is a modern approach designed to provide better environmental control. This technology allows for precise control of light, CO_2 supply, nutrients, and temperature. Culture and growth are more stable when there is minimal contamination, leading to the production of biomass with consistent quality (Figure 1). Photobioreactors are available in various designs, such as tubular, flat-panel, bubble column, and helical. Each design offers specific advantages that depend on the production objectives.

Studies have demonstrated the advantages of closed systems in various applications. For example, Vella et al. (2019) used a tubular photobioreactor to cultivate *Thalassiosira weissflogii*, successfully maintaining a high-density culture with stable growth throughout the observation period. Eilertsen et al. (2022) tested a vertical column airlift photobioreactor model with CO_2 sequestration for mass cultivation. Glockow et al. (2023) modified the helical photobioreactor's function by placing it inside a chicken coop. The system produced biomass and functioned as a biofilter, significantly reducing CO_2 and NH_3 emissions. These results confirm the dual potential of closed photobioreactors in biomass production and pollution mitigation. Conversely, Tam et al. (2021) emphasized the importance of closed systems for producing live feed in aquaculture, especially for vaname shrimp. In this context, cell density can be consistently maintained to meet the demands of hatcheries and commercial farms. These studies demonstrate that photobioreactors are superior for growth stability and flexible enough to adapt to various purposes, ranging from bioindustry to environmental applications.

Closed photobioreactors provide higher biomass productivity per unit area compared to open systems, along with improved environmental control and reduced risks of contamination (Sato et al., 2014). These advantages make them particularly suitable for high-value applications, such as the production of lipids, pigments, or bioenergy, where maintaining consistent biomass quality is crucial. However, these systems also have significant limitations. Initial investment costs and operational energy consumption, particularly for lighting and cooling, are higher. Additionally, certain designs can trigger dissolved oxygen accumulation or cause hydrodynamic stress on cells (Narala et al., 2016). Challenges associated with biomass harvesting continue to pose significant obstacles. The small size of algal cells makes processes like centrifugation, flocculation, and filtration expensive and energy-intensive (Mantzorou and Ververidis, 2019).

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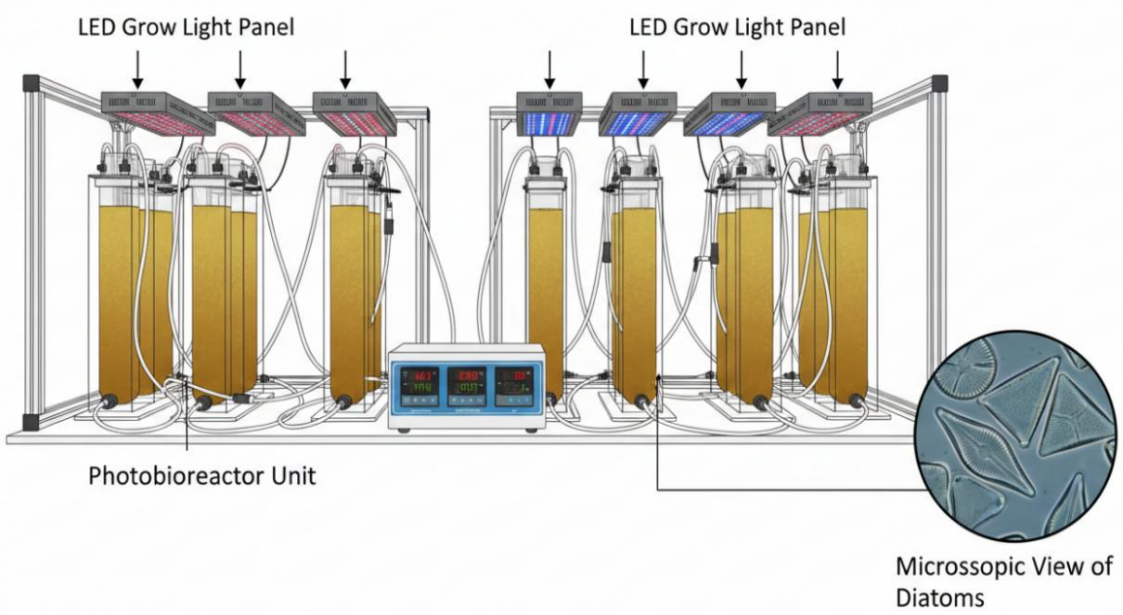

Figure-1. Photobioreactor system for closed cultivation of microalgae and diatoms.

Comparison between close and open cultivation

Compared to closed systems, other methods like open-field cultivation have lower productivity and makes it difficult to control environmental conditions; however, because investment and operating costs are significantly lower, it has become a highly attractive option for industrial-scale biomass production (Moejes and Moejes, 2017; Klein and Davis, 2022). Although this open system offers an excellent cost-benefit ratio, its success depends heavily on-site conditions and environmental factors and is susceptible to fluctuations in productivity as well as serious contamination issues, such as protozoan infestation during the large-scale cultivation of Phaeodactylum tricornutum (He et al., 2022). In addition, closed-system cultivation in photobioreactors offers excellent environmental control, stability, and biomass quality; however, its adoption remains limited due to high initial investment costs and high energy consumption.

A comparison of diatom cultivation methods is presented in Table 2. Consequently, future development efforts to address the limitations of individual systems will focus on innovative, energy-efficient designs for ponds and bioreactors, as well as their integration with wastewater sources and renewable energy, to improve the sustainability of this technology, particularly in regions with significant potential for aquaculture and the bioindustry.

Characterization of diatoms

Diatom characterization is an important step in identifying the species that have been isolated. The goal of this process is to obtain comprehensive information on diatom characteristics, including those visible morphologically and those confirmed by molecular analysis. Identification based solely on morphological observation is often insufficient due to species similarities and form variations influenced by environmental conditions. Therefore, an integrative approach combining morphological characterization with molecular analysis is necessary for more accurate, comprehensive identification. This section explains two main characterization methods: morphological characterization, which involves observing diatom cell characteristics using light and electron microscopes, and molecular characterization, which uses DNA analysis to determine phylogenetic relationships and confirm species. These two methods complement each other, providing a strong scientific basis for understanding the diversity and classification of the diatoms under study. Figure 2 shows a diagram of the diatom research workflow, including isolation, cultivation, and characterization.

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Table-2. Comparison of diatom cultivation methods.

Aspect	Open Cultivation (Open Pond/Raceway Pond)	Closed Cultivation (Photobioreactor)
Principle	Outdoor cultivation using open ponds, utilizing sunlight.	Culture in a closed system (tubular, flat-panel, helical, column) with full control.
Advantages	● Low operating costs ● Large production scale ● Simple design	● Precise environmental control (light, temperature, nutrients, CO_2) ● Minimal contamination ● Consistent biomass quality
Limitations	● Susceptible to contamination (microbes, predators) ● Fluctuations due to climate and temperature ● Low lipid productivity ● Water loss due to evaporation	● High investment and operational costs ● Risk of O_2 accumulation & hydrodynamic stress ● Expensive and complex biomass harvesting process
Main Applications	Mass biomass production at low cost; integration with liquid waste.	Production of lipids, pigments, bioenergy, aquaculture feed; biofilters for gas & waste.
Case Studies	• Matsumoto et al. (2017): seasonal strains for year-round production • Min et al. (2021): utilization of wastewater effluent & additional lighting	• Vella et al. (2019) : *T. weissflogii* in a stable tubular PBR • Glockow et al. (2023): Helical PBR + gas biofilter • Tam et al. (2021) : shrimp feed production

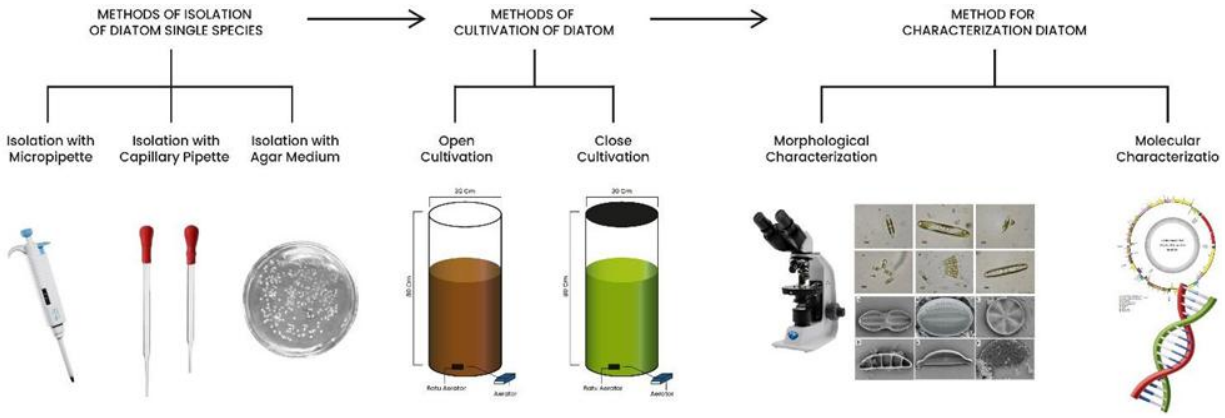

Figure-2. Flow diagram of diatom research workflow, including methods of single-species isolation, cultivation approaches, and characterization techniques.

Morphological characterization

Morphological characterization is an important method for identifying diatoms, as it provides a visual description of their external phenotype, including the shape, size, and structure of the frustules. This analysis is typically performed using a light microscope (LM) for general observation and an electron microscope (SEM/TEM) for the detailed examination of features as small as 5–7 nm, as shown in Figure 3. The morphological approach is important because it

remains the foundation of traditional classification systems and allows for comparison with previous taxonomic literature. However, this method has limitations because many species exhibit morphological similarity, or morphological plasticity, so identification cannot always be confirmed by visual observation alone.

The importance of removing organic material from frustules before conducting microscopic analysis is essential and the use of recently collected, versus old material, ensures the observation of fine features. There are several techniques available, each with their own advantages and disadvantages. Aggressive organic removal involves the use of acids like nitric and sulfuric with heating (Hamilton et al., 2015). These aggressive treatments are combined with hydrochloric acid carbonate removal in calcium rich samples. Another common less aggressive treatment uses heated hydrogen peroxide followed by potassium permanganate (Van der Werff, 1953). Heat treatment with hydrogen peroxide alone is also commonly used and considered less aggressive, potentially preserving fine morphological features. After removal of acids and treatment chemicals, the clean frustules are permanently mounted with Naphrax (refractive Index 1.64 in liquid, 1.74 solid) medium for observation. Light microscopy (LM) helps describe the size and shape of the valves, while scanning electron microscopy (SEM) allows for identification of detailed ornamentation, such as striae, raphe, or stigmata. This combined approach improves the reliability of morphological descriptions, especially for new species.

Studies such as those by Luthfi et al. (2024a, b) in Indonesian marine areas demonstrate the important role of morphological methods in exploring local biodiversity. Using samples from Bawean Island and Tomini Bay, light microscopy (LM) and scanning electron microscopy (SEM) were employed to describe morphological characteristics, including ornamentation details. As a result, the researchers were able to establish two new genera, *Paracatenula* and *Wallaceago*, and seven new species in the Catenulaceae family. These findings demonstrate the continued effectiveness of morphological approaches in detecting hidden diversity, particularly in tropical regions underrepresented in taxonomic studies. Despite limitations, morphological analysis remains a crucial starting point for discovering new species. Figure 3 shows various diatom species from the Indonesian archipelago that are new to science. Similar cases have been observed in studies by Hustedt (1942); Bramburger et al. (2006); Kulikovskiy et al. (2020b) in the Malili Lakes of Sulawesi, and Tseplik et al. (2021b) in Lake Matano. These studies used frustule-clean procedures followed by light microscopy (LM) and scanning electron microscopy (SEM) to describe the morphological variation of local species, including *Achnanthidium gladius* sp. nov. This research suggests that combining monoclonal strain isolation with micropipette techniques and morphological analysis provides a more accurate picture of species differentiation. Findings from Lake Malili reveal previously undocumented characteristics of the *Gomphonema longissimum* population (Kulikovskiy et al., 2020b). These results strengthen the evidence that the Sulawesi aquatic ecosystem is a diatom biodiversity hotspot with great potential for species new to science.

Overall, the morphological approach remains an essential first step in diatom taxonomy. Although interspecies similarities and environmental factors can hinder morphological identification, the combined use of light microscopy (LM) and scanning electron microscopy (SEM) can enrich our understanding of diagnostic features. However, morphological analysis, combined with molecular characterization, essentially improves identification accuracy and establishes a solid foundation for ecological, evolutionary and diatom application studies.

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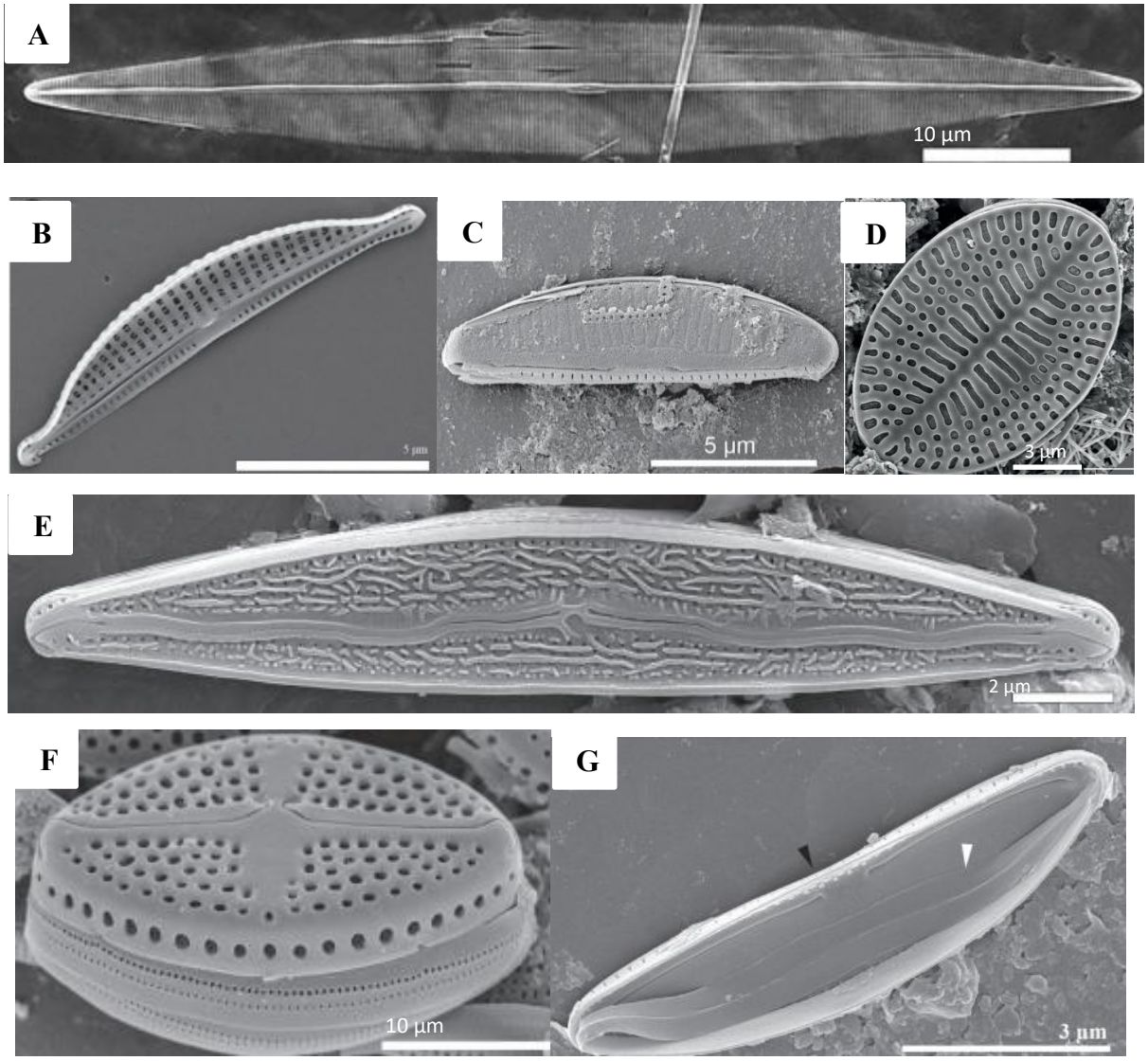

Figure-3. Various new diatom species found in Indonesia: (A) *Haslea nusantara* (Prasetiya et al., 2019); (B) *Halamphora lombokensis* (Pane et al., 2024); (C) *Catenula javanica* (Kryk et al., 2021); (D) *Upsilococconeis dapalistriata* (Riaux-Gobin et al., 2022); (E) *Encyonopsis indonesica* (Kapustin et al., 2021); (F) *Luticola cribriareolata* (Rybak et al., 2021); and (G) *Catenula densestriata* (Luthfi et al., 2024a).

Molecular characterization

Molecular characterization of taxa is an important complement to morphological identifications, especially in cryptic species. Marker genes, such as 18S rRNA (nuclear), rbcL (chloroplast), and cox1 (mitochondrial), are widely used in diatom phylogenetics because they are stable and available in international databases. By analyzing these genetic sequences, researchers can ensure accurate classifications and reveal genetic diversity and evolutionary relationships between species. Thus, molecular data forms an important foundation for studies of diatom biodiversity and ecology.

A study of new species *Haslea nusantara* by Prasetiya et al. (2019) demonstrates how analyzing organelle genomes (chloroplast and mitochondrial) can clarify the taxonomic position of new species. Using the Illumina HiSeq sequencing approach and rbcL- and cox1-based phylogenetic analyses, *H. nusantara* was found to form a separate clade within the blue *Haslea* group. Kulikovskiy et al. (2020b) used a combination of morphological analysis and DNA sequencing of the SSU rDNA, rbcL, and cox1 genes to characterize

Gomphonema longissimum populations from Lake Malili. Maximum likelihood (ML) and Bayesian inference (BI) analyses revealed genetic variation between populations that was not always apparent from morphological characteristics. This integrative approach strengthens taxonomic assignments and provides new insights into the evolutionary trajectories of diatoms in ancient systems. Another example comes from the study of *Achnanthidium gladius* sp. nov. by Tseplik et al. (2021b). Using 18S rDNA fragments (V4 domain), (Tseplik et al., 2021b) confirmed the phylogenetic position of *A. gladius* using ML and BI approaches.

DNA-based molecular identification, which uses genetic markers such as 18S and 28S ribosomal DNA, ITS, rbcL, and cox1, achieves far higher identification accuracy than purely morphological approaches. Using the full-length 18S rDNA-based metabarcoding method with Nanopore MinION technology, long-read sequences identified 298 genera of protozoa, including diatoms. In comparison, full-length 18S rDNA detected 250 genera (84%), whereas the short-read V4 sequence identified only 226 genera (76%) and the V8-V9 sequence only 213 genera (71%). This demonstrated the superiority of metabarcoding's taxonomic resolution (Gaonkar and Campbell, 2024). Among the available markers, the chloroplast rbcL marker is frequently used for metabarcoding diatoms, as it can distinguish taxonomic groups down to the species and even subspecies level. At the same time, the V4 region of 18S ribosomal DNA remains the first choice, as reference libraries are widely available and it exhibits sufficient variation (Kezlya et al., 2023). Analyses of the rbcL marker in the metabarcoding of marine diatoms have shown that this marker is suitable not only for monitoring community diversity but also for inferring population structure within certain genera, such as *Pseudo nitzschia* (Turk Dermastia et al., 2023).

DNA-based molecular identification methods provide more detailed information for identifying diatom species that are difficult to distinguish based on morphological characteristics alone (Bouazzara and Chaibi, 2025). Although high hopes are pinned on this method, there are still numerous limitations to molecular approaches in diatom taxonomy that must be considered. Even with common molecular markers such as 18S rDNA, 28S rDNA, ITS, rbcL, and cox1, it is difficult to distinguish closely related species from one another, let alone detect genetic variations within a species. Consequently, of the estimated 100,000 to 200,000 diatom species, only about 12,000 have been successfully identified to date (Wang et al., 2022). From a bioinformatics perspective, the DNA metabarcoding method introduces biases at every stage of the process, particularly during bioinformatics analysis phases such as OTU clustering, chimeric detection, and taxonomic assignment. The diversity of these methods complicates comparisons between different studies and hinders the development of standardized procedures for biomonitoring (Tapolczai et al., 2019). This limitation is further exacerbated by the lack of well-maintained reference libraries. Although some general libraries for protozoa are available, there is as yet no comprehensive library specifically focused on diatoms (Rimet et al., 2019). Therefore, the low taxonomic representativeness of diatoms in existing molecular databases underscores the need for a more integrated approach that combines morphological and molecular data in future diatom research (Gonzalez-Saldias et al., 2026).

Conclusion

Diatoms are single-celled microalgae with a silica shell; they are highly versatile ecologically, play a fundamental role as primary producers in aquatic ecosystems, and are of great value in biotechnology and materials science. The success of diatom collection depends largely on three fundamental pillars: precise separation using micropipettes, the selection of an efficient cultivation system (open tanks or photobioreactors), and the analysis of morphological and molecular characteristics (18S rRNA, rbcL, cox1). This integrated approach has proven indispensable for species classification, as demonstrated by the discovery of new taxonomic groups in Indonesian archipelago and other tropical regions worldwide.

However, since only about thousands of species worldwide have been identified, urgent research tasks remain. This means that the diversity of tropical diatoms in Indonesia and other regions is still significantly underestimated. Inadequacies in molecular databases exacerbate this problem, bioinformatic biases in metabarcoding analysis, and technical and economic constraints on large-scale sampling. Future research should prioritize the study of biodiversity in tropical regions by establishing a comprehensive DNA reference library based on third-generation sequencing (Nanopore). This initiative should be combined with innovations in energy-

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efficient hybrid cultivation systems and omics approaches to promote the biosynthesis of high-value compounds (fucosanthin, EPA, biosilica) and develop diatoms into a strategic and sustainable biotechnological platform.

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Contribution of Authors

Widayat C: Wrote the original draft of the manuscript.
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Nurdiani R: Served as supervisors, providing guidance and critical oversight of the research.
Hamilton PB & Solak CN: Reviewed literature, critiqued and edited the manuscript draft.

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