

Marine Mollusks are a Good Source of Natural Products

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Abstract

Almost all marine ecosystems have *Mollusca* members for their balanced continued functioning. While some may seem harmless or even gentle, others are equipped with powerful weapons like venomous tentacles to fend off predators. Mollusks are considered an important subject of research in various fields due to their unique biology and ecological significance. Their research has many implications in areas such as medicine and biotechnology. Numerous species have recently been the subject of research, with numerous secondary metabolites being isolated and their chemical structures being clarified. Natural substances that were poisonous to tumor cells, harmful bacteria, viruses, and others have been discovered. Some isolated compounds constituted the active ingredients in medicinal preparations used in clinical practice, while others are still in various stages of preclinical research. These substances often lacked equivalents in other naturally occurring substances with terrestrial origin. Metagenomics tools has simplified the discoveries in this field and with the help of metagenomics, we could identify new genes and proteins to be used in biotechnology applications, such as the development of new drugs.

Keywords: Marine mollusks; natural products; secondary metabolites; shotgun metagenomics; bioinformatics.

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1. Introduction

Mollusks are found in a wide range of habitats, from freshwater streams and lakes to the deep sea. They play important ecological roles in their respective environments (Proksch *et al.*, 2002; Pati *et al.*, 2015). These animals have evolved a variety of adaptations to survive in their ecosystems. Species of oysters and mussels, for example, can filter large amounts of water and help maintain water quality (Riisgård *et al.*, 2015). Snails and slugs are important decomposers and contribute to nutrient cycling (Smith, 2008). Therefore, they are crucial to maintain a healthy ocean

environment. Although they are a diverse group, almost all mollusks share some common characteristics that identify their morphologies as being mollusks.

They are classified into seven major classes by different morphologies (Parkhaev, 2017). These classes are seen in **Figure 1**. Many of them have been greatly studied, for example, The bivalve clam *Meretrix petechialis*, found abundantly in the shallow waters of Southeast Asia, can store and secrete erythromycin antibiotic (Yue *et al.*, 2022). Other recent studies have shown that *Hexabranhus sanguineus* nudibranch produces antibiotics and anti-inflammatory compounds that could potentially be

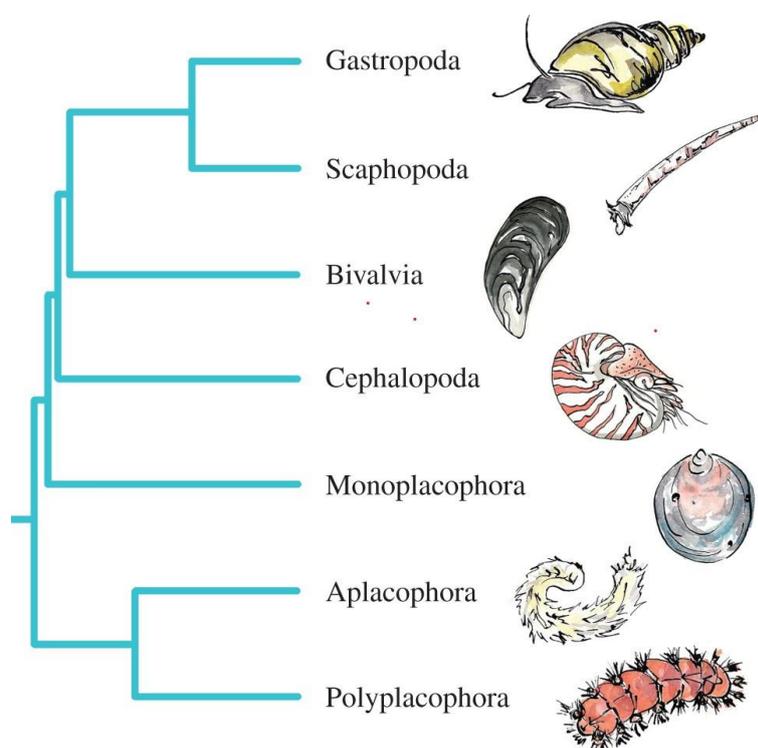


Figure 1. Major classes of *Mollusca* phylum. Copyright © 2021 the Royal Society Journal.

used to develop new drugs (Shou-Mao Shen *et al.*, 2023; S. M. Shen *et al.*, 2023). *Euprymna scolopes*, the Hawaiian bobtail squid was approved to have antimicrobial and antifungal compounds exhibited from its symbiotic bacterial communities. Such associated bacteria live in its female reproductive organ for the protection of its embryos from diseases by microorganisms to ensure successful development (Kerwin *et al.*, 2019; Suria *et al.*, 2020). Shipworms are other interesting members. Their bacterial species are also used in scientific research because shipworms cannot live without their endosymbiotic bacteria. It was found that these bacteria live in some specialized cells in shipworms' gills and produce wood-degrading enzymes. These enzymes are transported to the shipworm gut to digest wood there (Altamia *et al.*, 2020; Stravoravdis *et al.*, 2021). A study also demonstrated that some shipworm bacterial symbionts can produce promising new secondary metabolites with anti-parasitic activities (O'Connor *et al.*, 2020). Therefore, marine mollusks are an important resource for medical research.

2. Mollusks in scientific research

2.1. Pain management activity

Cone snails are a group of marine snails that have been known for their deadly venom. The venom of cone snails has been found to have various bioactive compounds that may have potential medical benefits

(Vetter *et al.*, 2012). The venom of cone snails is a complex mixture of peptides and proteins that targets specific receptors in the nervous system (Himaya *et al.*, 2018). These peptides and proteins have different pharmacological properties that make them possible candidates for drug development (Xiong *et al.*, 2020). One of the most promising applications of cone snail venom is pain management (Proksch *et al.*, 2002). The venom contains peptides that can selectively block certain types of pain receptors, such as those involved in chronic pain. Researchers have isolated and assessed several peptides from cone snail venom, some of which have already been approved for clinical use. For example, ziconotide is a peptide that has been approved by the FDA for the treatment of severe chronic pain in humans (Miljanich, 2004; Safavi-Hemami *et al.*, 2019).

In addition to pain management, cone snail venom has also shown the possibility to treat neurological disorders (Nguyen *et al.*, 2023). Some of the peptides in the venom can cross the blood-brain barrier and target specific receptors in the brain. For example, one peptide called conantokin-G has been shown to improve cognitive function in animal models of Alzheimer's disease (Vetter *et al.*, 2012). Another peptide called alpha-conotoxin has been tested in clinical trials for the treatment of multiple sclerosis (Vetter *et al.*, 2012).

2.2. Proteins with anti-inflammatory properties

Recent studies suggest that mollusks may also possess anti-inflammatory properties. Slugs and snails have long been recognized for their unique healing properties, particularly for their ability to provide relief from inflammation (Chakraborty *et al.*, 2017; Khan *et al.*, 2019). Recent studies have shown that snails contain a variety of anti-inflammatory compounds that have the ability to reduce the severity of numerous health conditions (Borquaye *et al.*, 2017; Ahmad *et al.*, 2019). These compounds, which include glycoproteins, HTI peptides, and enzymes, have been found to inhibit the activity of pro-inflammatory agents, including interleukin-6, interleukin-8, and tumor necrosis factor- α .

Different studies revealed the high degree of anti-inflammatory activity of snails' protein extract, and that the extract have immunomodulatory proteins that inhibit the production of inflammatory cytokines such as TNF- α , IL-1 β and IL-6 (Gubitosa *et al.*, 2020; El-Zawawy *et al.*, 2021). In addition, investigations revealed the presence of allantoin, which is a powerful anti-inflammatory agent (Wargala *et al.*, 2023). Allantoin has been shown to reduce inflammation and pain in the body by inhibiting the production of inflammatory cytokines (Gubitosa *et al.*, 2020; Wargala *et al.*, 2023). It also helps speed up the healing of wounds, making it a valuable compound for treating a range of skin conditions. Therefore, research recommends that snail peptides should be further investigated for their good anti-inflammatory potentials.

2.3. Mollusks' mucus

Mollusks' mucus has been studied as a source of new secondary metabolites with diverse biological activities. Antiviral activity of the mucus of a land slug *Phyllocaulis boraceiensis* has been analyzed which protects cells infected with measles virus (Toledo-Piza *et al.*, 2016). Another study approved its antiviral activity against influenza virus (de Toledo-Piza *et al.*, 2018). Snail mucus has also been used in traditional medicine for its healing properties (Gubitosa *et al.*, 2020; El-Zawawy *et al.*, 2021; Waluga Kozłowska *et al.*, 2021). In recent years, it has gained popularity as a key ingredient in skincare products. This slime-like substance contains a unique combination of compounds that make it highly beneficial for skin health (El Mubarak *et al.*, 2013; Laneri *et al.*, 2019). The mucus of the garden snail *Cornu aspersum* has been investigated, and has showed some biologically active natural substances that might be an important source for new drugs to treat human diseases (Dolashki *et al.*, 2020).

In general, mollusk mucus is rich in nutrients such as

hyaluronic acid, glycoprotein, and allantoin, which make it an effective moisturizer, exfoliant, and anti-aging agent (Dinica *et al.*, 2021). Scientific studies have shown promising results as it has been found to improve skin elasticity, reduce wrinkles, and fine lines, and even fade scars and hyperpigmentation (Dinica *et al.*, 2021). Furthermore, this mucus has antioxidant properties that protect the skin from damage caused by free radicals, environmental pollutants, and UV radiation (El Mubarak *et al.*, 2013).

2.4. Mollusks in neuroscience and developmental biology Research

Mollusk members have different contributions to biological research. Sea squids have improved our knowledge of the neurological system because they have one of the largest known nerve cells in the animal kingdom called the "giant squid axon" (Song *et al.*, 2016; DeGiorgis *et al.*, 2022). The giant squid axon has become a popular subject of study for neuroscientists due to its size and unique properties, which allow researchers to better understand the workings of the nervous system (Song *et al.*, 2013). This nerve fiber is almost 1 meter long and 1 mm in diameter. It helps the animal in rapid movement and escapes from danger (Song *et al.*, 2016).

In addition, the giant squid axon has served as a model system for studying the principles of electrical signaling in humans (Crispino *et al.*, 2018). It contains various proteins and ions that help regulate the flow of electrical signals through nerve fibers. Therefore, it has been used extensively to study action potentials and ion channels. Research on that nerve cell has led to numerous breakthroughs in the field of neuroscience (DeGiorgis *et al.*, 2022). This has helped researchers to better understand the mechanisms behind diseases such as epilepsy and multiple sclerosis (Moreno *et al.*, 2011).

Moreover, mollusks have unique developmental processes that make them valuable research subjects. The ability to regenerate lost body parts is an important adaptation that allows mollusks to survive in their environment. The mechanisms of regeneration in mollusks vary depending on the species and the type of tissue being regenerated (Bever *et al.*, 1988; Krois *et al.*, 2013). In general, it is a complex process that involves the activation of stem cells and the differentiation of these cells into specialized cell types. Studies have shown that mollusks can regenerate a wide range of structures, including limbs, eyes, and organs (Chase *et al.*, 1983; Bever *et al.*, 1988; Machałowski *et al.*, 2020).

The study of mollusk regeneration has potential applications in fields such as medicine and biotechnology. Researchers are exploring the use of

mollusk-derived compounds for wound healing and tissue regeneration in humans (Anjaneyulu *et al.*, 2016). For example, mollusk-derived stem cells could be used to regenerate damaged or diseased tissues, such as cartilage or nerves (Anjaneyulu *et al.*, 2016; Dhiman *et al.*, 2021). Additionally, some researchers are using snails as a model system to better understand the basic principles of regeneration, which could ultimately lead to new therapies for a wide range of diseases and injuries.

2.5. Anti-tumor activity

As sponges often contain unpleasant chemicals in their tissues. Many species of nudibranch mollusks accumulate secondary metabolites obtained from sponges on which they feed, including alkaloids, diterpenes, and sesquiterpenes (Blunt *et al.*, 2016). These chemicals are reused by the nudibranch to help them prevent predators from eating them (Huang *et al.*, 2016; Cheney *et al.*, 2018). *Jorunna funebris* nudibranch was discovered to have "jorumycin" chemical compound (Angelo Fontana *et al.*, 2000). It exhibits high antitumor activity in cells of breast, prostate, and renal cancers. This species' distribution was supported by the distribution of a sponge in the genus *Xestospongia* (Huang *et al.*, 2016). This nudibranch was found to prey exclusively on this kind of sponge (Huang *et al.*, 2016). These chemicals are stored in the nudibranch's body and are incorporated into its tissues, providing them with a good defense against predators.

Latrunculin-A, which is an important secondary metabolite, has been isolated from a Pacific nudibranch *Chromodoris* sp. (Cheney *et al.*, 2016; Hamilton *et al.*, 2022). It was previously described from a Red Sea sponge, *Latrunculia magnifica*, for which it serves as a defensive secondary metabolite (Okuda *et al.*, 1985). It is a 16-membered macrolide that prevents actin polymerization within cellular processes. A study showed the sequestration of this toxic compound by five closely related nudibranch mollusks of the genus *Chromodoris* (Cheney *et al.*, 2016). This compound was only present in the rim of the mantle of these species, where storage reservoirs containing secondary metabolites are located. It was assumed that these species selectively accumulate latrunculin A in the part of the mantle that is more exposed to possible predators for defense (Hertzer *et al.*, 2023). This was consistent with another study that showed the transfer of some secondary metabolites from sponges to the nudibranch *Hypserlodoris webbi* mantle dermal formations (A Fontana *et al.*, 1994). Moreover, *Nembrotha kubaryana*, *Hypselodoris infucata*, and *Chromodoris*

petechialis nudibranchs were also observed for producing some natural products from their diet (Karuso *et al.*, 2002).

2.6. Natural products of mollusks' bacterial symbionts

The bacterial symbionts associated with mollusks produce a wide range of compounds that protect the mollusk and provide it with essential nutrients. For example, *Anadara broughtoni* clam has been studied for exploring the antimicrobial activity of their associated bacteria. It was revealed that its bacterial members have a high number of antimicrobial compounds (Romanenko *et al.*, 2008). This can help protect the animal from infections caused by pathogens. Genome analysis for a novel bacterium isolated from the sea snail *Acmaea* species showed that this isolate has many biosynthetic gene clusters including bacteriocins antimicrobials (Yun *et al.*, 2021). These bacteriocins help the animal to maintain a healthy microbiome.

Another study showed that a cone snail contains abundant γ -pyrones known as nocapyrones (Lin *et al.*, 2013). These are secondary metabolites that are synthesized by symbiotic bacteria. The natural roles of nocapyrones are unknown, but they are active in neurological assays, showing that mollusks are an overlooked source of different secondary metabolites. *Lienardia* species, a marine gastropod mollusk, has *Streptomyces* members that contained lobophorins compounds. These are spirotetronate polyketides that have antimicrobial and antitumor activity. Researchers could isolate new and known lobophorins compounds which may participate in the discovery of new important drugs (Lin *et al.*, 2014).

Studying toxin production in the Hawaiian marine mollusk *Elysia rufescens*, showed that this mollusk is chemically defended against predators by a diverse group of lipopeptide toxins called kahalalides (Zan *et al.*, 2019). Investigations showed that one of these molecules, kahalalide F, is a potent antitumor agent which has been evaluated clinically. Researchers have combined different techniques to determine the molecular basis of kahalalides production in that mollusk. Results showed that the molecular structures of these kahalalides have several features of microbial biosynthesis and the bacterial source of these compounds has been acquired by the mollusk from its algal food (Zan *et al.*, 2019).

Other studies of bacterial communities associated with nudibranchs from Indonesia showed antibacterial activity against multidrug-resistant bacteria

(Böhringer *et al.*, 2017; Kristiana *et al.*, 2019). Another study from Indonesia described the anti-MRSA, cytotoxicity, and anti-HSV-1 activity of bacterial symbionts from the viscera of nudibranchs (Kristiana *et al.*, 2020). The presence of bacterial symbionts in a nudibranch to inhibit the growth of pathogenic *Vibrio* species (Prayitno *et al.*, 2020) was also revealed. In addition, the nudibranch *Rostanga alisae* harbors a high diversity of specific endo- and extracellular bacteria, and some of them are previously unknown as symbionts of marine invertebrates (Zhukova *et al.*, 2022). These bacterial symbionts provide the nudibranch with essential nutrients and chemical defense against predators.

Bacterial communities of *Chromodoris quadricolor* mantle, a Red Sea nudibranch, were detected to encode many potential BGCs that would help produce different natural products (Mahmoud *et al.*, 2023). The presence of several pathways related to antimicrobial and secondary metabolites biosynthesis suggested that the nudibranch host has these bacterial symbionts as protecting members. They use these compounds as a defensive mechanism against bacterial pathogens and host predators, promoting nudibranch immunity. Research into the bacterial symbiosis of nudibranchs is still ongoing, and there is still much that is not known about the relationship between nudibranchs and their associated bacteria. However, it becomes obvious that these bacteria play an important role in the ecology and biology of these interesting animals which we can benefit from.

3. Metagenomics and sequencing tools for exploring bioactive compounds

Metagenomics is a field of study that involves the genomic analysis of, for example, microbial communities, including those found in marine environments (Barone *et al.*, 2014). Starting from the characterization of community compositions by the analysis of 16S rRNA marker gene to whole metagenome shotgun sequencing which additionally allows for functional analysis. It has been confirmed to be one of the most valuable research fields for different ecologies in the last decades. Metagenomics has been used to study marine members in many ways. For example, researchers can use metagenomics to study the diversity of microbial communities of coral reefs or deep-sea hydrothermal vents (Eythorsdottir *et al.*, 2016; Dong *et al.*, 2023). They can also use metagenomics to detect new genes and enzymes that could be used in different applications (Barone *et al.*, 2014).

3.1. Sanger and Next-generation sequencing (NGS) instruments

Sanger sequencing is a method of DNA sequencing developed by Frederick Sanger in the 1970s. It is a

chain termination method that uses dideoxynucleotides (ddNTPs) to halt the extension of complementary strands during DNA synthesis. This allows for the determination of the nucleotide sequence of a DNA fragment (Sikkema-Raddatz *et al.*, 2013). However, Next-generation sequencing (NGS) refers to a high-throughput technology that enables rapid sequencing of millions of DNA or RNA molecules in a single sequencing effort (Schuster, 2008). NGS platforms use innovative technologies to sequence DNA or RNA samples in parallel, which is much faster and more cost effective than traditional Sanger sequencing methods. The most widely used NGS equipment is Illumina sequencing platform. It is a platform for large-scale sequencing. The device uses Illumina's sequencing by synthesis (SBS) chemistry and can massively and parallelly sequence billions of DNA fragments having bases between 50 and 500 with an output of up to 6 Tb (Modi *et al.*, 2021). Due to their better per-base cost-effectiveness and excellent sequencing accuracy, Illumina's sequencing instruments are currently the most extensively utilized ones (Quail *et al.*, 2008). They accomplish read lengths between 2 X 125 and 2 X 300 bp across their platforms and throughout with multiple gigabases and several terabases in a few hours and in almost a week, respectively (Myer *et al.*, 2016). General workflow for NGS is illustrated in Figure 2.

3.2. Bioinformatics

Sequencing-based research has gained power in all branches of biological science. However, technology advanced more quickly than its computational equivalent in addition to the cost reduction of sequencing tools. This resulted in unexpected volumes of sequencing data being produced, creating a significant challenge for data processing. Earlier, it was not available that complex bioinformatic tools may handle millions of readings in a reasonable amount of time. Thus, handling these enormous volumes of data was essentially the main emphasis of early bioinformatic processing. Therefore, to keep up with this quick development, bioinformatics also expanded. Thus, processing pipelines were about comparing 16S rRNA gene sequence tags to ribosomal databases or detecting environmental gene tags (EGTs) by searching reads against various nucleotide or protein databases using extensive BLAST searches on powerful computer clusters (Edwards *et al.*, 2006; Camacho *et al.*, 2009). Powerful computer clusters are groups of computers that work together to solve complex problems. They were used in scientific research, engineering simulations, and other applications that require large amounts of computing

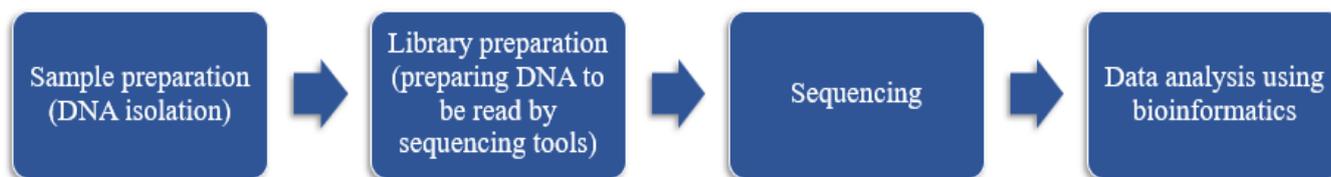


Figure 2. General steps for NGS workflow.

power (Stein, 2010; Yoshii *et al.*, 2012).

The error patterns of the emerging NGS devices presented another challenge. Not enough was known about potential biases and artifacts introduced during the sequencing process. However, it has been demonstrated that they can have an altering effect on study outcomes if left untreated (Huse *et al.*, 2007; Dohm *et al.*, 2008; Berry *et al.*, 2011). Now, many tools that can be used to analyze metagenomic datasets have been developed. It offers a wide range of algorithms, tools, and software. Bioinformatics tools have evolved into a necessary step which is the proper quality treatment, such as error filtering or error correction. This was very necessary, especially with the increased sequencing depth (Zhou *et al.*, 2014). The processing and analysis of metagenomic data sets have also seen the development of new tools, including pre-processing, various taxonomic classifications, the reconstruction of genomes by metagenome assembly, the clustering of assembled genomic fragments, the clustering of single reads into functional groups, and others (Teeling *et al.*, 2012; Kim *et al.*, 2013; Gauthier *et al.*, 2019).

3.3. Shotgun metagenomics

Traditional culturing techniques do not capture the full extent of microbial range present in environmental samples as the vast majority, or about 99 percent, cannot be easily cultivated. Shotgun metagenomics enables researchers to analyze the genetic makeup of the community without the need for culturing, which can be a limiting factor when it comes to identifying and characterizing microorganisms (Usyk *et al.*, 2023). Shotgun metagenomics is a powerful sequencing technique that allows the study of microbial communities in diverse environments (Sharpston, 2014; Sevim *et al.*, 2019). By capturing and analyzing the genomic content of microbial communities, shotgun metagenomics enables researchers to characterize the taxonomic and functional potential of microbiomes without the need for prior knowledge of the species present (Quince *et al.*, 2017). Different from traditional 16S rDNA amplicon sequencing that only targets specific genes, shotgun metagenomics provides a more comprehensive view of microbial communities by capturing both known and unknown species

(Durazzi *et al.*, 2021). Furthermore, shotgun metagenomic sequencing has a higher resolution and can provide insights into the strain-level differences within microbial communities (Durazzi *et al.*, 2021). Moreover, it can lead to the discovery of novel bacterial species, revealing metabolic pathways and allowing predictions of ecological interactions with symbiotic animals.

To perform shotgun metagenomic sequencing, the DNA of the entire microbial community is extracted from a sample, and fragmented into smaller pieces using physical or chemical methods such as sonication or nebulization (Quince *et al.*, 2017). Then using the fragmented DNA for library preparation. The prepared library is then sequenced using high-throughput sequencing technologies such as Illumina or PacBio. The microbial genomes are then bioinformatically reconstructed. The sequencing data is then processed and analyzed using various bioinformatics tools to identify and classify the microbial species present in the sample (Sharpston, 2014; Quince *et al.*, 2017).

4. Conclusion

Marine life is essential to the planet's ecosystem. It has been the subject of extensive research worldwide owing to the possibility to deliver diverse compounds. Marine mollusks combine different organisms with unique features and adaptations to their environment. Scientists have been interested in exploring and understanding the vast diversity of marine mollusks, and in recent years, the study of these animals has become an attractive field of research. With the help of metagenomics, we can discover more natural compounds from marine sources in the future.

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