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Recent molecular techniques to strengthen ecological studies in echinoderms

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ABSTRACT

Introduction: Echinoderms, an integral component of marine ecosystems worldwide, have captivated scientific interest for centuries. Despite this longstanding attention, comprehending key facets such as trophic relationships, diet composition, and host-microbiota relationships still represents a challenge using traditional techniques. Recent years, however, have witnessed a transformative shift, thanks to the emergence of advanced molecular techniques, offering new approaches to strengthen ecological studies in echinoderms.

Objective: Explore how recent advancements in molecular tools have impacted ecological research on echinoderms. Specifically, we aim to investigate the potential of these tools to shed light on trophic interactions, diet composition, and the characterization of gut microbial communities in these organisms.

Methods: Available literature was used to clarify how novel molecular techniques can improve ecological studies. The focus is diet, trophic relationships, and gut microbiota.

Results: Traditionally, studies of stomach contents using compound microscopy have provided an idea of ingested material; nevertheless, sometimes a simple magnified visualization of dietary content does not allow exhaustive identification of the entire food spectrum, as it is limited due to the rapid digestion and maceration of food items within the echinoderm's digestive tract. The use of DNA-metabarcoding, targeting specific DNA regions, such as the mitochondrial COI gene, has allowed us to enhance the accuracy and precision of diet characterization by enabling the identification of prey items down to the species or even genetic variant level, providing valuable insights into specific dietary preferences. Another approach is the use of stable isotopes, particularly carbon and nitrogen, which provide a powerful tool to trace the origin and flow of nutrients through food webs. By analyzing the isotopic signatures in muscular tissues and food items, we can discern the sources of their primary food items and gain insights into their trophic position within the ecosystem. Lastly, a third new technique used to elucidate the characterization of the prokaryotic community is 16S rRNA sequencing. This method allows us to explore the composition and dynamics of the digestive tract microbial communities.

Conclusions: This is a promising era for ecological research on echinoderms, where advances of molecular tools have enabled an unprecedented level of detail, resolving longstanding challenges in comprehending their trophic interactions, diet composition, and host-microbiota relationships, and opening new avenues of investigation in ecological studies.

Key words: echinoderms; stable isotopes; 16S rRNA; DNA-metabarcoding; ecology; review.

RESUMEN

Técnicas moleculares recientes que fortalecen los estudios ecológicos de equinodermos

Introducción: Los equinodermos, un componente integral de los ecosistemas marinos en todo el mundo, han captado el interés científico durante siglos. A pesar de esta prolongada atención, el comprender facetas clave

como las relaciones tróficas, la composición de la dieta y las relaciones huésped-microbiota todavía representa un desafío utilizando técnicas tradicionales. Sin embargo, los últimos años han sido testigos de un cambio transformador, gracias a la aparición de técnicas moleculares avanzadas, que ofrecen nuevos enfoques para fortalecer los estudios ecológicos en equinodermos.

Objetivo: Explorar cómo los avances recientes en herramientas moleculares han impactado la investigación ecológica sobre equinodermos. Específicamente, nuestro objetivo es investigar el potencial de estas herramientas para arrojar luz sobre las interacciones tróficas, la composición de la dieta y la caracterización de las comunidades microbianas intestinales en estos organismos.

Métodos: Se utilizó la literatura disponible para aclarar cómo las nuevas técnicas moleculares pueden mejorar los estudios ecológicos. La atención se centra en la dieta, las relaciones tróficas y la microbiota intestinal.

Resultados: Tradicionalmente, los estudios del contenido estomacal mediante microscopía compuesta han proporcionado una idea del material ingerido; Sin embargo, a veces una simple visualización ampliada del contenido dietético no permite una identificación exhaustiva de todo el espectro alimentario, ya que está limitado debido a la rápida digestión y maceración de los alimentos dentro del tracto digestivo del equinodermo. El uso de metabarcoding de ADN, dirigidos a regiones específicas del ADN, como el gen COI mitocondrial, nos ha permitido mejorar la exactitud y precisión de la caracterización de la dieta al permitir la identificación de presas hasta el nivel de especie o incluso de variante genética, lo que proporciona valiosos resultados sobre preferencias dietéticas específicas. Otro enfoque es el uso de isótopos estables, en particular carbono y nitrógeno, que proporcionan una poderosa herramienta para rastrear el origen y el flujo de nutrientes a través de las redes alimentarias. Al analizar las firmas isotópicas en los tejidos musculares y los alimentos, podemos discernir las fuentes de sus alimentos primarios y obtener información sobre su posición trófica dentro del ecosistema. Por último, una tercera técnica nueva utilizada para dilucidar la caracterización de la comunidad procariótica es la secuenciación del ARNr 16S. Este método nos permite explorar la composición y dinámica de las comunidades microbianas del tracto digestivo.

Conclusiones: Esta es una era prometedora para la investigación ecológica sobre equinodermos, donde los avances de las herramientas moleculares han permitido un nivel de detalle sin precedentes, resolviendo desafíos de larga data en la comprensión de sus interacciones tróficas, composición de la dieta y relaciones huésped-microbiota, y abriendo nuevas vías de investigación. en estudios ecológicos.

Palabras clave: equinodermos; isótopos estables; ARNr 16S; metabarcoding de ADN; ecología; revisión.

INTRODUCTION

The use of novel technologies in ecological studies on echinoderms and other marine organisms has significantly advanced our understanding of ecology, physiology, and interactions within marine ecosystems. Among these techniques, stable isotope analysis provides valuable insights into trophic relationships and nutrient flow within marine ecosystems (Phillips & Gregg, 2003). The use of stable isotopes in ecological studies has its origins in the field of geochemistry, where isotopic analysis was initially used to understand the composition and age of rocks and minerals (O'Connell et al., 2012). The application of stable isotopes in ecology began in the 1960s when researchers realized that isotopic signatures could be used to trace the movement of elements through ecosystems, using stable carbon isotopes to study the carbon cycle and its impact on climate change (Keeling et al., 1995). Since then, stable isotopes have become valuable tools in the study of ecological processes, such as trophic interactions, nutrient cycling, and migration patterns. The development of new analytical techniques and advancements in mass spectrometry have further enhanced the application of stable isotopes in the field of ecology. The Isotope ratio mass spectrometry (IRMS) method allows researchers to measure the ratios of isotopes in a sample with high precision and accuracy (Godin & McCullagh, 2011; Hayes, 2001). The development of compound-specific isotope analysis (CSIA) has allowed researchers to examine isotopic signatures at the molecular level, providing more detailed insights into ecological processes (Chikaraishi et al., 2007). These advancements have opened new avenues for studying ecological dynamics and have

contributed significantly to our understanding of complex ecological systems."

Another powerful technique that has significantly advanced ecological studies involving echinoderms is the application of DNA sequencing methods for prokaryotic and eukaryotic organisms. This molecular tool allows researchers to explore the diversity and composition of microbial communities associated with echinoderms (Leray & Knowlton, 2016; Silva et al., 2021). The use of 16S rRNA in ecological studies has its origins in genetics and molecular biology. 16S rRNA is a ribosomal RNA sequence present in the DNA of prokaryotic and some eukaryotic cells, which encodes for structural components of the ribosome. In the 1980s, the technique of 16S rRNA sequencing was first used to investigate the diversity and phylogenetic relationships among different bacterial species (Oren, 2004; Whitman et al., 2022). This approach revolutionized the field of microbial ecology by providing a molecular tool to identify and classify microorganisms based on their genetic relatedness. By comparing the sequences of 16S rRNA genes from different organisms, researchers can reconstruct the evolutionary history of bacteria and infer their ecological roles in various ecosystems. Advancements in DNA sequencing technologies have further propelled the use of 16S rRNA in ecological studies. The development of high-throughput sequencing platforms, such as next-generation sequencing, has enabled researchers to analyze large volumes of 16S rRNA sequences from environmental samples in a cost-effective and time-efficient manner (Caporaso et al., 2010; Muhamad-Rizal et al., 2020). This has facilitated the exploration of microbial diversity and community composition in several ecological settings, ranging from soil and marine environments to the human gut microbiome. Additionally, bioinformatics tools and databases specifically designed for 16S rRNA analysis, such as QIIME (Quantitative Insights Into Microbial Ecology) and Greengenes, have emerged, providing researchers with computational resources to process and interpret vast amounts of sequencing data (DeSantis et al., 2006; Gilbert et al., 2014).

DNA metabarcoding, is a third novel technique that has emerged as a transformative tool for ecological studies in echinoderms. This technique allows for high-throughput identification of species based on DNA sequences extracted from environmental samples (Leray & Knowlton, 2015). By targeting specific DNA regions, such as the mitochondrial COI gene, researchers can rapidly assess echinoderm diversity, community structure, and species interactions in various marine habitats (Leray & Knowlton, 2016; Leray et al., 2013). DNA metabarcoding involves the amplification and sequencing of specific DNA regions, such as the barcode regions, to identify and quantify the diversity of organisms present in environmental samples. The concept of DNA barcoding was initially proposed to identify species based on short, standardized DNA sequences (Antil et al., 2023; Hebert et al., 2003). However, it was soon realized that this approach could be extended to ecological studies by simultaneously sequencing multiple DNA samples and generating large datasets for species identification and community analysis. The development of next-generation sequencing platforms, such as Illumina sequencing, provided the technological capacity to process a massive number of DNA sequences at a reasonable cost, making DNA metabarcoding a powerful tool in ecological research (Hassan et al., 2022; Pawlowski et al., 2022). By targeting different barcode regions, DNA metabarcoding can provide a comprehensive and high-resolution assessment of biological communities. Furthermore, it can be applied to other organisms, including plants, animals, prokaryotes, and microeukaryotes by selecting appropriate barcode regions. The interpretation of DNA metabarcoding data relies on reference databases that contain known DNA sequences from a wide range of organisms. These databases, such as GenBank and the Barcode of Life Data Systems (BOLD), allow for the identification and taxonomic assignment of the sequenced DNA fragments (Taberlet et al., 2012). With

advances in bioinformatics tools and analytical pipelines designed specifically for DNA metabarcoding analysis, researchers can now extract valuable ecological information from complex and diverse environmental samples (Hassan et al., 2022; Zhang et al., 2023).

These novel techniques and their multiple variations have important implications for conservation and ecosystem management, contributing to our understanding of echinoderm species and their roles within marine ecosystems. Therefore, the main goal of this review is to analyze the fast advance of new molecular tools that allows a level of detail never seen before, clarifying old doubts, and opening new research avenues in ecological studies.

Stable isotopes

The complexity of trophic relationships exceeds common perception. Simply examining the contents of an organism's stomach does not reveal which specific items are being absorbed by the consumer (Peterson & Fry, 1987; Phillips et al., 2014). Stable isotopes have emerged as a valuable tool for studying the ecology and physiology of echinoderms, a marine group of invertebrates that includes sea lilies, brittle stars, starfish, sea urchins, and sea cucumbers, providing insights into the trophic interactions, migration patterns, and habitat preferences of echinoderms (Fry & Sherr, 1984; Sturbois et al., 2022). Carbon, nitrogen, oxygen, and sulfur isotopes are commonly employed to unravel several aspects of echinoderm biology (Cabanillas-Terán et al., 2016; Rodríguez-Barreras et al., 2015; Wangensteen et al., 2011). In recent years, stable isotope analysis has been extensively used to examine the trophic ecology of echinoderms, shedding light on their feeding preferences, diet breadth, and interactions with other organisms (Cabanillas-Terán et al., 2019; Pérez-Posada et al., 2023). For instance, Rodríguez-Barreras et al. (2016) presented the ellipses-based metrics of niche width for five Caribbean sea urchins based on habitat preferences where each species displayed a particular isotopic signature of carbon and nitrogen

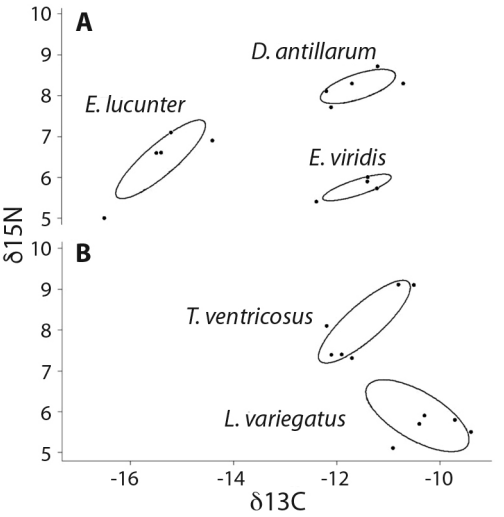

Fig. 1. Results of the ellipses-based metrics of niche width for two echinoids groups based on habitat preferences where circles represent the potential niche of each species. **A.** Represents three species from reefs biotopes, and **B.** represents two species from seagrass beds.

indicating no food overlapping among them, despite of species from the same habitat can be found inhabiting close to each other (Fig. 1).

Among stable isotopes, Carbon, and particularly carbon-13 ($\delta^{13}C$), play a crucial role in understanding the feeding ecology of echinoderms (Purcell et al., 2007). By analyzing the carbon isotopic signatures in muscular tissues, researchers can decipher the primary carbon sources they consume, providing valuable information about their dietary preferences and trophic positions within marine food webs (Burton et al., 2011; Carney, 2010; Gale et al., 2013). Moreover, nitrogen isotopes, such as nitrogen-15 ($\delta^{15}N$), have proven instrumental in studying the trophic levels and nutrient sources (Hobson et al., 1995; Nilsen et al., 2008). The $\delta^{15}N$ values in their tissues increase with higher trophic positions, offering insights into their position in the food chain and potential dietary shifts (Gurney et al., 2001). Furthermore, stable nitrogen isotopes have been also used to explore the effects of anthropogenic nutrient inputs on echinoderm communities and to assess the ecological impacts of

eutrophication (Radabaugh et al., 2013; Yatsuya & Nakahara, 2004). The ratios of ^{13}C to ^{12}C and ^{15}N to ^{14}N in the tissues compared to their algae food resources is referred to as Trophic Enrichment Factor (Parnell et al., 2010). Thus, we can also calculate the trophic level for every individual, using the equation proposed by Hobson and Welch (1992).

Additionally, oxygen isotopes, such as oxygen-18 ($\delta^{18}O$), are employed to determine the thermal history and evolutionary patterns of dispersal (Gerringer 2019; Zenteno et al., 2013). Changes in $\delta^{18}O$ values in skeletal structures can indicate temperature changes or movements across different water conditions (Killingley & Rex, 1985). This isotope also provides insights into the thermal ecology and habitat preferences, helping to understand their responses to climate change and oceanographic processes (Luo et al., 2023; Tiwari et al., 2013; Zhao et al., 2020). The fourth most common used stable isotope are sulfur isotopes, particularly sulfur-34 ($\delta^{34}S$), contribute to understanding the nutrient sources and sulfur cycling in food webs (Connolly et al., 2004). The $\delta^{34}S$ values in their tissues can reflect the use of different sulfur sources, such as marine organic matter or sulfide-rich sediments (Vaslet et al., 2012), but also, researchers can gain insights into the influence of the prokaryotic community in the diet of echinoderms (Mendes et al., 1963; Pascal et al., 2017).

Stable isotopes have also proven valuable for studying the physiology and metabolic processes of echinoderms. For example, carbon isotopes have been used to investigate metabolic rates and energy allocation in these organisms (Sun et al., 2012). By tracing the input of isotopically labeled compounds, obtaining insights into nutrient uptake, assimilation, and utilization (Walters et al., 2021). Thus, stable isotope analysis has also been employed to study the reproductive biology and larval ecology of echinoderms. Nitrogen isotopes have been used to trace the transfer of maternal nutrients to developing embryos and larvae (Reitzel & Miner, 2007). By analyzing the isotopic composition of different life stages, marine ecologist can gain insights into larval dispersal patterns, connectivity between populations, and the influence of local versus distant sources of larvae (Lester et al., 2007; Levin, 2006). Lastly, the combination of stable isotope analysis with other techniques, such as fatty acid analysis and genetic markers, has provided a comprehensive understanding of the feeding ecology and population structure in echinoderms (McKenzie et al., 2000; North et al., 2019). These integrated approaches allow researchers to examine the dietary preferences of different echinoderm species, identify potential competition among species, and investigate the drivers of population dynamics (Howell et al., 2003; Rossi & Elias-Piera, 2018).

Eukaryotic DNA-metabarcoding

This method provides a comprehensive and efficient approach to studying the biodiversity and ecological patterns of echinoderms, aiding in conservation efforts, and informing ecosystem management strategies (Leray & Knowlton, 2015; Leray et al., 2013; Riccioni et al., 2022). DNA metabarcoding, a high-throughput sequencing technique, has revolutionized the field of biodiversity assessment and ecological studies (Barnes & Turner, 2016). This powerful tool allows for the rapid identification of species and the analysis of complex biological communities based on DNA markers. In recent years, DNA metabarcoding has emerged as a valuable approach in echinoderm research, enabling scientists to gain insights into the diversity, community structure, and ecological roles of these marine organisms (Licuanan & Matias, 2022; Okanishi et al., 2023; Rodriguez-Barreras et al., 2020). This essay aims to explore the applications of DNA metabarcoding in echinoderm studies, highlighting its contributions to taxonomy, community ecology, trophic interactions, and conservation. In addition, DNA metabarcoding has proven to be a reliable method for species identification and taxonomy in echinoderms. By sequencing specific gene regions, such as the mitochondrial cytochrome c oxidase subunit I (COI) gene, researchers can accurately identify

and differentiate echinoderm species, including cryptic and morphologically similar taxa (Wangensteen et al., 2018). This non-invasive approach overcomes the limitations of traditional morphological identification and has led to the discovery of new species and the revision of taxonomic classifications in echinoderms (Borrero-Pérez et al., 2019; Trivedi et al., 2016).

DNA metabarcoding has also been instrumental in elucidating the community structure and diversity of echinoderm assemblages. By analyzing environmental DNA (eDNA) extracted from seawater or sediment samples, researchers can assess the presence and abundance of different echinoderm species in each habitat (Deiner et al., 2016; Leray et al., 2013). This approach provides a comprehensive and efficient method for studying echinoderm biodiversity, particularly in hard-to-access or cryptic habitats and allows for the monitoring of community dynamics and changes over time (Boissin et al., 2017; Deiner et al., 2016). Moreover, DNA metabarcoding enables the identification of rare or endangered species, contributing to their conservation and management. On the other hand, DNA metabarcoding has shed light on the trophic interactions and feeding ecology of echinoderms. By analyzing the gut contents or fecal material of echinoderms, researchers can identify the prey items consumed by these organisms and investigate their dietary preferences and ecological roles (Jia et al., 2022; Rodríguez-Barreras et al., 2020). This information is crucial for understanding energy flow and nutrient cycling in marine ecosystems and can contribute to the assessment of ecosystem health and functioning (Bourlat et al., 2013; Compson et al., 2018). Likewise, DNA metabarcoding can reveal the occurrence of symbiotic or commensal organisms associated with echinoderms, providing insights into their ecological interactions and mutualistic relationships (Kodama et al., 2023; Toju, 2015).

DNA metabarcoding has proven particularly valuable in studying the trophic ecology of echinoderms, shedding light on the trophic interactions and food web dynamics involving echinoderms (Baure et al., 2023; Redd et al., 2014). Thus, a recent study described the food items found in four Caribbean sea urchins (Rodríguez-Barreras et al., 2020). Authors also compared diet similarities among species from the same habitat (Fig. 2). Through the analysis of gut contents from 60 individuals across three collection sites, we found that these four sea urchins primarily feed on seaweeds, with additional consumption of small protists, fungi, and metazoans. Notably, variations in diet were observed both between species and among collection sites, suggesting potential inter-specific competition and niche overlap among these generalist omnivores. Hence, genetic studies have been performed in echinoderms (Alcudia-Catalma et al., 2020; Layton et al., 2016). For example, the study of 19 sea cucumber species, finding low genetic variation within most species (Alcudia-Catalma et al., 2020). Another study analyzed the effectiveness of DNA barcoding for species identification in a diverse range of marine invertebrates, including crustaceans, mollusks, polychaetas, and echinoderms. By analyzing nearly half of the 300 known Echinodermata species in Canadian waters, the research highlighted six species requiring further taxonomic investigation due to their specimens falling into two or three distinct sequence clusters. The study also explored the potential impacts of larval dispersal and glacial events on genetic diversity patterns in 19 trans-oceanic species (Layton et al., 2016).

Prokaryotic 16S metabarcoding

Traditional methods used in microbiology, such as culture and microscopy, have provided evidence of the presence of bacteria within the gut microbiota of sea urchins. Many of these bacteria are associated with ecological interactions and metabolic processes (Marangon et al., 2023; McCracken et al., 2023; Temara et al., 1993). However, recent advancements in molecular sequencing techniques have emerged as powerful tools that are revolutionizing the characterization of microbiota in marine organisms (Hakim et al., 2016; Nelson

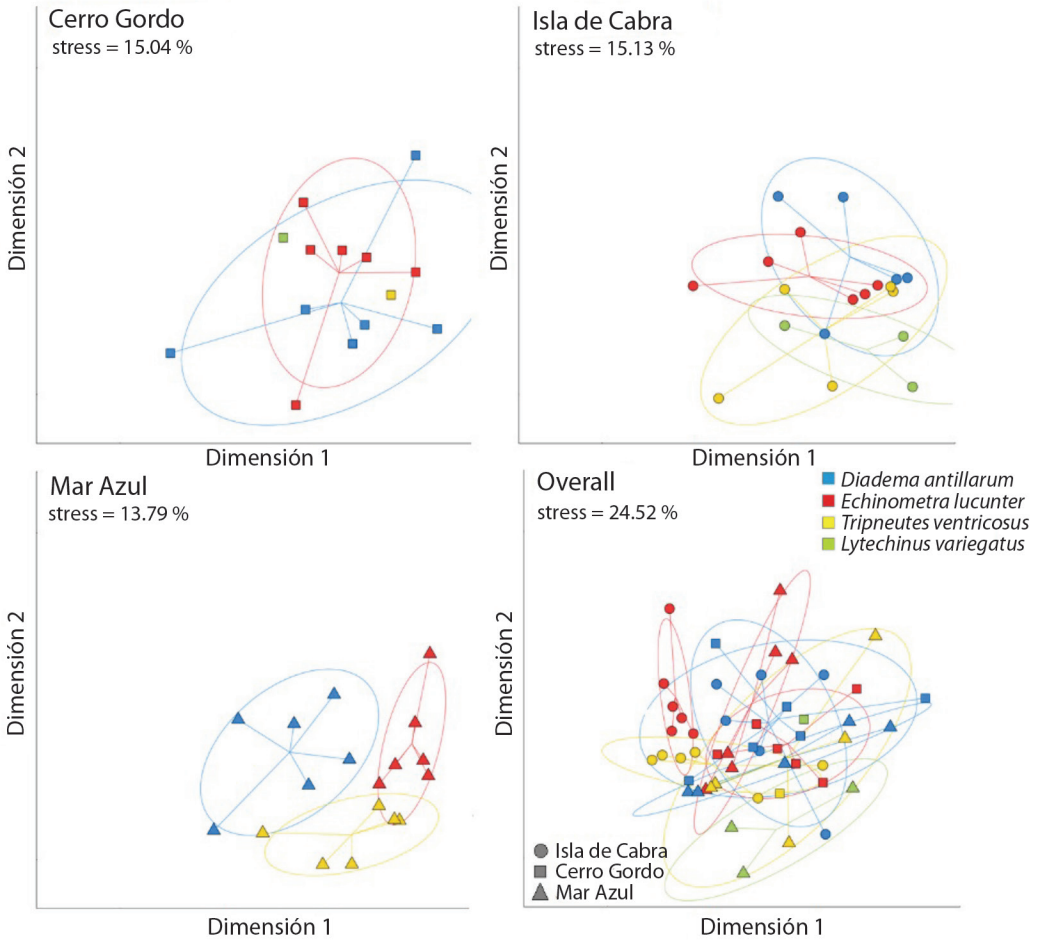

Fig. 2. A nMDS ordination (Bray–Curtis) for COI samples of four sea urchins *Diadema antillarum* (Philippi, 1845), Echinometra *lucunter* (Linnaeus, 1758), *Lytechinus variegatus* (Lamarck, 1816) and *Tripneustes ventricosus* (Lamarck, 1816), collected from three sites of Puerto Rico. Circles, triangles, and squares identify individuals of each species. In each case, individuals of the same species are grouped together.

et al., 2010; Petti et al., 2005; Rodríguez-Barreras et al., 2021). These molecular approaches have significantly enhanced our understanding of the microbial communities associated with echinoderms and other marine invertebrates, enabling a more comprehensive analysis of their composition and function.

The 16S ribosomal RNA (16S rRNA), present in all bacteria and archaea, serves as a molecular marker to identify and classify microbial taxa (Hakim et al., 2019). By analyzing the 16S rRNA gene sequences, researchers can unravel the intricate relationships between echinoderms and their associated microbial communities, shedding light on the potential roles of these microbes in echinoderm health, nutrition, and overall ecological functioning (Lima-Mendez et al., 2015; Rodríguez-Barreras et al., 2023). The 16S rRNA gene is a widely used molecular marker for studying microbial diversity and community structure. In recent years, the application of this marker sequencing has expanded to include investigations of microbial associations in various ecosystems, including the marine environment. This essay explores the applications of 16S rRNA

sequencing in echinoderm research, highlighting its contributions to understanding the microbial interactions, symbiosis, and environmental adaptations.

The use of 16S rRNA sequencing has revealed valuable insights into the microbial communities associated with echinoderms. By analyzing the V4 region of the 16S rRNA gene, researchers have identified diverse bacterial taxa that inhabit the surfaces, tissues, and gut of echinoderms (Jackson et al., 2018). This approach has enabled the characterization of core microbiomes in echinoderms across different species, providing a foundation for understanding the functional roles of microorganisms in echinoderm health, development, and physiology. In addition, the use of 16S rRNA sequencing has provided insights into the microbial contributions to echinoderm physiology and environmental adaptations. Studies have identified specific bacterial taxa associated with nutrient metabolism, detoxification processes, and resistance to environmental stressors in echinoderms (Franzenburg et al., 2012; Marangon et al., 2021). This knowledge enhances our understanding of the functional capabilities of echinoderm-associated microorganisms and their potential roles in host health, resilience, and acclimation to changing environmental conditions.

Furthermore, through 16S rRNA sequencing, researchers can decipher intriguing microbial interactions within echinoderms. For instance, recent studies revealed the occurrence of potential symbiotic relationships between echinoderms and specific bacterial taxa, suggesting mutualistic or commensal associations (Brigmon & De Ridder, 1998; Schuh et al., 2020). These bacterial partnerships may contribute to nutrient acquisition, defense against pathogens, and overall host fitness. Understanding the dynamics and functional roles of these symbiotic interactions can provide valuable insights into the ecology and evolution of echinoderms (Carrier et al., 2021). In addition to symbiotic interactions, 16S rRNA sequencing has provided insights into the dynamic nature of the microbiome in echinoderms. Studies have revealed variations in the microbial community composition across different developmental stages, habitats, and environmental conditions (Ketchum et al., 2021; Marangon et al., 2023; Rodríguez-Barreras et al., 2023). These findings suggest that the echinoderm microbiome is influenced by both intrinsic factors and extrinsic environmental factors, highlighting the importance of considering the context-dependent nature of microbial associations in echinoderm research.

The study of the microbial diversity associated with echinoderms using 16S rRNA sequencing has also provided valuable insights into host-microbe interactions and disease dynamics. By examining the microbiomes of diseased echinoderms, researchers have identified shifts in microbial composition and potential pathogens associated with disease symptoms (Galac et al., 2016; Marangon et al., 2023). This knowledge contributes to our understanding of the etiology and progression of diseases affecting echinoderms, enabling the development of targeted management strategies and conservation efforts. Another significant application of 16S rRNA sequencing in echinoderm research is the investigation of microbial-mediated ecological processes, such as nutrient cycling and organic matter degradation. By characterizing the functional potential of the echinoderm-associated microbiota, researchers can gain insights into the roles of microorganisms in nutrient transformations, carbon fluxes, and the overall functioning of marine ecosystems (Masasa et al., 2023; Thompson & Polz, 2006). These findings highlight the interconnectedness between echinoderms and their associated microbiomes in ecosystem processes.

The integration of 16S rRNA sequencing with other omics approaches, such as metagenomics and metatranscriptomics, has further expanded our understanding of the functional potential and metabolic activities of invertebrates-associated microbiomes (Gudenkauf & Hewson, 2015). These multi-omics approaches enable the exploration of gene functions, metabolic pathways, and ecological interactions within the microbial communities associated

with echinoderms (Rey-Campos et al., 2022). By combining different molecular techniques, researchers can unravel the intricate relationships between echinoderms and their microbial partners, shedding light on the mechanisms driving their symbiotic associations and ecological contributions (Lowe et al., 2017; Voronov et al., 2023). On the other hand, the use of 16S rRNA sequencing has significantly advanced our understanding of the microbiota residing in the digestive systems. This novel technique. For instance, recent study revealed the gut microbiota of sea cucumbers (Pagán-Jiménez et al., 2019), revealing distinct microbial profiles among different species of sea cucumbers, and suggesting the influence of host-specific factors in shaping the composition of the gut microbiota. Another study examined the gut microbiota of a sea star and found the existence of specific microbial taxa involved in nutrient metabolism and the breakdown of complex organic compounds, highlighting the symbiotic relationship between the host and its gut microbiota in the digestive processes of sea stars (McCracken et al., 2023). In another study conducted with four Caribbean sea urchins, authors found similar gut microbiotas among the species, but one of them (*Lytechinus variegatus*) displayed specific microbiota profiles (Rodríguez-Barreras et al., 2021) (Fig. 3).

16S rRNA sequencing has also revealed the diversity and dynamics of the digestive system microbiota in echinoderms (Masasa et al., 2021; Masasa et al., 2023). For instance, a recent study confirmed that the microbiome of algae-eating sea urchins like *Tripneustes gratilla* (Linnaeus, 1758) plays a significant role in digesting fiber-rich seaweed, but also the study revealed unique characteristics in the microbial communities, particularly in the esophagus and

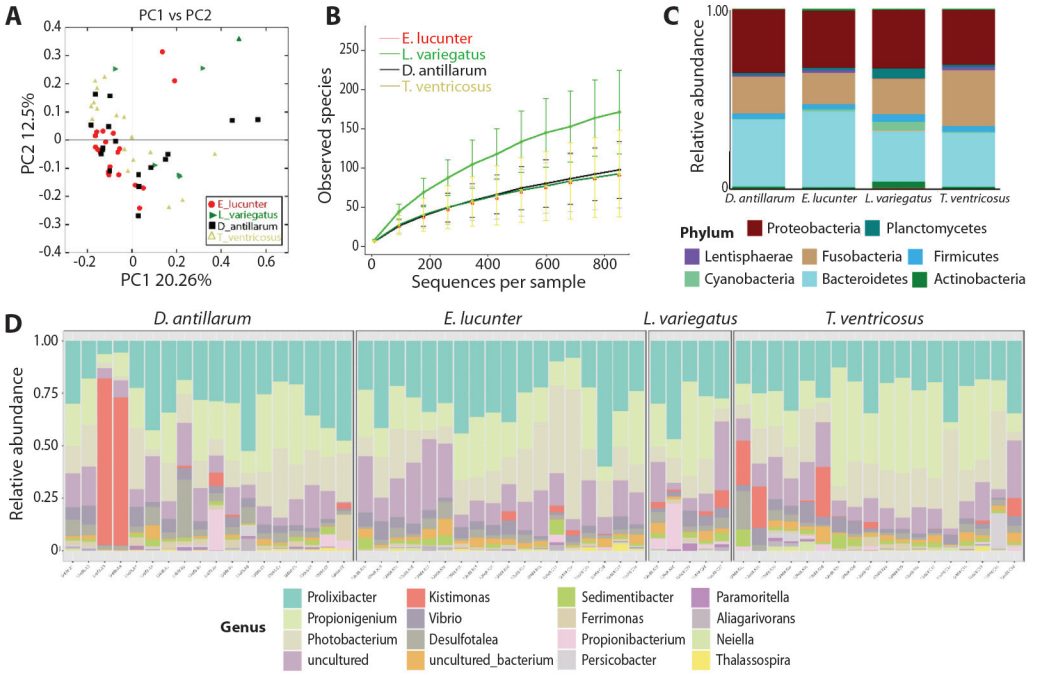

Fig. 3. Global analyses comparing the four sea urchin species. **A.** Bray-Curtis analysis represented in a 2D Principal Coordinates Analysis (PCoA) using species as metadata categories, depicts distinct species clustering with permanova P_{value}= 0.004; Anosim P_{value}< 0.001, **B.** Rarefaction curves of Chao1 index demonstrated significant differences between green (*Lytechinus_variegatus*) and red (*Echinometra_lucunter*) sea urchin (P_{value}= 0.006) and between red (*Echinometra_lucunter*) and black (*Diadema_antillarum*) sea urchin (P_{value}= 0.048), **C.** Species relative abundance at phyla, and **D.** genus levels are depicted by the bar plots.

intestine, concluding that the shifting microbial communities within the digestive tract provide strong evidence supporting the idea of bacteria playing a crucial role in food digestion (Masasa et al., 2023). Furthermore, environmental factors have been shown to influence the composition of gut microbiota. A study found that the relative abundance of certain microbial taxa with changing temperatures, indicating the sensitivity of the gut microbiota to environmental variations and the potential impact on host health and physiology (Dong et al., 2021; Zhang et al., 2013; Zhang et al., 2019). However, it is important to remark that these powerful molecular techniques have some limitations. For example, the 16S rRNA sequencing only provides information on Bacteria and some Archae but fails to detect other members of the microbiota (fungi, viruses, and unicellular eukaryotes). This limitation is particularly striking in the recent publication that a ciliated eukaryote is the culprit of sea urchin mass mortality (Hewson et al., 2023) a finding that would not have been possible using 16S technology.

DISCUSSION

The integration of stable isotopes, DNA-metabarcoding, both eukaryotic and prokaryotic, has revolutionized ecological studies with echinoderms, providing valuable insights into their trophic interactions, biodiversity, and microbial associations. Stable isotopes have been instrumental in unraveling the trophic ecology of echinoderms, shedding light on their feeding habits, diet preferences, and ecological roles within marine ecosystems (González-De Zayas et al., 2020; Hobson, 2023). DNA-metabarcoding has transformed the way we assess echinoderm biodiversity, allowing for efficient species identification and revealing hidden diversity and community dynamics (Leray et al., 2013; Sinniger et al., 2016). Additionally, 16S rRNA sequencing has provided a deeper understanding of the microbial associations and functional roles of microorganisms associated with echinoderms, unraveling the intricate symbiotic relationships and their ecological contributions (García-Aljaro et al., 2017; Webster et al., 2019). By leveraging these advanced techniques, researchers can gain comprehensive insights into the ecology and conservation of echinoderms. The knowledge obtained through stable isotopes, DNA-metabarcoding, and 16S rRNA sequencing contributes to our understanding of ecosystem functioning, trophic dynamics, and the impacts of environmental changes on echinoderm populations. This information is crucial for effective management and conservation strategies aimed at protecting these ecologically important organisms and the marine habitats they inhabit.

Ethical statement: the author declares that he agrees with this publication; that there is no conflict of interest of any kind; and that he followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgements section. A signed document has been filed in the journal archives.

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