



## INVESTIGATING THE DIVERSITY OF SEA URCHINS (*Echinoidea*) IN YOGYAKARTA, INDONESIA USING MOLECULAR APPROACHES, ABUNDANCE, AND ECOLOGICAL INDEX

Putri Ayu Hia, Ratih Ida Adharini\*, Eko Setyobudi, Eko Hardianto

Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora Bulaksumur, Depok, Sleman, Yogyakarta 55281, Indonesia

\*Corresponding Author: ratih.adharini@ugm.ac.id

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### ABSTRACT

Sea urchins are invertebrates playing a crucial ecological role in maintaining a balance within intertidal ecosystems and serve as bioindicators, in addition to their significant economic value as marine resources. This study aims to investigate sea urchins at Ngrumput Beach, Yogyakarta through ecological index analysis, abundance measurements, and morphological and molecular identification using the DNA barcoding approach. Sampling was from November to December 2023, using a 1x1 m quadrat transect method. Molecular identification targeted the *mitochondrial DNA cytochrome oxidase subunit I* (mtDNA COI) gene. On the basis of morphological and molecular identification, the identified sea urchin species were *Echinometra oblonga*, *Echinometra mathaei*, *Heterocentrotus trigonarius*, and *Stomopneustes variolaris*. The results indicated that the sea urchins at Ngrumput Beach had an abundance of 74.14 individuals/m<sup>2</sup>, a species diversity index of 1.07 (moderate), an evenness index of 0.83 (high), and a dominance index of 0.23 (low). Distribution pattern analysis revealed that *Echinometra oblonga* and *Stomopneustes variolaris* exhibited clumped distributions, while *Heterocentrotus trigonarius* and *Echinometra mathaei* displayed both uniform and clumped distributions. The highest sea urchin abundance was observed 30 meters from the shoreline. Molecular identification revealed a DNA sequence length of 630 bp. DNA barcoding analysis, including phylogenetic tree construction using the neighbour-joining method and genetic distance evaluation, supported the morphological analysis results.

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## INTRODUCTION

Sea urchins are invertebrate species classified under the phylum Echinodermata and the class Echinoidea. They inhabit intertidal zones and deep-sea environments across a wide geographic range (Crespi-Abril and Rubilar, 2023; Rahim and Nurhasan, 2016). Sea urchins occupy diverse habitats, including live and dead coral colonies, coral reef flats, soft corals, and areas with algal growth (Suryanti et al., 2020; Yiu and Chung, 2024; Goh and Lim, 2015). They play an essential role as grazers, contributing to the regulation of macroalgae abundance, composition, and distribution (Jeon et al., 2015; Boudouresque and Verlaque, 2020). Consequently, sea urchins serve as bioindicators of macroalgae abundance in intertidal ecosystems.

Sea urchins are critical to the functioning of benthic marine ecosystems and influence coral reef communities by controlling macroalgae growth (Rahim and Nurhasan, 2016; Westlake et al., 2021; Angellia and Nugrahapraja, 2023). High sea urchin abundance is typically associated with a decline in macroalgae populations due to their grazing activities (Suarez et al., 2021). Hermosillo-Núñez (2020) also reported that sea urchins are highly sensitive to environmental changes, making them keystone species in kelp forest ecosystems. They may live either in groups or in solitude. Group-living sea urchins are better able to defend themselves against predators, while solitary individuals are more vulnerable to predation threats (Kurniati et al., 2023).

In addition to their ecological importance, sea urchins hold significant economic value due to their gonads, commonly known as uni or roe, which are highly nutritious and widely consumed as a seafood delicacy. These gonads are sold at relatively high prices in several countries worldwide (Archana and Babu, 2016; Gobala Krishnan et al., 2020). Sea urchins are regarded as a high-value food product, with strong domestic markets in countries such as the Philippines, South Korea, Japan, China, and several nations in East Asia, Europe, and the Americas. The estimated global demand for sea urchins is approximately 60,000–70,000 tons annually (Stefansson et al., 2017). Beyond their culinary appeal, sea urchins are rich in essential nutrients and active compounds with potential applications in pharmaceuticals, including antifungal, anti-inflammatory, antiviral, and antidiabetic properties, among others (Moreno-García et al., 2022; Sabilu et al., 2022).

Sea urchin populations are commonly found in the intertidal areas along the southern coast of Yogyakarta. The intertidal zone of this region is dominated by rocky substrates with high macroalgae abundance and diversity (Susanti et al., 2022; Susanti et al., 2023; Saraswati et al., 2024), making it an ideal habitat for supporting sea urchin life. Local coastal communities have widely utilised sea urchins for personal consumption and for sale to tourists,

harvesting them at several beaches in Yogyakarta. However, uncontrolled exploitation of sea urchins raises concerns about the potential decline in their abundance and diversity, which could disrupt the balance of intertidal and deep-sea ecosystems.

Overexploitation of sea urchins has been reported on the Mediterranean coast of Egypt, where management is challenging due to extensive harvesting during their reproductive phase (Elmasry et al., 2023). This situation is further exacerbated by global climate change and human interventions that impact water quality, predator populations, and food availability (Bernal-Ibáñez et al., 2021). Despite these challenges, sea urchins play a critical role in regulating benthic communities from intertidal zones to deep-sea ecosystems.

Therefore, this study aims to investigate the abundance, morphological and molecular identification, and diversity of sea urchins through ecological index analysis, including species diversity, evenness, dominance, and distribution patterns at Ngrumput Beach, Yogyakarta. The results of this research are expected to provide fundamental data to support coastal ecosystem management and conservation efforts, as well as promote the sustainable utilisation of sea urchins.

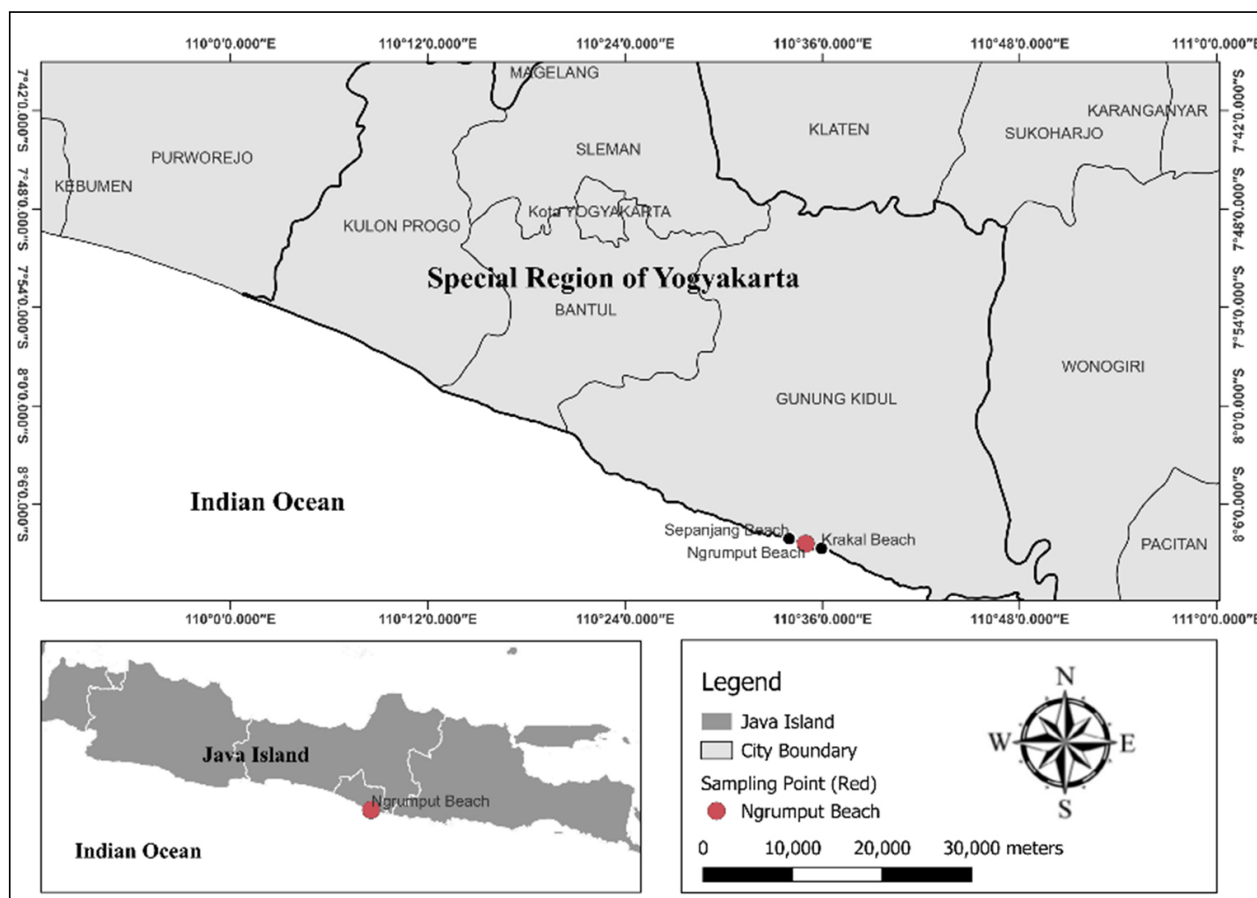
## MATERIALS AND METHODS

Samples were collected from November to December 2023 in the Ngrumput Beach area, Gunungkidul Regency, Yogyakarta (Fig. 1). Field activities included photographing sea urchin samples, observing sea urchins, counting individuals to determine ecological indices, and collecting water quality parameter data.

Sampling was at four stations, using transects and 1 × 1 m quadrats placed perpendicular to the shoreline during low tide. Sea urchin samples were preserved in 96% alcohol and transported to the laboratory for further identification. Water quality parameters—including temperature, salinity, and pH—were measured using a Water Quality Checker (Hanna H198194, made in Romania).

### *Morphological identification*

Sea urchins were identified morphologically by observing their morphological characteristics and comparing them with references from the *World Register of Marine Species* and the identification guide by Clark and Rowe (1971). The observations focused on primary spine colour, secondary spine colour, body shape, body diameter, height, and the colour of the skeletal structure. Sea urchin specimens were photographed, and the resulting images were compared with identification guidebooks or reference sources.



**Fig 1.** Map of the sampling locations for sea urchins in the intertidal zone of Ngrumput Beach, Yogyakarta

### Molecular identification

Sea urchin samples with different morphological characteristics were further identified through molecular analysis. A 10-mg sample of the sea urchin's tube feet was taken for DNA extraction, using the Favoragen Tissue Genomic DNA Extraction Mini Kit protocol. DNA was amplified, using a PCR machine (*Polymerase Chain Reaction; Thermal Cycler T100 Biorad Combi Block, Whatman-Biometra Germany*). The primers used were EchinoF1 (5'-TTTCAACTAATCATAAGGACATTGG-3') and EchinoR1 (5'-CTTCAGGGTGTCCAAAAAATCA-3'; (Ward et al., 2008). These primers target the mtDNA COI region, amplifying approximately 800 bp. The PCR process began with a pre-denaturation step (98 °C for 1 minute), followed by denaturation (98 °C for 10 seconds), annealing (48 °C for 5 seconds), and extension (68 °C for 5 seconds) for 30 cycles. A post-extension step was performed at 68°C for 5 minutes, using KOD one PCR *Master Mix-Blue* (Tokyo, Japan). DNA was electrophoresed, using a 1% agarose gel to assess DNA quality. Sequencing was by First Base, Singapore.

### Molecular data analysis

The sequencing results were analysed using the Multiple Sequence Comparison by Log-Expectation method, which offers high accuracy and speed, through MEGA-X software.

Comparative sequence analysis was performed to identify genomic differences (Edgar, 2004; Kumar et al., 2016). The nucleotide sequences were further analysed using the Basic Local Alignment Search Tool (BLAST) program, accessed through the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), to apply the DNA barcoding approach. This step allowed species identification by comparing the sequencing results of this study with the data stored in GenBank. Phylogenetic relationships were analysed using the Neighbour-Joining Tree method available in MEGA-X, with bootstrap test phylogeny (1000 replicates) and the Kimura 2-parameter model. Genetic distance analysis was also performed using MEGA-X software.

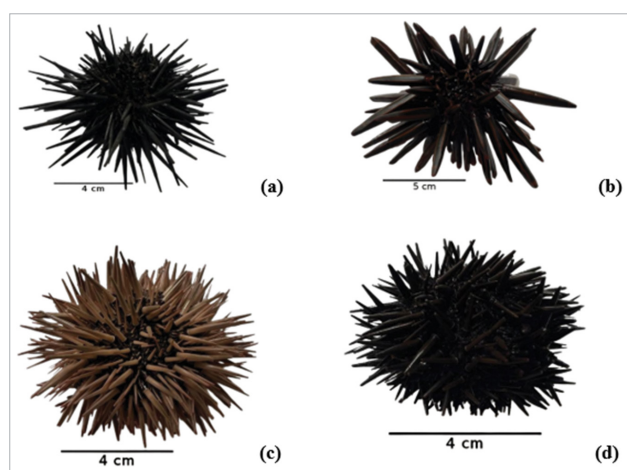
### Ecological index analysis

The observed parameters for the ecological index analysis included abundance, species diversity index, dominance index, evenness index, and distribution patterns using the Morisita index. The species diversity index (H) was calculated using the Shannon-Wiener index (Odum, 1983). Abundance was determined based on the method outlined by Cox (1972). The evenness index (E) was calculated using Pielou's evenness index (Odum, 1983). The dominance index (C) was calculated using Margalef's method (Odum, 1983), while the Morisita index was determined based on Brower's method (1990).

## RESULTS AND DISCUSSION

### Morphological identification

The diversity of species within the class Echinoidea found in the intertidal zone of Ngrumput Beach consists of four species from two families and three different genera. The identified species include *Stomopneustes variolaris*, *Heterocentrotus trigonarius*, *Echinometra mathaei*, and *Echinometra oblonga*. According to Brusca et al. (2016), members of the class Echinoidea have a body shape ranging from round to flattened, surrounded by calcium-containing spines. The species *Heterocentrotus trigonarius*, *Echinometra mathaei*, and *Echinometra oblonga* belong to the family Echinometridae, while *Stomopneustes variolaris* belongs to the family Stomopneustidae (Fig. 2).



**Fig 2.** Sea urchins found at Ngrumput Beach: **(a)** *Stomopneustes variolaris* (NG1), **(b)** *Heterocentrotus trigonarius* (NG2), **(c)** *Echinometra mathaei* (NG3), and **(d)** *Echinometra oblonga* (NG4)

The genus *Stomopneustes* exhibits an irregular, pentagonal body shape with an entirely black colouration. However, it has unique characteristics: when submerged in water and exposed to sunlight, it appears greenish. *S. variolaris* possesses both primary and secondary spines. The primary spines are relatively thick with blunt tips and are entirely black. In contrast, the secondary spines are black with a slightly brownish hue and are located near the oral region, close to the mouth. *S. variolaris* has tube feet that function as locomotory organs, situated within the pore fields on its body. According to Clark and Rowe (1971), the family Stomopneustidae consists of only one genus, *Stomopneustes*, with a single species, *Stomopneustes variolaris*. The spines of *S. variolaris* are characterised as sharp, black, and thick. Toha et al. (2015) noted that the habitat of sea urchins influences their diverse colouration, serving as an adaptive mechanism to their environment.

The genus *Heterocentrotus* has a round body shape with a flattened oral region, and its body is dark brown. Its shell is very hard and thick. It possesses both primary and secondary spines on its body. The primary spines are dark green with brown streaks, thick like pencils, and not pointed. In contrast, the secondary spines are flattened, purple, and brown-tipped. The tube feet are clearly visible between the secondary spines.

According to Clark and Rowe (1971), this genus comprises two species: *Heterocentrotus mammillatus* and *Heterocentrotus trigonarius*. These species can be distinguished by the number of pore pairs per arcus in the ambulacral area and the shape of the primary spines on the aboral side of the ambulacral plate. *Heterocentrotus mammillatus* has 9–11 pore pairs per arcus in the ambulacral region, with primary spines resembling truncated secondary spines. In contrast, *Heterocentrotus trigonarius* has 15–16 pore pairs per arcus in the ambulacral region, and its primary spines are not truncated, meaning the spine tips are not cut off at the apex. Based on observations, the second sample was identified as *Heterocentrotus trigonarius*.

The genus *Echinometra* is characterised by an oval body shape and the presence of both primary and secondary spines. The primary spines of *Echinometra mathaei* are distinctive, displaying a gradient coloration with a light brown base transitioning to a darker brown tip. In contrast, *Echinometra oblonga* has uniformly coloured spines that are dark brown to black from base to tip. A key characteristic of this genus is a white ring-like structure at the base of the primary spines. The secondary spines of *E. mathaei*, near the oral region, also exhibit a gradient coloration, starting with a light brown base and transitioning to a pinkish hue at the tips. In contrast, *E. oblonga* has secondary spines that are uniformly brown along their entire length. The tube feet of this genus are clearly visible between the secondary spines. According to Clark and Rowe (1971), the genus *Echinometra* is characterised by having four pore pairs per arcus and a distinctive white ring-like structure at the base of the spines.

### Molecular identification

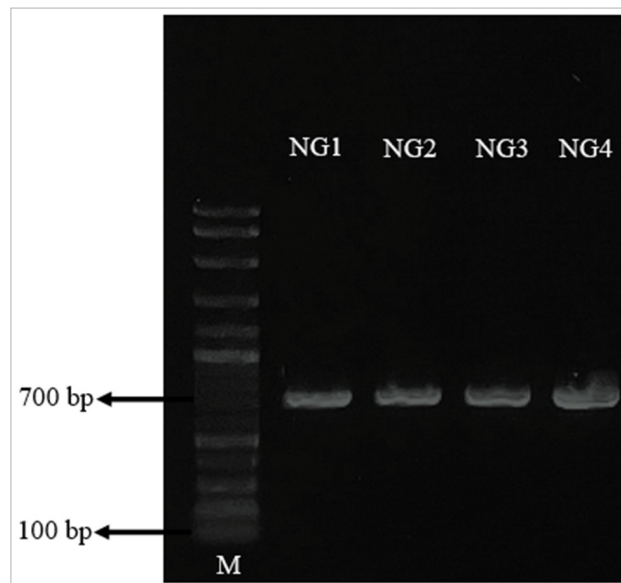
Based on sequence editing using MEGA-X software, the DNA sequencing results showed a band length of 630 bp, which was further confirmed by the electrophoresis results indicating successful DNA amplification suitable for sequencing (Fig. 3). The DNA sequencing results were subsequently examined by comparing the samples with sequences available in GenBank, which was analysed using the BLAST tool on the NCBI website. The BLAST results are presented in Table 1.

The BLAST analysis results (Table 1) showed a Query Cover range of 78–100%, with percent identity values ranging from 99.68–100%. The molecular identification results from this study support the morphological analysis and further confirm the sea urchin species found at Ngrumput

**Table 1.** Percent identity of nucleotide bases for sea urchins from BLAST (NCBI)

Sample code	Morphological identification	Molecular identification	Query cover (%)	Percent identity (%)	References Accession Number	Sampling Location
NG1	<i>S. variolaris</i>	<i>S. variolaris</i>	100	99.52	OP898242.1	Ngrumput
NG2	<i>H. trigonarius</i>	<i>H. trigonarius</i>	78	100	GU480563.1	Ngrumput
NG3	<i>E. mathaei</i>	<i>E. mathaei</i>	99	99.84	AY262912.1	Ngrumput
NG4	<i>E. oblonga</i>	<i>E. mathaei</i>	100	99.84	AY262868.1	Ngrumput

Beach. According to Wangenstein et al. (2018), a percent identity value higher than 97% using the COI gene generally indicates accurate species identification or confirms that the species are the same. Therefore, samples NG1-NG4 were successfully identified. Specifically, the sea urchin sample NG1 was identified as *Stomopneustes variolaris*, NG2 as *Heterocentrotus trigonarius*, NG3 as *Echinometra mathaei*, and NG4 as *Echinometra oblonga*.

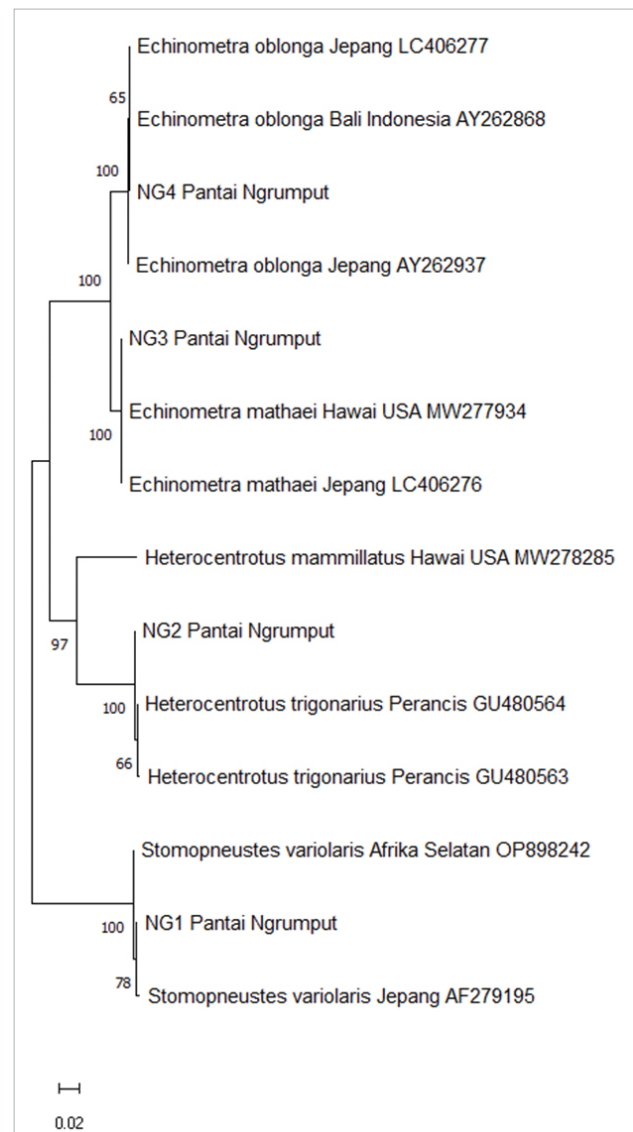


**Fig 3.** Visualisation of electrophoresis results for the COI gene of sea urchins (M = DNA ladder; NG1= sea urchin sample 1; NG2 = sea urchin sample 2; NG3 = sea urchin sample 3; NG4= sea urchin sample 4)

### Phylogenetic analysis

The results of the phylogenetic tree analysis (Fig. 4) indicate that sample NG1 is closely related to *Stomopneustes variolaris* from South Africa and Japan. Sample NG2 is closely related to *Heterocentrotus trigonarius* from France but is not closely related to *Heterocentrotus mammillatus* from Hawaii, as these species are significantly different. Sample NG3 shows a close relationship with *Echinometra mathaei* from Hawaii and Japan, while sample NG4 is closely related to *Echinometra oblonga* from Japan and Bali. The phylogenetic tree construction yielded a scale bar value of 0.02.

According to Hardianto and Satriyo (2023), a scale bar value of 0.02 in phylogenetic tree construction indicates a genetic distance corresponding to two nucleotide changes per 100 bp.



**Fig 4.** Phylogenetic tree of sea urchins from the Southern Coast of Java and other sea urchins using the Neighbour-Joining method with 1000X bootstrap replications

## Abundance

The average abundance of sea urchin individuals in November and December is presented in Fig. 5.

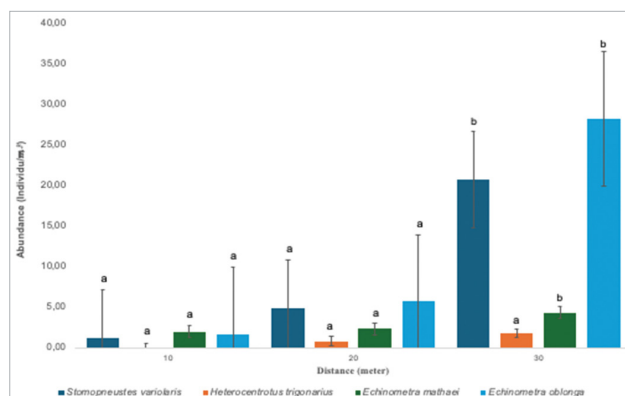


Fig 5. Average abundance of sea urchin individuals

The abundance of sea urchin individuals increased with increasing distance from the shoreline, with the highest abundance observed 30 meters from the shoreline. The average total abundance of Echinoidea species was 74.14 individuals/m<sup>2</sup>. At distances of 10, 20, and 30 meters from the shoreline, the abundance of sea urchins varied significantly, primarily influenced by substrate differences at each distance. At 10 meters, the substrate consisted of sand and rocky coral, while at 20 and 30 meters, extending towards the open sea, dead coral and coral reefs dominated. Sea urchins at Ngrumput Beach were predominantly found at 20 and 30 meters, where the substrate characteristics of dead coral and rocky coral provided favourable habitats.

The substrate plays a crucial role in influencing the abundance and distribution of sea urchins, as it serves as both a refuge and a feeding ground (Beddingfield and McClintock, 2000). Substrate crevices are particularly significant, with a positive correlation to sea urchin distribution and abundance (Clemente and Hernandez, 2008). Dead coral substrates with crevices help sea urchins survive predation, which can otherwise reduce their population (Hereu et al., 2004). Yiu and Chung (2024) noted that substrate type substantially impacts the presence and abundance of sea urchins.

Additionally, depth and location are critical factors influencing sea urchin abundance. Depth directly affects light penetration, sedimentation, nutrient concentrations, and water temperature (Cordeiro et al., 2014). This study found that the highest sea urchin abundance was observed at 30 meters from the shoreline, compared to the shallower depths of 10 and 20 meters. In shallower waters, sea urchins experience greater physical stress compared to deeper waters, including fluctuations in temperature, wave action, and salinity.

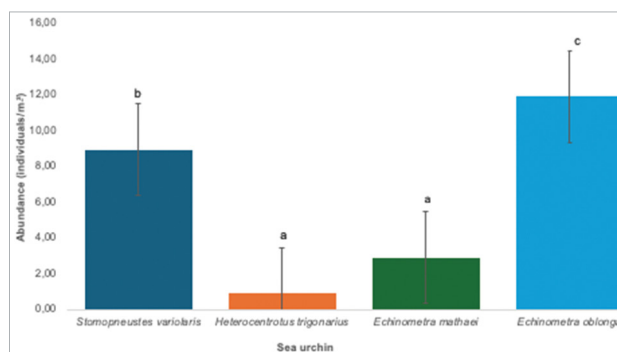


Fig 6. Average abundance among sea urchin species

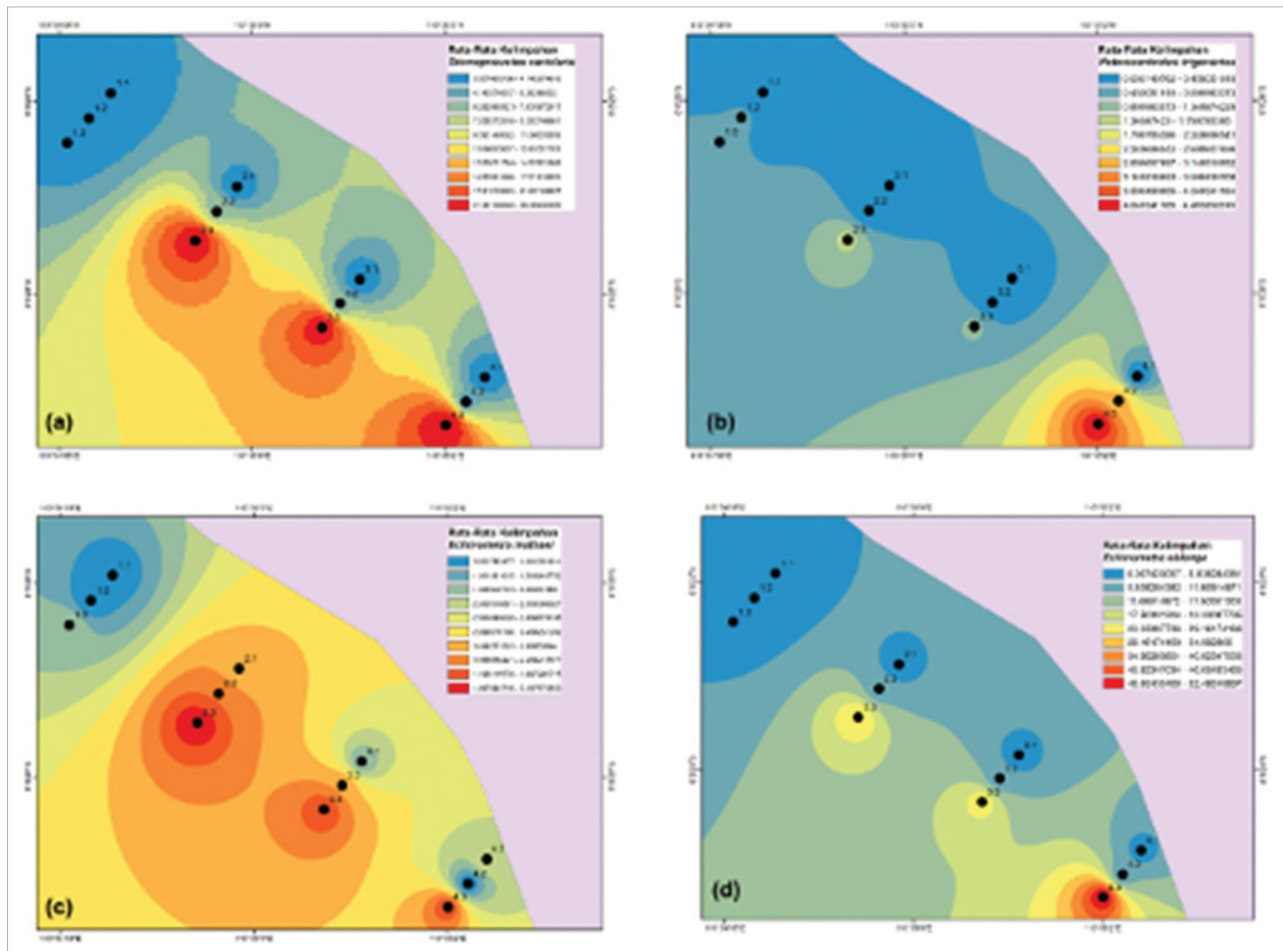
The average abundance of sea urchin species at Ngrumput Beach varied significantly (Fig. 6). The highest average abundance was recorded for *Echinometra oblonga* at 11.92 individuals/m<sup>2</sup>, while the lowest was for *Heterocentrotus trigonarius* at 0.92 individuals/m<sup>2</sup>. ANOVA analysis revealed a *p*-value of 0.001, indicating a statistically significant difference in average abundance among species.

These differences are likely due to the varying environmental adaptations of each species. Besides their tolerance to aquatic conditions, body size also plays a crucial role in sea urchin abundance. *Echinometra oblonga*, the species with the highest abundance, has the smallest body size among the identified species (Fig. 2). Smaller body size allows *E. oblonga* to hide in narrow crevices within rocky coral substrates, protecting it from predators and increasing its population. Additionally, *E. oblonga* exhibits a clumped distribution pattern, which further contributes to its higher population density. In contrast, the abundance patterns differ from those observed on the southern coast of Lombok Island, where *Diadema setosum* and *Tripneustes gratilla* showed the highest abundance (Bahri et al., 2021). Rahim and Nurhasan (2017) suggest that differences in species composition and abundance of sea urchins are influenced by water quality parameters, food availability, and predator presence.

## Distribution map of sea urchin abundance

A distribution map of sea urchin abundance illustrates the spatial variation in population density across different distances from the shoreline at Ngrumput Beach. The distribution map of sea urchins at Ngrumput Beach, Yogyakarta is shown in Fig. 7.

The colours on the map represent the abundance of sea urchins at each location. Red indicates higher abundance values, while blue represents lower values. Overall, the highest abundance of all sea urchin species was observed 30 meters from the shoreline towards the open sea. The distribution of sea urchin abundance is influenced by various factors, including substrate conditions, water quality parameters, tidal patterns, predators, and the availability of natural food sources.



**Fig 7.** Distribution map of sea urchin abundance at Ngrumput Beach: **(a)** *Stomopneustes variolaris*, **(b)** *Heterocentrotus trigonarius*, **(c)** *Echinometra mathaei*, and **(d)** *Echinometra oblonga*

Additionally, water conditions affect the abundance and diversity of marine organisms; polluted waters can reduce the tolerance and survival capability of marine life (Novinta and Adharini, 2022).

#### Ecological index of sea urchins

The ecological index is used to assess the balance of an ecosystem within a community structure. The analysed ecological indices include the species diversity index ( $H'$ ), evenness index ( $E$ ), and dominance index ( $C$ ), as presented in Table 2.

The lowest species diversity index ( $H'$ ) for sea urchins was recorded at 10 meters from the shoreline, with a value of 0.99, categorised as low (Fig. 8). This value is attributed to the smaller number of species or individuals found at this distance compared to 20 and 30 meters. The highest diversity index was observed at 20 meters, with a value of 1.19, categorised as moderate. A moderate diversity index may result from a limited number of individuals or uneven distribution among species. This observation aligns with the findings by Arthaz et al. (2015) who reported that moderate diversity values were caused by the low number of species and uneven distribution of individuals.

**Table 2.** Ecological index of sea urchins in Ngrumput Beach, Yogyakarta

Distance (m)	H	Category	E	Category	C	Category
10	0.99	Low	0.90	High	0.41	Low
20	1.19	Moderate	0.86	High	0.13	Low
30	1.02	Moderate	0.74	High	0.14	Low

The number and distribution of individuals within a species can be influenced by environmental conditions, food availability, and substrate type. The species diversity of sea urchins at Ngrumput Beach is lower compared to that at the southern coast of Lombok Island, where 11 species have been identified (Bahri et al., 2021), and the eastern waters of Sabah, Malaysia, which host ten species of sea urchins (Rahim and Nurhasan, 2016). According to Odum (1993), the level of species diversity is influenced by the distribution of individuals within each species. A community may have many species, but if individuals are unevenly distributed, the species diversity is considered low. ANOVA analysis revealed a  $p$ -value of 0.16 for the diversity index ( $H'$ ), indicating that the mean diversity index across different distances was not significantly different.

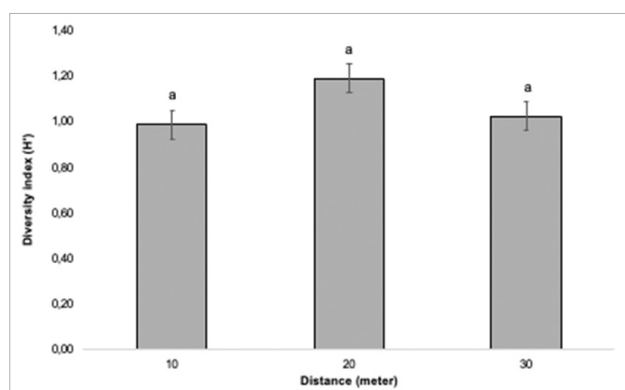


Fig 8. Species diversity index ( $H'$ ) of sea urchins at Ngrumput Beach

The sea urchins at Ngrumput Beach exhibited a high evenness index ( $E$ ) across all distances of 10, 20, and 30 meters from the shoreline (Fig. 9). This value indicates a relatively stable distribution of individuals among species.

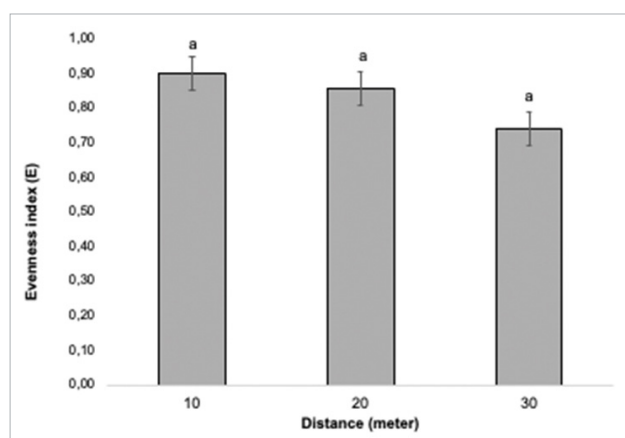


Fig 9. Evenness index ( $E$ ) of sea urchins at Ngrumput Beach

The high evenness index is attributed to the presence of multiple species, with a relatively balanced number of individuals across species. ANOVA analysis revealed a  $p$ -value of 0.20 for the evenness index ( $E$ ), indicating that the mean evenness index values did not significantly differ based on the distance from the shoreline. This stability suggests that the ecosystem at Ngrumput Beach supports a balanced distribution of sea urchins across species and distances.

The sea urchins at Ngrumput Beach exhibited a low dominance index ( $C$ ) across all distances from the shoreline (Fig. 10). This value indicates that the community of sea urchins is stable, as no single species dominates the ecosystem. ANOVA analysis revealed a  $p$ -value of 0.01 for the dominance index ( $C$ ), indicating that the mean dominance index values differed significantly based on the distance from the shoreline.

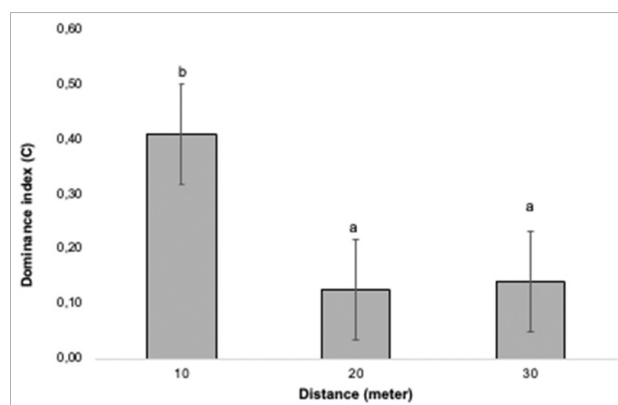


Fig 10. Dominance index ( $C$ ) of sea urchins at Ngrumput Beach

### Sea urchin distribution patterns

The distribution patterns of sea urchins based on their distance from the shoreline are presented in Table 3.

The species *Stomopneustes variolaris* and *Echinometra oblonga* exhibit a clumped distribution pattern at distances of 10, 20, and 30 meters towards the open sea. This pattern is attributed to environmental conditions that support their survival. Additionally, clumped distribution serves as a defensive strategy against predators (Kurniati et al., 2023). However, *S. variolaris* occasionally displays a uniform distribution pattern, probably due to competition among individuals or species that drives the division of shared space. Studies by Putri et al. (2019) and Triatmojo et al. (2018) reported that *S. variolaris* exhibits both clumped and uniform distribution patterns. Meanwhile, the genus *Echinometra* often forms cohesive units or colonies, further promoting a clumped distribution pattern.

At 10 meters from the shoreline, *Heterocentrotus trigonarius* was not found. However, at 20 meters, *H. trigonarius* exhibited a uniform distribution pattern, while at 30 meters, it showed a clumped distribution

**Table 3.** Sea urchin distribution patterns at Ngrumput Beach, Yogyakarta

Species	10 meters	20 meters	30 meters
<i>S. variolaris</i>	2.64 (clumped)	1.11 (clumped)	1.22 (clumped)
<i>H. trigonarius</i>	0.00 (-)	0.00 (uniform)	1.29 (clumped)
<i>E. mathaei</i>	0.98 (uniform)	1.94 (clumped)	0.94 (uniform)
<i>E. oblonga</i>	1.53 (clumped)	1.10 (clumped)	1.36 (clumped)

Notes: (-) sign indicates that no sea urchins were found

pattern. The clumped pattern is associated with defence against predators and facilitates reproductive processes by increasing the likelihood of fertilisation. In contrast, the uniform distribution of *H. trigonarius* is likely driven by strong competition among individuals or species to secure substrate crevices as shelter. According to Latuconsina (2021), the uniform distribution pattern of *Heterocentrotus trigonarius* is due to competition in a very large population that encourages the same division of space; this can be seen at a distance of 20 m where several types of sea urchins with a clumped pattern exist, causing the distribution of *Heterocentrotus trigonarius* to become uniform due to competition for space. The clumped distribution pattern of *Heterocentrotus trigonarius* is also caused by the substrate at Ngrumput Beach, which is very supportive, as the substrate at a distance of 30 m is rocky and exposed to strong waves. This is also supported by the research of Nurcahyo et al. (2024), which found that *Heterocentrotus trigonarius* is often found at a distance of 30 m. According to Coppard and Campbell (2005), high wave energy and coral reef substrates or coral reefs favour the existence of certain species in these habitats, including *Heterocentrotus trigonarius* which likes coral reef substrates with high wave exposure.

The distribution pattern of *Echinometra mathaei* was uniform at 10 meters and 30 meters from the shoreline. This uniform pattern is attributed to competition among individuals for space, food, and shelter. The uniform distribution at these distances is also influenced by competition with *Stomopneustes variolaris* and *Echinometra oblonga*, both of which exhibit clumped distribution patterns at the same distances. At 20 meters, however, *E. mathaei* displayed a clumped distribution

pattern. This clustering occurs because *E. mathaei* can compete effectively for space, food, and shelter, allowing it to establish groups in suitable habitats. According to Coppard and Campbell (2005), the *Echinometra mathaei* species occupy the most at a distance of 15–20 m and form a clumped distribution. Different distribution patterns at varying distances can be caused by environmental interactions and affect the behaviour and distribution patterns of the genus *Echinometra* species (McClanahan and Muthiga, 2020).

#### Water quality parameters

The water-quality parameters at Ngrumput Beach, Yogyakarta are shown in Table 4. The water-quality parameters at Ngrumput Beach during the study period showed an average temperature of 27.18 °C. According to Toha et al. (2012), the normal temperature range for benthic organisms, particularly sea urchins, is 25–33 °C. Temperatures above 35 °C can stress sea urchins (Haurissa et al., 2021). The temperature range observed at Ngrumput Beach supports the growth and reproduction of sea urchins. The average pH parameter at Ngrumput Beach was 8.75. pH is a limiting factor for organisms in aquatic environments. While the recorded pH value slightly exceeded the threshold of 8.5, sea urchins at Ngrumput Beach could still thrive well in the observed pH range.

The average salinity during the study was 34.80 ppt, which is considered optimal for supporting the growth and development of sea urchins. According to Nomleni et al. (2020), sea urchins are intolerant of low salinity and cannot survive in estuarine environments due to freshwater input. Hypo-salinity conditions (24–28‰)

**Table 4.** Average water quality parameters at Ngrumput Beach, Yogyakarta

No	Parameter	Results	Range	Reference
1	Temperature (°C)	27.18 ± 2.06	25–33	Toha et al. (2012)
2	pH	8.75 ± 0.16	6–8,5	Rahim & Nurhasan (2016)
3	Salinity (ppt)	34.26 ± 0.58	30–35	Rahim & Nurhasan (2016)

reduce sea urchin responses and movement, while hyper-salinity significantly impacts the coordination and adhesive ability of their tube feet on substrates (Moura et al., 2023). In general, the temperature, pH, and salinity parameters at Ngrumput Beach fall within the optimal range, making it a suitable and healthy habitat for the life and sustainability of sea urchins.

## CONCLUSION

The sea urchins identified at Ngrumput Beach through morphological and molecular analysis comprised four species: *Stomopneustes variolaris*, *Heterocentrotus trigonarius*, *Echinometra mathaei*, and *Echinometra oblonga*. The DNA band length was measured at 630 bp. The phylogenetic tree construction showed a scale bar of 0.02, indicating a genetic distance with nucleotide changes occurring twice per 100 bp. The total abundance of sea urchins in the intertidal zone of Ngrumput Beach was 74.14 individuals/m<sup>2</sup>, with the highest abundance observed 30 meters from the shoreline. The average species-diversity index was 1.07, categorised as moderate. The evenness index was 0.83, classified as high, while the dominance index was 0.23, categorised as low. The distribution patterns of *Echinometra oblonga* and *Stomopneustes variolaris* were clumped, while *Heterocentrotus trigonarius* and *Echinometra mathaei* exhibited both uniform and clumped distribution.

## ISTRAŽIVANJE RAZNOLIKOSTI MORSKIH JEŽINACA (*Echinoidea*) U YOGYAKARTA I INDONESIA PRIMJENOM MOLEKULARNIH METODA, ABUNDANCE I EKOLOŠKIH INDEKSA

### SAŽETAK

Morski ježinci su beskralješnjaci koji imaju ključnu ekološku ulogu u održavanju ravnoteže u međuplimnim ekosustavima, a ujedno služe i kao bioindikator, uz značajnu gospodarsku vrijednost kao morskih resursa. Cilj ovog istraživanja bio je ispitati morske ježince na području Ngrumput u Yogyakarta kroz analizu ekoloških indeksa, mjerenja abundance te morfološku i molekularnu identifikaciju primjenom metode DNA barkodiranja. Uzorkovanje je provedeno od studenog do prosinca 2023. godine, koristeći metodu transekta s kvadratom veličine 1 x 1 m. Molekularna identifikacija bila je usmjerena na mitohondrijski gen citokrom oksidazu podjedinicu I (mtDNA COI). Na temelju morfološke i molekularne identifikacije, identificirane su sljedeće vrste morskih ježinaca: *Echinometra oblonga*, *Echinometra mathaei*, *Heterocentrotus trigonarius* i *Stomopneustes variolaris*. Rezultati su pokazali da su morski ježinci na području Ngrumput imali abundancu od 74,14 jedinki/m<sup>2</sup>, indeks

raznolikosti vrsta 1,07 (umjeren), indeks ravnostnosti 0,83 (visok) te indeks dominacije 0,23 (nizak). Analiza obrasca raspodjele pokazala je da su *Echinometra oblonga* i *Stomopneustes variolaris* imali skupnu raspodjelu, dok su *Heterocentrotus trigonarius* i *Echinometra mathaei* pokazivali i ujednačene i skupne obrasce raspodjele. Najveće obilje morskih ježinaca zabilježeno je na udaljenosti od 30 metara od obale. Molekularna identifikacija otkrila je duljinu DNA sekvence od 630 bp. Analiza DNA barkodiranja, uključujući konstrukciju filogenetskog stabla *neighbour-joining* metodom i procjenu genetske udaljenosti, potvrdila je rezultate morfološke analize.

**Ključne riječi:** beskralješnjaci, raznolikost, gustoća, mtDNA COI

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