



Beyond Morphology: COI-Based Molecular Identification of Sea Urchins from Kumu Beach, North Sulawesi

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Abstract

Sea urchins (Echinoidea) play important ecological and economic roles in coastal ecosystems, but morphological identification is often unreliable due to overlapping diagnostic traits among closely related taxa. This study used DNA barcoding of the mitochondrial COI gene to identify a single sea urchin specimen (NA1) from Kumu Beach, North Sulawesi, and determine its phylogenetic relationship with GenBank reference sequences. DNA was extracted from gonad tissue, amplified via PCR using the *BK-COI-AntF&AntR* primers, sequenced, and analyzed through BLAST, MUSCLE alignment, and Maximum Likelihood phylogenetic reconstruction (Kimura 2-Parameter model, 1000 bootstrap replications). PCR yielded a clear ~709 bp fragment, producing a 658 bp COI consensus sequence (A+T content: 56.84%). BLAST analysis showed 100% query coverage and identity with multiple *Tripneustes gratilla* reference sequences, and phylogenetic analysis placed the specimen within the *Tripneustes* clade, closely nested among *T. gratilla* sequences. These results support the identification of the specimen as *T. gratilla* and demonstrate the utility of COI-based DNA barcoding for resolving morphologically ambiguous sea urchin specimens, while underscoring the need for additional specimens from Kumu Beach to assess genetic diversity at the population level.



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1. Introduction

Sea urchins (Echinoidea) are macrobenthic marine organisms that occupy a strategic position in maintaining the balance and productivity of shallow coastal ecosystems, particularly coral reefs and seagrass beds. As key herbivores and detritivores, sea urchins regulate macroalgal growth through grazing activity, preventing phase shifts from coral-dominated to fleshy macroalgae-dominated ecosystems, which makes their presence an important indicator of coastal ecosystem resilience [1]. Beyond their ecological function, several sea urchin species also hold considerable socioeconomic value, as their gonads are harvested as a premium seafood commodity rich in protein and essential fatty acids [2].

The coastal waters of North Sulawesi lie within the Coral Triangle, one of the richest marine biodiversity regions in the world, and consequently harbour a high diversity of Echinoidea [3, 4]. Previous surveys have recorded numerous sea urchin taxa in this region, including *Astropyga radiata*, *Diadema setosum*, *Diadema savignyi*, *Tripneustes gratilla*, *Mespilia globulus*, *Echinothrix calamaris*, and *Echinometra mathaei*, among others [5–9].

Despite this species richness, accurate taxonomic identification of Echinoidea in the field remains challenging. High phenotypic plasticity and overlapping morphological characters among closely related genera, such as *Diadema* and *Echinothrix*, frequently give rise to cryptic species visually indistinguishable and genetically

distinct lineages [10]. Such taxonomic ambiguity can lead to misidentification during harvesting, which is especially concerning given that edible and non-edible sea urchin taxa may coexist in the same habitat, and can also compromise the accuracy of species-level biodiversity and fisheries records [11]. In the present case, field identification based on external morphology alone was complicated by well-documented phenotypic plasticity within the genus *Tripneustes*, in which spine colour pattern, test size, and overall body shape vary considerably even within a single species and overlap extensively between congeners [10]. This morphological ambiguity prevented confident species-level assignment in the field and necessitated molecular confirmation.

To overcome the limitations of conventional morphological identification, integrative taxonomy combines morphological observation with molecular identification. DNA barcoding using the mitochondrial *Cytochrome c Oxidase Subunit I* (COI) gene has been widely adopted as a reliable standard for confirming animal species identity, owing to its relatively conserved intraspecific variation and comparatively high interspecific divergence [12]. Beyond species determination through sequence matching (BLAST search), molecular characterisation can be extended through phylogenetic reconstruction, which places a specimen objectively within a taxonomic clade relative to reference sequences deposited in global databases such as GenBank.

A literature search conducted in December 2025 using Google Scholar, ScienceDirect, and GenBank's nucleotide database, with the keywords "COI," "DNA barcoding," "Echinoidea," and "Kumu Beach" / "Tombariri," returned COI-based barcoding records for Echinoidea from several other North Sulawesi localities [5–9], but no comparable molecular record for Kumu Beach specifically. This absence is significant not merely as a geographic gap but as a methodological one, without a molecular reference point, morphologically ambiguous specimens collected from this site such as the one motivating this study, which could not be confidently assigned to species level based on external morphology alone cannot be reliably confirmed to species level, leaving both taxonomic records and downstream fisheries or conservation assessments vulnerable to misidentification.

This study was conducted at Kumu Beach, Tombariri Subdistrict, Minahasa Regency, North Sulawesi, a coastal area with a mosaic of sandy substrate, seagrass beds, and coral reef habitats. The area is subject to informal sea urchin gonad harvesting by local coastal communities, making accurate species-level identification directly relevant to sustainable local fisheries management and

conservation planning. Based on external morphological characters observed in the field, the specimen was tentatively identified as a member of the genus *Tripneustes*. This hypothesis required molecular confirmation given the identification challenges outlined above. Given this data gap, the present study aimed to molecularly identify a single sea urchin specimen (NA1) collected from Kumu Beach using COI gene DNA barcoding, to test this presumptive field identification, and to analyse its phylogenetic relationship with reference sequences from GenBank, thereby providing a molecular (COI) record for the analysed specimen from Kumu Beach, offering an initial reference point for future, larger-scale biodiversity assessment at this site.

2. Materials and Methods

2.1. Research Design and Location

This study used a descriptive exploratory design integrating field sampling with a molecular approach based on the mitochondrial *Cytochrome c Oxidase Subunit I* (COI) gene, followed by phylogenetic analysis against GenBank reference sequences. Field sampling was carried out in the coastal waters of Kumu Beach, Kumu Village, Tombariri Subdistrict, Minahasa Regency, North Sulawesi. At the same time, laboratory analyses were performed at the Molecular Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi, Manado.

2.2. Sample Collection and DNA Extraction

Field sampling was carried out between 15 December 2025 and 25 February 2026 in the intertidal to shallow subtidal zone (± 1 m depth) of Kumu Beach, Kumu Village, Tombariri Subdistrict, Minahasa Regency, North Sulawesi (approximately $1^{\circ}23'50''$ – $1^{\circ}24'00''$ N, $124^{\circ}34'15''$ – $124^{\circ}34'20''$ E), using purposive sampling during stable tidal conditions, consistent with the known clumped microhabitat distribution of sea urchins in coral reef crevices and sandy substrate [13]. During the survey period, three individuals were hand-collected by snorkelling from the site. Of these, one specimen meeting all inclusion criteria (adult stage, alive, fully intact test and spines, no physical damage) was selected and designated NA1 for DNA extraction; the remaining two individuals were excluded from molecular analysis, one being in the juvenile stage and considered too small to meet the inclusion criteria, and the other showing damaged/broken spines. The collection point of NA1 was georeferenced using a GPS timestamp application. This study was approved by the Ethics Committee of the Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Department of Biology (July 18, 2026).

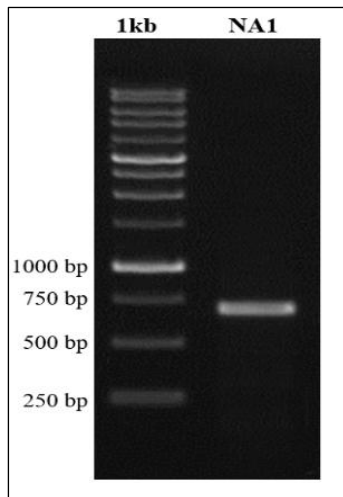


Figure 1. Agarose gel electrophoresis (0.8%) of the PCR product of sample NA1, showing a single ~709 bp band relative to a 1 kb DNA ladder (Vivantis).

Approximately 15 mg of gonad tissue, selected over test or spine tissue due to its higher cellular density and DNA yield [14], was excised aseptically from NA1, preserved in 95% ethanol, and stored at -20°C . Genomic DNA was extracted using the Tissue Genomic DNA Mini Kit (Geneaid) following the manufacturer's protocol, involving tissue lysis (GT buffer/Proteinase K, 60°C), silica membrane binding (GBT buffer, 60°C), ethanol washing, and elution, extracted DNA was stored at -20°C until further analysis.

2.3. PCR Amplification, Electrophoresis, and Sequencing

PCR amplification targeting the COI gene was performed in triplicate, each in a $40\ \mu\text{L}$ reaction volume consisting of $20\ \mu\text{L}$ MyTaq HS Red Mix (Bioline), $3\ \mu\text{L}$ of the BK-COI-AntF/AntR primer pair, $2\ \mu\text{L}$ of DNA template, and $15\ \mu\text{L}$ of sterile Milli-Q water, using thermal cycling conditions adapted from standard COI barcoding protocols [15], initial denaturation at 94°C (2 min), 35 cycles of 94°C (30 s), 52°C (40 s), 72°C (1 min) and final extension at 72°C (10 min). Amplification success (~650 bp target fragment) was confirmed by 0.8% agarose gel electrophoresis under UV transillumination. Among the three replicates, the amplicon producing the sharpest, most specific single band without smearing was selected for sequencing. The selected amplicon was sequenced bidirectionally at First Base, Malaysia, using the same primer pair. Chromatograms were quality-checked in BioEdit [16] following Phred score conventions [17], applying a minimum quality threshold of Phred ≥ 20 [18], with low-quality 5'/3' ends trimmed prior to downstream analysis.

2.3. Data Analysis

Sequencing data were analysed using Geneious Prime (version 2025.1.1) to assess sequence quality, remove

noise, and assemble the COI consensus sequence. The resulting sequence was compared against GenBank reference data using the Basic Local Alignment Search Tool (BLAST), with species-level identity assigned to matches with $\geq 97\%$ sequence identity [19]. Prior to phylogenetic tree construction, the COI sequence of sample NA1 was aligned together with GenBank reference sequences using Multiple Sequence Alignment (MSA) with the MUSCLE algorithm [20], which is critical because a poor alignment can lead to an erroneous tree topology and incorrect taxon identification. Phylogenetic relationships were then reconstructed using the Maximum Likelihood (ML) method in MEGA under the Kimura 2-Parameter (K2P) substitution model, with *Dendroaster excentricus* (HM542162) set as the outgroup and node support assessed through 1,000 bootstrap replications.

3. Results and Discussion

3.1. PCR Amplification and COI Sequencing

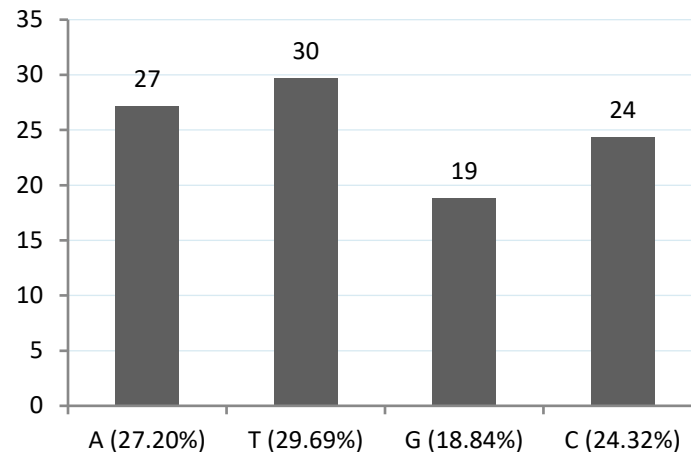
Agarose gel electrophoresis (0.8%) of the PCR product from sample NA1 showed a single, clearly visible DNA band of approximately 709 bp compared with a 1 kb DNA ladder Figure 1, indicating successful and specific amplification of the Cytochrome c Oxidase Subunit I (COI) gene. This fragment size falls within the typical range reported for the universal COI barcoding region [5, 21]. The absence of smearing or additional non-specific bands indicated good DNA quality and optimal PCR conditions, consistent with the reliability of COI as a standard DNA barcoding marker for animal species identification [12].

Following quality control of the chromatogram data using Geneious Prime and BioEdit, sequencing of sample NA1 yielded a consensus COI fragment of 658 bp Table 1, consistent with the standard length of the COI barcode region amplified using Folmer type primers for marine invertebrate identification [12]. Nucleotide composition analysis in Figure 2 showed that thymine (T) was the most abundant base (29.64%), followed by adenine (27.20%), cytosine (24.32%), and guanine (18.84%), giving a combined A+T content of 56.84%, higher than the G+C content (43.16%). This A+T-rich pattern is characteristic of Echinoidea mitochondrial DNA and is consistent with the mitogenome of *Diadema antillarum* (57.84% A+T) [22] and broader patterns reported across Echinoidea mtDNA [23]. Minor asymmetries between A/T and G/C are expected in single-stranded sequencing data and do not contradict Chargaff's rule, which strictly applies to double-stranded DNA [24].

BLASTn analysis of the NA1 sequence against the GenBank database Table 2 showed 100% query coverage and 100% identity with multiple *Tripneustes gratilla*

Table 1. Nucleotide sequence of the COI gene obtained from sequencing of sample NA1. The raw sequence shown represents the forward/consensus read prior to trimming and alignment.

Sample	Sequencing Results
NA1	AACCTCTATTTAATCTTCGAGCCTGAGCCGGAATGGTAGGAACGCCATGAGTGTAAATTATACGAGCCGAAGTAGCTCAACCGGGATCTCTTTTAAAGACGACCAAATATACAAAGTAGTCGTTACTGCACATGCCCTAGTAATGATCTTTTTATGGTAATGCCAATAATGATTGGAGGATTTGGTAAATGACTCATTCCACTAATGATTGGCGCTCCAGATATGGCTTTTCCCCGAATGAAAAAATGAGCTTTTGACTTGTTCCAACATCTTTTATACTCCTTTTAGCTCCGCCGGAGTTGAAAGAGGAGCAGGAACAGGATGAACAATCTACCCTCCACTTTCAAGAAAAATTGCACACGCGGGGGGATCCGTTGACTTGGCCATCTTTCACTTCATCTTGCCGGGGCTTCCTCTATTTAGCCTCCATTAACCTTCATCACAAATCATAAATATGCGAACACCAGGAATGTCTTTTGACCGACTTCCACTATTTGTATGGTCTGTATTTGTTACGGCATTTCTCCTTGTCTTCTTACCAGTTCTAGCAGGAGCAATCACCATGCTGCTGACGGACCGAAACATTAATACAACCTTCTTCGACCCAGCAGGCGGAGGAGATCCTATCCTATTCAACATCTATTC

**Figure 2.** Nucleotide composition of the COI gene sequence from sample NA1. The sequence shows an AT rich pattern (56.89% A+T vs. 43.16% G+C), typical of mitochondrial DNA.**Table 2.** BLAST results for the COI sequence of sample NA1 against GenBank reference sequences (top 10 matches).

No.	Scientific Name	Query Cover	Percent Identity	Accession
1	<i>T. gratilla</i>	100%	100%	MK751627.1
2	<i>T. gratilla</i>	100%	100%	MK084941.1
3	<i>T. gratilla</i>	100%	100%	PQ347805.1
4	<i>T. gratilla</i>	100%	100%	PQ347807.1
5	<i>T. gratilla</i>	100%	100%	NC_034770.1
6	<i>T. gratilla</i>	100%	100%	KU314830.1
7	<i>T. gratilla</i>	100%	100%	GU480557.1
8	<i>T. gratilla</i>	100%	100%	OP898253.1
9	<i>T. gratilla</i>	100%	100%	KU314836.1
10	<i>T. gratilla</i>	100%	100%	KU314838.1

reference sequences, indicating that the entire length of the query sequence aligned with the reference sequences without gaps and that every nucleotide position matched the reference. This result strongly supports the identification of sample NA1 as *T. gratilla* at the species level [19].

Although the 100% BLAST identity strongly supports species-level identification, pairwise similarity searches alone do not reveal the evolutionary branching structure among related sequences and cannot fully exclude the possibility of intraspecific variation or cryptic species [12]. Cryptic diversity has indeed been reported within *Tripneustes*; a Kermadec Islands population initially

identified as *T. gratilla* based on morphology and COI similarity was later shown to form a genetically distinct clade [25–27]. Phylogenetic reconstruction was therefore performed as a complementary analysis to clarify the position of sample NA1 within a broader evolutionary context.

3.2. Multiple Sequence Alignment (MSA) of the COI Gene

Multiple Sequence Alignment (MSA) was performed to align the COI gene sequences and determine the degree of similarity and nucleotide variation among sea urchin species, using the MUSCLE algorithm [20]. The alignment, spanning the full length of the 658 bp COI sequence, is presented in Figure 3.

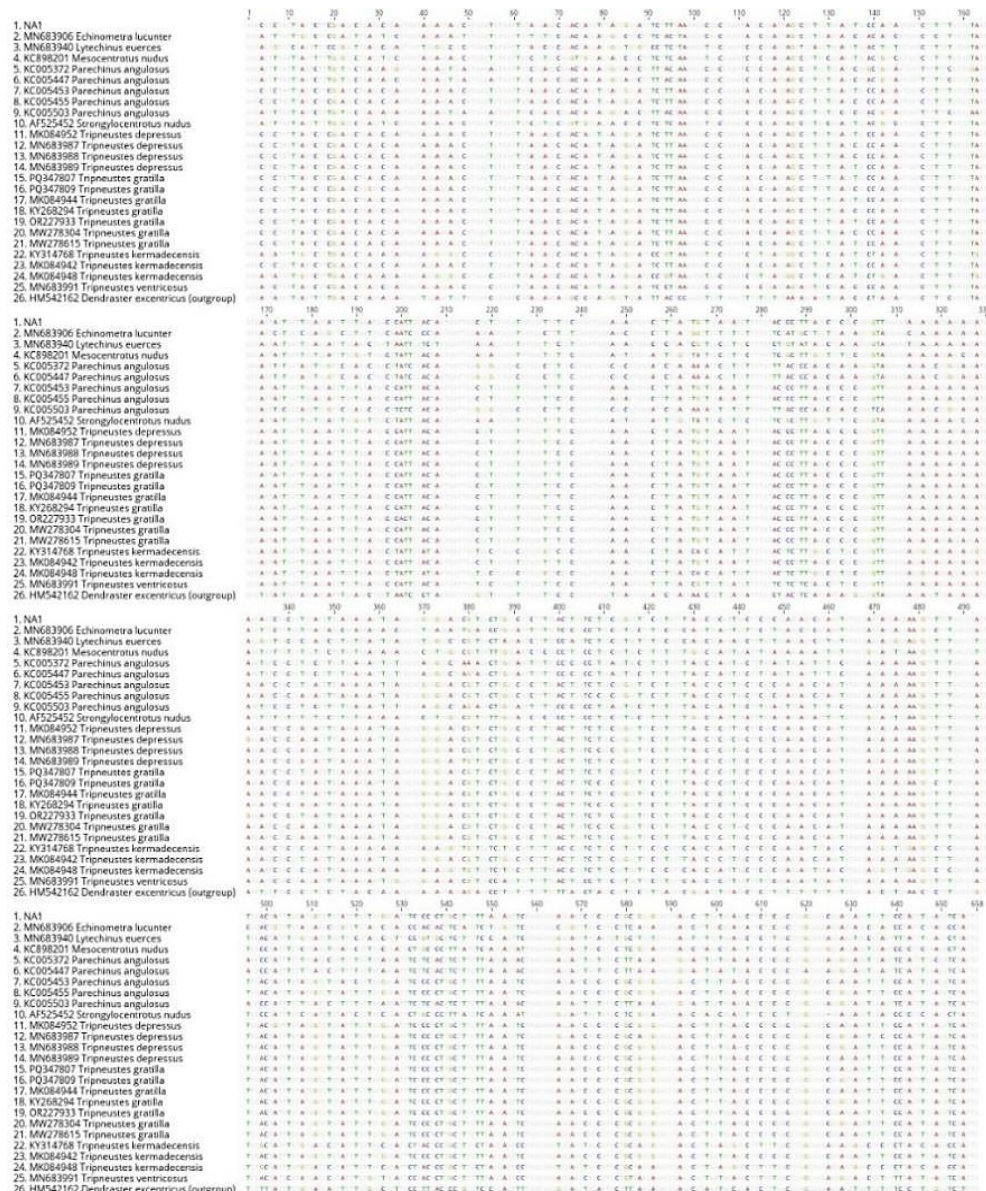


Figure 3. Multiple Sequence Alignment (MSA) of the COI gene generated using the MUSCLE algorithm, showing a high degree of sequence similarity between specimen NA1 and reference sequences of *Tripneustes gratilla* and related taxa.

The alignment showed that the majority of nucleotide positions across the compared sequences were identical, indicating conserved sites that reflect evolutionarily stable segments maintained across Echinoidea species; such conserved regions enable the design of universal primers and support COI's established use as a DNA barcoding marker [12, 21]. The alignment also revealed several variable nucleotide differences at specific positions that allow molecular differentiation between species within the class [15]. No significant insertions or deletions (indels) were observed across the analysed fragment, indicating that the observed variation was predominantly attributable to nucleotide substitutions rather than length changes. Specimen NA1 exhibited a nucleotide pattern highly similar to the *T. gratilla* reference sequences, with a large number of identical

sites and minimal base differences, supporting a close genetic relationship between specimen NA1 and *T. gratilla* [15].

3.3 Phylogenetic Analysis with GenBank Reference Sequences

Phylogenetic reconstruction was performed using the Maximum Likelihood (ML) method [28] under the Kimura 2-Parameter (K2P) substitution model implemented in MEGA X [29], with 1,000 bootstrap replicates. The K2P model was adopted following standard convention in DNA barcoding studies rather than through a formal model-selection procedure, consistent with widely used barcoding protocols that pair K2P with COI-based species discrimination [15]. *Dendroaster excentricus* (HM542162) was designated as the outgroup, as this irregular echinoid (order Clypeasteroidea) is phylogenetically more

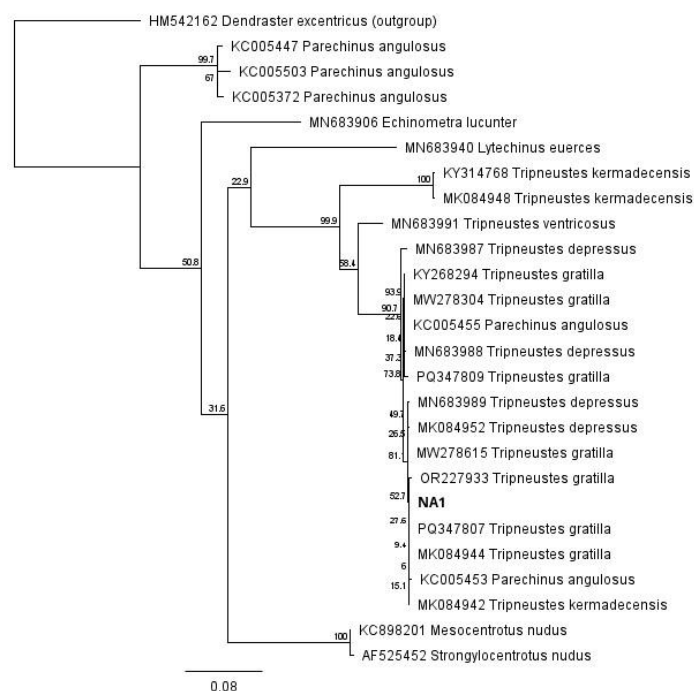


Figure 4. Phylogenetic tree based on the COI gene, constructed using the Maximum Likelihood method with the Kimura 2-Parameter model in MEGA (1,000 bootstrap replicates). NA1 (bold) clusters within the *Tripneustes gratilla* clade.

distant from the regular echinoid ingroup taxa, enabling unambiguous rooting of the tree. A total of 25 GenBank reference sequences spanning nine species across five genera were included alongside sample NA1 Table 3, selected to provide adequate congener, confamilial, and outgroup representation for robust tree construction; note that this reference set is broader than the top 10 BLAST hits reported in Table 2, which were selected specifically to demonstrate closest sequence matches.

The resulting tree Figure 4 revealed several well-supported clades. *Parechinus angulosus* (KC005447, KC005503, KC005372, KC005455, KC005453) formed a monophyletic clade with 99.7% bootstrap support. *Tripneustes kermadecensis* (KY314768, MK084948, MK084942) clustered together with 100% support. A clade grouping *Mesocentrotus nudus* (KC898201) with a sequence deposited under the earlier taxonomic name *Strongylocentrotus nudus* (AF525452) also received 100% support; this is consistent with *Strongylocentrotus nudus* being a recognized taxonomic synonym of *Mesocentrotus nudus*, rather than reflecting a grouping of two distinct species. The two accessions represent the same species under its pre- and post-revision genus names. *Echinometra lucunter* (MN683906) and *Lytechinus euerces* (MN683940) each formed distinct branches outside the *Tripneustes* clade, consistent with substantial genetic divergence between these genera and the ingroup.

The largest clade comprised multiple sequences of *T. gratilla*, *T. depressus*, and *P. angulosus*, with comparatively

low to moderate internal bootstrap support at several nodes (18.4–93.5%). This reduced resolution likely reflects genetic similarity resulting from high gene flow among populations, a pattern previously reported in pantropical *Tripneustes* [26]. Consequently, while the overall tree topology is broadly consistent with established taxonomy, the fine-scale relationships within this clade should not be overinterpreted as fully resolved, and any evolutionary inferences beyond species-level placement should be treated as provisional.

Within this broader clade, specimen NA1 was nested specifically within the *T. gratilla* subclade, positioned near reference sequences OR227933, PQ347809, and MK084944, at a node supported by a bootstrap value of 52.7%. This clustering pattern is consistent with previous COI-based phylogenetic studies on Indonesian *T. gratilla* populations [29]. This relatively low support likely reflects the limited number of phylogenetically informative sites among closely related, near-identical *T. gratilla* sequences at this shallow phylogenetic depth, a well-documented limitation of COI-based markers, which resolve species-level divergence far more reliably than fine-scale relationships among conspecific or recently diverged sequences [12]. This limitation does not undermine the species-level identification itself, which is independently and more robustly supported by the 100% BLAST identity reported in Section 3.1. Within this subclade, NA1 showed clear separation from congeners such as *T. depressus*, *T. ventricosus*, and *T. kermadecensis*, consistent with the principle of DNA barcoding whereby conspecific

Table 3. Reference sequences used for phylogenetic analysis.

No.	Species	Accessions	Group / Rationale for Inclusion
1	<i>Dendraster excentricus</i>	HM542162	Outgroup, irregular echinoid (Clypeasteroidea), distantly related to ingroup, used to root the tree
2–6	<i>Parechinus angulosus</i>	KC005447, KC005503, KC005372, KC005455, KC005453	Comparative genus within regular echinoids (Camarodonta), tests resolving power between closely related but distinct genera
7	<i>Echinometra lucunter</i>	MN683906	Distinct genus (Echinometridae) for broader family level phylogenetic context
8	<i>Lytechinus euerces</i>	MN683940	Distinct genus (Toxopneustidae) for broader family level phylogenetic context
9–11	<i>Tripneustes kermadecensis</i>	KY314768, MK084948, MK084942	Congener with documented cryptic divergence from <i>T. gratilla</i> [27–29], tests species level resolution within <i>Tripneustes</i>
12	<i>Tripneustes ventricosus</i>	MN683991	Atlantic congener, included for genus wide comparative context
13–16	<i>Tripneustes depressus</i>	MN683987, MN683988, MN683989, MK084952	Congener included to test whether NA1 groups with <i>T. gratilla</i> or <i>T. depressus</i>
17–23	<i>Tripneustes gratilla</i>	KY268294, MW278304, PQ347809, MW278615, OR227933, PQ347807, MK084944	Presumptive target species, multiple GenBank accessions used to assess intraspecific consistency and NA1's placement
24–25	<i>Mesocentrotus nudus</i> (= <i>Strongylocentrotus nudus</i> , syn.)	KC898201, AF525452	Conspecific pair under pre and post revision nomenclature, internal validation check of tree topology and bootstrap reliability
—	<i>Tripneustes</i> sp. (query)	NA1 (this study)	Specimen under investigation

individuals are expected to form a distinguishable monophyletic group [12].

3.4 Summary of Molecular and Phylogenetic Evidence

Taken together, BLASTn analysis (100% identity), MSA (high conservation with *T.* references), and phylogenetic placement of *gratilla* nested within the *T. gratilla* subclade as constructed via Maximum Likelihood [28, 30] and aligned with Indonesian *T. gratilla* lineages [29] consistently support the species-level identification of specimen NA1 as *T. gratilla*. Because this conclusion rests on a single specimen and a moderately supported phylogenetic node (52.7%), it is presented as strong molecular support for species-level identification rather than definitive confirmation of population-level genetic status at Kumu Beach.

4. Conclusions

This study molecularly identified a single sea urchin specimen (NA1) collected from Kumu Beach, North Sulawesi, whose field morphology could not be confidently resolved to species level due to intrageneric morphological plasticity, using COI-based DNA barcoding and phylogenetic analysis. BLASTn analysis showed 100% query coverage and 100% identity to *T. gratilla* reference sequences, and Multiple Sequence Alignment confirmed a high degree of nucleotide conservation with these references. Phylogenetic reconstruction placed sample NA1 within a *T. gratilla* subclade, clearly separated from other congeners, thereby resolving the initial field-based

morphological ambiguity and supporting the specimen's identification as *T. gratilla*.

The internal node placing NA1 within this subclade received only moderate bootstrap support (52.7%), a level of resolution consistent with the limited number of phylogenetically informative COI sites among closely related, recently diverged *Tripneustes* sequences rather than any weakness in the underlying data. This finding is consistent with reports elsewhere of cryptic lineages within nominal *T. gratilla* populations, underscoring that while COI is highly reliable for species-level discrimination, as reflected by the unambiguous 100% BLAST identity, it offers comparatively limited resolution for fine-scale relationships within closely related taxa. The species-level identification of NA1 as *T. gratilla* is therefore best supported by the convergence of BLAST, alignment, and phylogenetic evidence taken together, rather than by any single line of evidence in isolation.

This study is subject to important limitations. Only a single specimen was analysed molecularly, out of three individuals collected during the survey period; the other two did not meet the inclusion criteria (one juvenile, one with damaged spines). The findings therefore represent a species-level identification of one individual. They should not be interpreted as a characterisation of sea urchin species diversity or population-level genetic structure at Kumu Beach. Broader ecological inferences await sampling of additional individuals.

Despite this narrow scope, the study provides the first COI-based molecular record for the Echinoidea fauna of Kumu Beach, filling a locality-specific gap identified through a literature search of Google Scholar, ScienceDirect, and GenBank. Given that the area supports informal sea urchin gonad harvesting by local coastal communities, an accurate, molecularly verified reference point for at least one locally occurring species offers a concrete baseline for future fisheries monitoring and conservation planning. We recommend that future work expand sampling effort at Kumu Beach to include multiple individuals and, where possible, additional Echinoidea taxa, in order to build a locality-specific DNA barcode reference library and assess genetic diversity at the population level.

Author Contributions: Conceptualization, N.A., T.E.T. and B.J.K.; methodology, N.A. and B.J.K.; software, N.A.; validation, T.E.T. and B.J.K.; formal analysis, N.A.; investigation, N.A.; resources, T.E.T. and B.J.K.; data curation, N.A.; writing original draft preparation, N.A.; writing review and editing, T.E.T. and B.J.K.; supervision, T.E.T. and B.J.K. All authors have read and agreed to the published version of the manuscript.

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Ethical Clearance: This study was conducted with field sampling of marine invertebrates and did not involve vertebrate animals or human subjects. Ethical clearance was obtained from the Faculty of Mathematics and Natural Sciences, Sam Ratulangi University.

Informed Consent Statement: Not applicable.

Data Availability Statement: The COI gene sequence data generated in this study is available in GenBank NCBI. Raw sequence chromatograms are available upon request from the corresponding author.

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Conflicts of Interest: All the authors declare no conflicts of interest.

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