Philippine Journal of Science
153 (5): 1569-1577, October 2024
ISSN 0031 - 7683
Date Received: 01 Feb 2024

Genetic Diversity of the White Teatfish *Holothuria fuscogilva* Cherbonnier, 1980 Based on mtDNA *COI* Gene: Starting Point for its Conservation Management in the Philippines

**Nonillon M. Aspe[1,2]*, Christian Jay R. Nob[2], Venus E. Leopardas[1,2],
Sitti Zayda B. Halun[3], Mariefe B. Quiñones[1,2], Sandra M. Moscoso[1,2],
Dan M. Arriesgado[2,4], Oliver Ratunil T. Paderanga[2,5],
Harry Kenn T. dela Rosa[2], Kevin G. Natingga[2], Abner C. Evasco Jr.[2],
Jackie Lou G. Empron[2], and Wilfredo H. Uy[1,2]**

[1]College of Marine and Allied Sciences, Mindanao State University at Naawan,
Naawan 9023, Misamis Oriental, Philippines
[2]Sea Cucumber Research and Development Center, Mindanao State University at Naawan,
Naawan 9023, Misamis Oriental, Philippines
[3]Institute of Oceanography and Environmental Science,
Mindanao State University–Tawi-Tawi College of Technology and Oceanography,
Bongao 7500, Tawi-Tawi, Philippines
[4]School of Marine Fisheries and Technology, Mindanao State University at Naawan,
Naawan 9023, Misamis Oriental, Philippines
[5]Institute of Arts and Sciences, Camiguin Polytechnic State College,
Mambajao 9100, Camiguin, Philippines

Assessing the genetic information of natural populations of fishery species is crucial for enhancing management strategies. Here, we examined the genetic variability of the white teatfish *Holothuria fuscogilva* from the Philippines and neighboring countries in the Pacific using the mitochondrial DNA *COI* gene marker. The species has a declining population with a "vulnerable" status based on the International Union for Conservation of Nature. Our analysis revealed a high haplotype diversity within *H. fuscogilva* populations across the Pacific region (Hd = 0.95658). Notably, we observed significant partitioning in haplotype frequencies (Fst = 0.05800, p-value = 0.01139), indicating pronounced genetic differentiation among Pacific populations (5.8%), albeit the majority remains within populations (94.2%). Restocking efforts in response to the declining population should be carefully considered to safeguard the genetic integrity of local populations. However, strict adherence to protocol is necessary to safeguard the genetic integrity of each local population. Hence, country-specific diversity analysis of *H. fuscogilva* population is necessary as a basis to ensure long-term sustainability and conservation of the species.

Keywords: conservation, genetic diversity, mtDNA COI, Philippines, sea cucumber

---

*Corresponding author: nonillon.aspe@msunaawan.edu.ph

INTRODUCTION

Broadcast spawners like sea cucumbers generally have high dispersal capacity and display weaker genetic structure and higher gene flow among populations (Borrero-Pérez *et al.* 2011). Under certain conditions, however, the presumption is not always the case because the gene flow which influences species' dispersal and distribution of marine species is affected by other environmental factors such as ocean currents, habitat conditions for larval settlement, and complexity of topography and geological structure due to continental breaks (Mercier *et al.* 2000; Juinio-Meñez 2015; Ravago-Gotanco and Kim 2019).

The importance of gene flow can be understood from various perspectives. It is possible that limited gene flow can facilitate local adaptation, which can lead to small-scale evolutionary improvements in the fitness of local populations. Conversely, gene flow becomes essential when the population size is small to preserve genetic diversity (Slatkin 1985, 1987). As a result, reproductive strategies, dispersal, and distributions in sea cucumbers have an impact on genetic differentiation as these may either restrict or enhance gene flow among populations (Arndt *et al.* 1996; Chang *et al.* 2009; Park *et al.* 2019). In the case of the sandfish *Holothuria scabra*, a significant signal of isolation-by-distance was observed as their dispersal and distribution are greatly influenced by the surrounding ocean currents and delayed larval settlement (up to 4 days), coupled with a low survival rate without a suitable substrate (Mercier *et al.* 2000; Ravago-Gotanco and Kim 2019). In fact, the different water currents within an archipelagic setting like the Philippines have demonstrated a discernible influence on molding genetic variation, particularly in slow-moving and sedentary species, which contribute valuable insights to the field of phylogeography (Lourie *et al.* 2005; de Boer *et al.* 2014; Raynal *et al.* 2014; Ravago-Gotanco and Kim 2019).

Neglecting the significance of genetic aspects, particularly phylogeography, and failing to prioritize the genetic integrity of local populations in mariculture efforts may result in detrimental consequences. Research has demonstrated that inadequate broodstock management and the use of low-quality hatchery- produced seeds stocks can result in loss of genetic diversity and may facilitate the transfer of diseases from the hatchery to the wild (Purcell 2012; Brown *et al.* 2024). Consequently, there is a growing effort to incorporate genetic data into stock assessments and conservation practices for commercially exploited sea cucumber species (Uthicke *et al.* 2010; Wen *et al.* 2011; Alcudia-Catalma *et al.* 2020). However, the genetic diversity of the white teatfish *Holothuria fuscogilva* in different regions, particularly in the Philippines – remains undocumented, highlighting the need for further research in this area.

Holothuria fuscogilva has been reported in the Indo-Pacific biogeographic region, including the Philippines (de Guzman and Quiñones 2021), Australia and Fiji (Uthicke *et al.* 2004); Wolfe and Byrne 2022; Purcell *et al.* 2023), Japan (Tanita *et al.* 2021), Samoa (Uthicke *et al.* 2010), New Caledonia (Conand 1981, 1988, 1989, 1993), Solomon Islands (Ramofafia *et al.* 2000) and other areas in Western Central Pacific region (Kinch *et al.* 2008), as well as in Red Sea (Ahmed *et al.* 2016), Seychelles (Oury *et al.* 2019), and other areas in Indian Ocean (Conand *et al.* 2022). Within the Philippines, *H. fuscogilva* has been documented in Laguindingan in Misamis Oriental, Lopez Jaena in Misamis Occidental, Hinatuan in Surigao Del del Sur, Tabina in Zamboanga Del del Sur, as well as various islands in Palawan, including the Tubbataha Reefs, Zamboanguita in Negros Oriental, and Apo Reef Natural Park in Sablayan, Occidental Mindoro (Jun 2001, 2002; Kerr *et al.* 2006; Dolorosa 2015; Jontila *et al.* 2018; Quimpo *et al.* 2018; *Ardines et al. 2020*; de Guzman and Quiñones 2021; Leopardas *et al.* 2021; Jontila 2023). The Mindanao State University at Naawan has successfully conducted spawning and restocking of hatchery-produced *H. fuscogilva*. Recently, there have been reports on the reproductive traits, captive breeding, hatchery techniques, and the first successful mass production of the *H. fuscogilva* in the Philippines (Leopardas *et al.* 2021; Arriesgado *et al.* 2022a).

Holothuria fuscogilva is classified as "vulnerable" by the International Union for Conservation of Nature (IUCN) due to its decreasing number of mature individuals in the Indo-Pacific regions as a result of its high demand in the *trepang* industry, making its conservation a matter of concern (Conand 1981; Conand *et al.* 2013; Arriesgado *et al.* 2022b). The fishing and trading of *H. fuscogilva* are controlled under the Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (di Simone *et al.* 2019, 2021) and as such, appropriate management measures are being sought to promote the recovery of heavily exploited stocks (Shedrawi *et al.* 2019). In the Philippines, research on the ecology of this species is notably scarce, with only a few studies addressing this aspect (de Guzman and Quiñones 2021; Paderanga *et al.* 2023). Furthermore, the utilization of molecular data for the conservation management of the species remains understudied (von der Heyden *et al.* 2014).

At present, there is insufficient baseline information on the population genetics of *H. fuscogilva,* hindering the implementation of sustainable mariculture initiatives and conservation units for effective management. To address this gap, we analyzed the genetic variability of the white teatfish using the mtDNA *COI* gene marker. By doing so, we seek to provide valuable insights for the management of restocking efforts, ensuring the preservation of its natural genetic diversity at both regional and local levels.

MATERIALS AND METHODS

Sample Collection

Prior to the collection of *Holothuria fuscogilva* samples from selected areas in Mindanao, southern Philippines, we secured a gratuitous permit (No. 0220-21) from the Department of Agriculture–Bureau of Fisheries and Aquatic Resources (DA-BFAR) in the Philippines. However, the collection of *H. fuscogilva* individuals in the country was restricted due to the prohibitions imposed by the DA-BFAR on capturing and trading of this sea cucumber species. The restriction is in place because *H. fuscogilva* holds a "vulnerable" IUCN and is listed in CITES Appendix II, in addition to being generally rare in the wild.

For molecular analysis, we obtained tissue samples from 15 *H. fuscogilva* individuals using a sterilized scalpel blade, collecting only 3–20 g of tissues from the body wall of each specimen. Subsequently, the individuals were carefully released back into their natural habitat following the tissue collection. The collected tissue samples were then preserved in 95% ethanol in 2-mL microtubes and stored at–20 °C until analysis.

DNA Extraction, PCR Amplification, and Sequencing

We extracted the total gDNA using the MG Tissue gDNA Extraction SV Mini Kit (Doctor Protein Inc., Korea), following the manufacturer's protocol. The *cytochrome c oxidase subunit 1* mitochondrial genes (*cox1* or *COI*) of the samples were amplified using the primers COIeF (5′-ATA ATG ATA GGA GGR TTT GG-3′) and COIeR (5′-GCT CGT GTR TCT ACR TCC AT-3′), amplifying 674 nucleotides of the echinoderm *COI* gene (Arndt *et al.* 1996). PCR conditions followed the protocol of Uthicke and Benzie (2003) using the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD). The PCR products were purified using the multiscreen filter plate (Millipore Corp., USA) and were sequenced by Macrogen, Inc. in Korea using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in ABI PRISM 3730XL Analyzer (96 capillary type).

Data Analysis

A total of 67 *COI* sequences were analyzed to assess the genetic diversity of the *Holothuria fuscogilva* population across the Pacific region. These sequences, which include those obtained from GenBank, originated from different geographical locations, including the Philippines (n = 16), Australia (n = 6), Japan (n = 5), and Vietnam (n = 40) (Table 1). Countries with < 5 *H. fuscogilva COI* sequences were excluded from the analysis.

We edited and visually optimized the *COI* bidirectional sequences of 15 *H. fuscogilva* Philippine samples using BioEdit Sequence Alignment Editor software v7.2.5 and subsequently generated consensus sequences for each specimen (Hall 1999). Then, we subjected the consensus

Table 1. List of *H. fuscogilva* mtDNA *COI* sequences used in the analysis.

Ecoregion	Location	Specific site	Group	GenBank accession	Reference
Pacific	Philippines	LMOR	1	OP921577–OP921581	This study
Pacific	Philippines	LMOR	1	MH834582	Alcudia-Catalma *et al*. (2020)
Pacific	Philippines	LJMOC	1	OP921582–OP921585	This study
Pacific	Philippines	HSDS	1	OP921586–OP921589	This study
Pacific	Philippines	BTWI	1	OP921590–OP921591	This study
Pacific	Australia	RI	2	AY700765–AY700767	Uthicke *et al.* (2004)
Pacific	Australia	WR	2	AY700763–AY700764	Uthicke *et al.* (2004)
Pacific	Australia	CI	2	EU848254	Uthicke *et al.* (2010)
Pacific	Japan	YI	3	LC593260, LC593267–LC593268	Tanita *et al.* (2021)
Pacific	Japan	O	3	LC593261, LC593266	Tanita *et al.* (2021)
Pacific	Vietnam	KH	4	MZ677066–MZ677085	Nguyen *et al.* (unpub.)
Pacific	Vietnam	KH	4	OM904542–OM904561	Nguyen *et al.* (unpub.)

Abbreviation: [LMOR] Laguindingan, Misamis Oriental; [LJMOC] Lopez Jaena, Misamis Occidental; [HSDS] Hinatuan, Surigao del Sur; [BTWI] Bongao, Tawi-Tawi; [RI] Rain Island; [CI] Cook Island; [YI] Yaeyama Islands; [O] Okinawa; [KH] Khanh Hoa. Note: groupings were used for the analysis of *H. fuscogilva* Pacific populations.

sequences to multiple sequence alignment using the MUSCLE algorithm in MEGA (Molecular Evolutionary Genetics Analysis (MEGA) v11 software (Tamura *et al.* 2021).

To evaluate the molecular indices of the samples and assess genetic diversity, we employed DnaSP v6 software (Rozas *et al.* 2017). The molecular indices, included sequence polymorphic sites (S), the number of haplotypes (Hn), haplotype diversity (Hd), average nucleotide differences (K), and nucleotide diversity (π). Additionally, we analyzed molecular variance (AMOVA) and fixation index (F_{st}) in Arlequin v3.5 software to investigate the genetic structure among species' populations (Excoffier and Lischer 2010). All parameters were assessed using 10,000 data permutations.

To ascertain the evolutionary relationships among haplotypes of *H. fuscogilva* populations, we generated a haplotype network and distribution using the minimum spanning network method (Bandelt *et al.* 1999) in PopART v1.7 software (http://popart.otago.ac.nz).

RESULTS

Among the *COI* sequences of *Holothuria fuscogilva* (n = 67), 33 haplotypes were detected. The haplotype diversity (Hd = 0.95658) was high, whereas the nucleotide diversity (π = 0.00704) was moderate (Table 1). All *H. fuscogilva* populations from different countries exhibited a quite high haplotype diversity (Hd = 0.9–1), suggesting a rich array of unique haplotypes within each population (Table 2).

AMOVA reveals that there was indeed a significant genetic differentiation among populations (5.8%), albeit the majority remains within populations (94.2%) (Table 3). This finding was corroborated by the significant F_{st} (p-value < 0.05) (Table 3). While notable genetic diversity and differentiation are observed among *H. fuscogilva* populations, the sharing of haplotypes is also evident across the majority of its Pacific populations (Figure 1).

DISCUSSION

This research explored the genetic variability of *H. fuscogilva* populations in the Philippines from its neighboring geographic regions, employing the mtDNA *COI* gene – a recognized tool for estimating genetic variation and delineating spatial genetic groups in sea cucumber populations, as well as in other species [*e.g.* Williams *et al.* (2002), Tanita *et al.* (2021), Panithanarak (2022), Sulardiono *et al.* (2022)]. Occupying a central position within the Coral Triangle, the Philippines, as an archipelago, is a hotspot for biodiversity (Sanciangco *et al.* 2013). This is due in part to its unique geography, which involves a number of islands forming a distinct entity with associated substructures (Kool *et al.* 2011). Also, the vast expanses of open ocean separating populations, coupled with limited islands acting as dispersal corridors, may contribute to greater genetic differentiation (Kimura and Weiss 1964; Sanciangco *et al.* 2013; Ravago-Gotanco and Kim 2019). In addition, the existence of favorable habitats coupled with the country's dominant currents facilitate species dispersion (Kool *et al.* 2011; Sanciangco *et al.* 2013).

The sharing of haplotypes across the majority of global populations (*e.g.* between the Philippines and Vietnam; Figure 1) suggests a potential historical gene flow among the populations. Despite the shared haplotypes, the network analysis highlights distinct characteristics within each population. Notably, the Japanese and Australian populations exhibit a more differentiated network

Table 2. Genetic (haplotype) diversity of *H. fuscogilva* from Mindanao, the Philippines, and in the other regions in the Pacific.

Pacific populations	n	S	Hn	Hd	K	π
Philippines	16	19	14	e	3.9	0.00853
Australia	6	9	6	1	3.8	0.00832
Japan	5	7	4	0.9	3.6	0.00788
Vietnam	40	18	14	0.92308	2.64103	0.00578
Total data estimates	67	38	33	0.95658	3.20443	0.00704

Table 3. Analysis of molecular variation (AMOVA). Statistical significance at $p < 0.05$.

Source of variation	df	Sum of squares	Variance components	Variation (%)
Among populations	3	8.296	0.09523 Va	5.8
Within populations	63	97.45	1.54683 Vb	94.2
Total	66	105.746	1.64206	
Fixation index (F_{st})	:	0.05800 (p-value = 0.01139 +– 0.00108)		

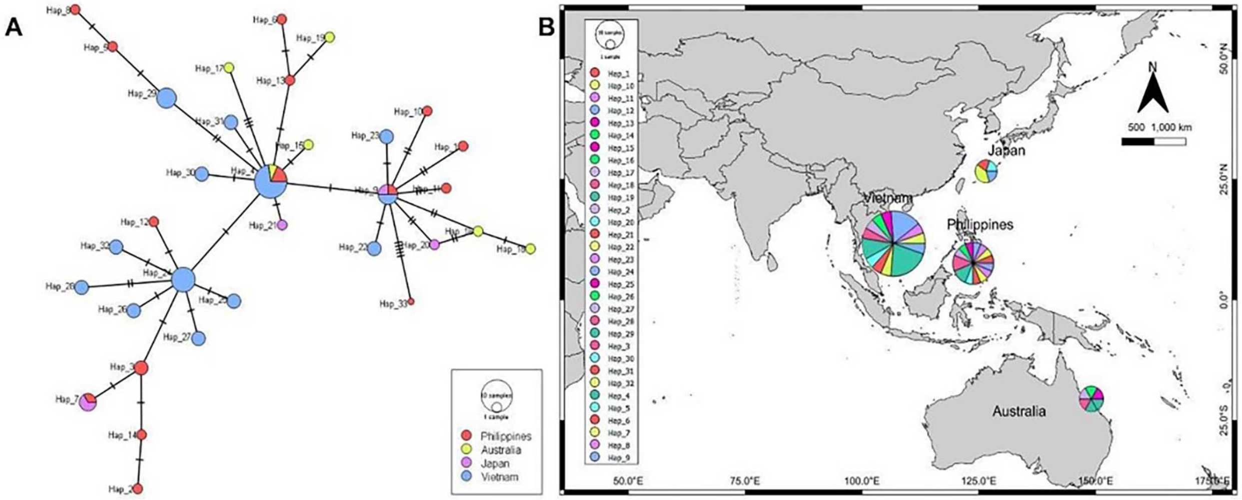

Figure 1. The minimum spanning network (A) and haplotype distribution (B) of *H. fuscogilva* Pacific populations. The circle size of each group is relative to the number of haplotype copies present in the dataset. A branch represents a single nucleotide change. The number of mutations for each branch is represented by hatch marks.

topology compared to other populations, lacking shared haplotypes that set them apart from other populations. It is essential to highlight that the absence of shared haplotypes may also be influenced by sample size, and a more comprehensive dataset could potentially reveal a more robust and nuanced understanding of the genetic relationships among populations.

The substantial genetic diversity observed in *H. fuscogilva* population, as highlighted by high haplotype diversity (Hd = 0.95658) and moderate nucleotide diversity (π = 0.00704), has critical implications for conservation management (Soliman *et al.* 2016; Hamamoto *et al.* 2021). This genetic richness not only indicates the population's ability to adapt to varying environmental conditions but also suggests a potential reservoir of unique traits that may be crucial for its long-term survival (Brown *et al.* 2024). The significant genetic differentiation among populations, as revealed by AMOVA and supported by the F_{st} value, underscores the need for tailored conservation strategies. Factors like genetic drift in small populations, barriers to gene flow, and water currents may contribute to the accumulation of genetic differences over time (Uthicke and Benzei 2003; Lohr 2003; Lermusiaux *et al.* 2011; Ravago-Gotanco and Kim 2019). Settlement preferences and early migration behaviors of sea cucumbers, particularly their affinity for specific substrates, may also be factors that can contribute to their high levels of genetic differentiation (Mercier *et al.* 2000).

Preserving the unique genetic makeup of populations is essential for ensuring the resilience of species amid environmental changes, habitat loss, and threats like overexploitation for the *trepang* industry (Lohr 2003; Lowell *et al.* 2023; Brown *et al.* 2024). Therefore, integrating these genetic insights into conservation management plans is vital to enhance the adaptive potential and long-term viability of *H. fuscogilva* populations, not only in the Philippines but also in other regions. For example, adherence to restocking protocol should strictly be followed to safeguard the genetic integrity of each local population. This study recommends conducting a diversity analysis of *H. fuscogilva* from different localities in the Philippines, given its archipelagic setting with diverse water currents known to influence the genetic structures of several species (Lourie *et al.* 2005; de Boer *et al.* 2014; Raynal *et al.* 2014; Ravago-Gotanco and Kim 2019).

While mitochondrial DNA provides an important preliminary glimpse into genetic structure, diversity, and connectivity, larger sets of genome-wide markers (*e.g.* microsatellites and SNPs) can offer a more comprehensive picture, overcoming the limitations of mtDNA to some extent and would provide a more accurate assessment of genetic pattern (Eckert *et al.* 2008; Nielsen *et al.* 2009). Hence, further investigations are needed to unravel the underlying mechanisms driving this genetic cline of *H. fuscogilva* and to gain insights into the connectivity and isolation of different populations. Such knowledge will provide a basis for the development of effective management and conservation strategies.

CONCLUSION

This research on *Holothuria fuscogilva*'s genetic variability in the Philippines and neighboring countries, using the mtDNA *COI* gene marker, reveals significant genetic diversity. This substantial genetic differentiation among

H. fuscogilva populations calls for tailored conservation strategies to preserve the unique local traits of the species. Protecting these distinct genetic structures is vital for local adaptations, resilience against environmental changes, and mitigating threats like habitat loss and overexploitation. Integrating these findings into conservation plans is key for adaptive potential and long-term viability, with future research needed to understand underlying mechanisms and enhance conservation strategies.

While the findings may provide an understanding of genetic differences, further research with larger sample sizes and covering more sites would ideally be preferred to gain a more accurate and comprehensive understanding of the genetic diversity within the *H. fuscogilva* species. This may include a broader set of gene markers, such as mitochondrial genome, genome-wide SNPs, and numerous microsatellite loci. By expanding the genetic toolkit, we can augment the study's resolution and reliability, facilitating a comprehensive approach to devising effective, sustainable management strategies for the species.

ACKNOWLEDGMENTS

This research was financially supported by the DOST (Department of Science and Technology) under the S4CP (Science for Change Program (S4CP) and was monitored by the DOST-PCAARRD (Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development) with (Project No. 3562). The authors express gratitude to the project collaborators from various state universities and colleges (SUCs), including Mindanao State University–Tawi-Tawi College of Technology and Oceanography, Mindanao State University–Lanao del Norte Agricultural College, University of Science and Technology of Southern Philippines–Panaon, and Camiguin Polytechnic State College, and the Local Government Units of Laguindingan in Misamis Oriental, Lopez Jaena in Misamis Occidental, Hinatuan in Surigao del Sur, and Bongao in Tawi-Tawi, for their invaluable support during the research process. Special thanks are extended to Mindanao State University at Naawan for providing office and laboratory support, as well as administrative assistance.

STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

AHMED MI, AAMER M, LAWRENCE AJ. 2016. Identification of the Holothurian species of the Red Sea and Gulf of Aqaba using DNA barcoding technique. Egyptian Journal of Aquatic Biology and Fisheries 20(4): 1–7.

ALCUDIA-CATALMA MN, DIAZ MGQ, GARCIA RN, OCAMPO PP, LAURENA AC, TECSON-MENDOZA EM. 2020. DNA barcoding and diversity analysis of 19 economically important Philippine sea cucumbers (Holothuroidea). Philippine Journal of Science 149(2): 335–346.

ARDINES R, MECHA NJM, DOLOROSA R. 2020. Commonly gleaned macro-benthic invertebrates in a small offshore island of Cawili, Cagayancillo, Palawan, the Philippines. The Palawan Scientist 12: 102–125.

ARNDT A, MARQUEZ C, LAMBERT P, SMITH MJ. 1996. Molecular phylogeny of eastern Pacific sea cucumbers (Echinodermata: Holothuroidea) based on mitochondrial DNA sequence. Molecular Phylogenetics and Evolution 6(3): 425–437.

ARRIESGADO DA, UBA KIN, TUBIO EG, NAVARRO VR, BUCAY DM, BESOÑA JF, MAGCANTA-MORTOS MLM, UY WH. 2022a. Spawning, larval development, and juvenile rearing of white teatfish *Holothuria fuscogilva* in the hatchery in the Philippines. Philippine Journal of Science 151(6B): 2555–2566.

ARRIESGADO EM, SORNITO MB, ZALSOS JD, BESOÑA JF, ALIA LC, CADELIÑA FA, MAGCANTA-MORTOS MLM, UY WH. 2022b. Diversity and abundance of sea cucumbers in selected areas of Mindanao, the Philippines. Philippine Journal of Science 151(3): 863–877.

BANDELT HJ, FORSTER P, RÖHL A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1): 37–48.

BORRERO-PÉREZ GH, GONZÁLEZ-WANGÜEMERT M, MARCOS C, PÉREZ-RUZAFA A. 2011. Phylogeography of the Atlanto-Mediterranean sea cucumber *Holothuria (Holothuria) mammata*: the combined effects of historical processes and current oceanographical pattern. Molecular Ecology 20(9): 1964–1975.

BROWN KT, SOUTHGATE PC, LOGANIMOCE EM, KAURE T, STOCKWELL B, LAL MM. 2024. Sandfish generations: loss of genetic diversity due to hatchery practices in the sea cucumber *Holothuria (Metriatyla) scabra*. Aquaculture 578: 740048.

CHANG Y, FENG Z, YU J, DING J. 2009. Genetic variability analysis in five populations of the sea cucumber *Stichopus (Apostichopus) japonicus* from China, Russia, South Korea, and Japan as revealed by microsatellite markers. Marine Ecology 30(4): 455–461.

CONAND C, CLAEREBOUDT M, DISSAYANAKE C, EBRAHIM A, FERNANDO S, GODVINDEN R, LAVITRA T, LEOPOLD M, MMBAGA TK, MULOCHAU T, NAAEM S, SHEA S, VAITILINGON D, YAHYA S, FRIEDMAN K. 2022. Review of fisheries and management of sea cucumbers in the Indian Ocean. Western Indian Ocean Journal of Marine Science 21(1): 125–148.

CONAND C, PURCELL S, GAMBOA R. 2013. *Holothuria fuscogilva*. The IUCN Red List of Threatened Species: e.T200715A2681354. Accessed on 13 Jun 2022.

CONAND C. 1981. Sexual cycle of three commercially important holothurian species (Echinodermata) from the lagoon of New Caledonia. Bulletin of Marine Science 31(3): 523–543.

CONAND C. 1988. Beche-de-mer in New Caledonia: biology and fishing. Paper prepared for the South Pacific Commission's Workshop on Pacific Inshore Fishery Resources.

CONAND C. 1989. Les holothuries aspidochirotes du lagon de Nouvelle-Caledonie: biologie, ecologie, et exploitation [Etudes et Thesses]. ORSTROM, Paris, France. (in French – the 'ecology' and 'biology' sections translated into English by the SPC).

CONAND C. 1993. Reproductive biology of the holothurians from the major communities of the New Caledonian lagoon. Marine Biology 116: 439–450.

DE BOER TS, NAGUIT MRA, ERDMANN MV, ABLAN-LAGMAN MCA, CARPENTER KE, TOHA AHA, BARBER PH. 2014. Concordance between phylogeographic and biogeographic boundaries in the Coral Triangle: conservation implications based on comparative analyses of multiple giant clam species. Bulletin of Marine Science 90(1): 277–300.

DE GUZMAN AB, QUIÑONES MB. 2021. Sea cucumbers (Holothuroidea) of northeastern and western Mindanao, the Philippines: the potential role of marine protected areas in maintaining diversity and abundance. Journal of Environment and Aquatic Resources 6: 47–70.

DI SIMONE M, HORELLOU A, CONAND C. 2019. Towards a CITES listing of teatfish. SPC Bêche-de-mer Information Bulletin 39: 76–78.

DI SIMONE M, HORELLOU A, CONAND C. 2021. The listing of three sea cucumber species in CITES Appendix II enters into force. SPC Beche-de-mer Information Bulletin 41: 3–4.

DOLOROSA RG. 2015. The sea cucumbers (Echinodermata: Holothuroidea) of Tubbataha Reefs Natural Park, the Philippines. SPC Beche-demer Information Bulletin 35: 10–18.

ECKERT CG, SAMIS KE, LOUGHEED SC. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17(5): 1170–1188.

EXCOFFIER L, LISCHER HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3): 564–567.

HALL TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic Acids Symposium Series 41(41): 95–98.

HAMAMOTO K, SOLIMAN T, POLISENO A, FERNANDEZ-SILVA I, REIMER JD. 2021. Higher genetic diversity of the common sea cucumber *Holothuria* (*Halodeima*) *atra* in marine protected areas of the central and southern Ryukyu Islands. Frontiers in Conservation Science, Vol. 53.

JONTILA JBS, MONTECLARO HM, QUINITIO GF, SANTANDER-DE LEON SM, ALTAMIRANO JP. 2018. Status of sea cucumber fishery and populations across sites with different levels of management in Palawan, the Philippines. Ocean and Coastal Management 165: 225–234.

JONTILA JBS. 2023. The sea cucumbers of Palawan, Philippines: a field guide. Dolorosa G ed. Western Philippines University. 92p.

JUINIO-MEÑEZ MA. 2015. Biophysical and genetic connectivity considerations in marine biodiversity conservation and management in the South China Sea. Journal of International Wildlife Law and Policy 18(2): 110–119.

JUN A. 2001. Holothurian exploitation in the Philippines: continuities and discontinuities. Tropics 10(4): 591–607.

JUN A. 2002. Trepang exploitation in the Philippines: updated information. SPC Bêche-de-mer Information Bulletin 17: 17–21.

KERR AM, NETCHY K, GAWEL AM. 2006. Survey of the shallow-water sea cucumbers of the central Philippines. A report to the Municipalities of Negros Oriental, Cebu, and Bohol, local *Bantay Dagat* groups, Coastal Conservation and Education Foundation, Inc., and Silliman University–Angelo King Center for Research and Environmental Management. University of Guam Marine Laboratory.

KIMURA M, WEISS GH. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49: 561–576.

KINCH J, PURCELL S, UTHICKE S, FRIEDMAN K. 2008. Population status, fisheries, and trade of sea cucumbers in the Western Central Pacific. In: Sea cucumbers: a global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper No. 516. p. 7–55.

KOOL JT, PARIS CB, BARBER PH, COWEN RK. 2011. Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. Global Ecology and Biogeography 20(5): 695–706.

LEOPARDAS VE, QUIÑONES MB, CALALA LR, MANULAT SL, DELA ROSA HKT, NOB CJR, EMPRON JL, NATINGGA KG. 2021. Notes on the reproductive traits of *Holothuria fuscogilva* Cherbonnier, 1980 from Laguindingan, Misamis Oriental, the Philippines. Journal of Environment and Aquatic Resources 6: 1–15.

LERMUSIAUX PF, HALEY JR PJ, LESLIE WG, AGARWAL A, LOGUTOV OG, BURTON LJ. 2011. Multiscale physical and biological dynamics in the Philippine archipelago: predictions and processes. Oceanography 24(1): 70–89.

LOHR HR. 2003. Genetic variation among geographically isolated populations of the commercially important sea cucumber *Isostichopus fuscus* in the Eastern Pacific [Doctoral Dissertation]. San Francisco State University, San Francisco, CA, USA.

LOURIE SA, GREEN DM, VINCENT ACJ. 2005. Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Molecular Ecology 14(4): 1073–1094.

LOWELL N, SUHRBIER A, TARPEY C, MAY S, CARSON H, HAUSER L. 2023. Population structure and adaptive differentiation in the sea cucumber *Apostichopus californicus* and implications for spatial resource management. PLOS One 18(3): e0280500.

MERCIER A, BATTAGLENE SC, HAMEL JF. 2000. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. Journal of Experimental Marine Biology and Ecology 249(1): 89–110.

NIELSEN EE, HEMMER-HANSEN J, POULSEN NA, LOESCHCKE V, MOEN T, JOHANSEN T, MITTLEHOZER C, TARANGER GL, OGDEN R, CARVALHO GR. 2009. Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). BMC Evolutionary Biology 9(1): 1–11.

OURY N, LÉOPOLD M, MAGALON H. 2019. Isolation and characterization of microsatellite loci from three widespread tropical sea cucumbers of the genus *Holothuria* (Echinodermata, Holothuroidea), and cross-amplification among them. Molecular Biology Reports 46(3): 3501–3510.

PADERANGA ORT, LEOPARDAS VE, NOB CJR, ANTINERO AT, NATINGGA KG, EVASCO AC JR., MANULAT-MOSCOSO SL, EMPRON JLG, CALALA LR, QUIÑONES MB, DELA ROSA HKT, ASPE NM, UY WH. 2023. Notes on the distribution and abundance of white teatfish – *Holothuria fuscogilva* Cherbonnier, 1980 – at White Island, the Philippines. SPC Beche-de-mer Information Bulletin 43: 11–16.

PANITHANARAK T. 2022. Genetic diversity and population differentiation of Ball Sea Cucumber *Phyllophorella kohkutiensis* in Thai Waters derived from *COI* and *16S* rDNA. Journal of Fisheries and Environment 46(1): 66–79.

PARK J, XI H, PARK C. 2019. The complete mitochondrial genomes from three body color variants of sea cucumbers, *Apostichopus japonicus* (Selenka, 1867). Mitochondrial DNA Part B 4(1): 836–837.

PURCELL SW, LOVATELLI A, GONZÁLEZ-WANGÜEMERT M, SOLÍS-MARÍN FA, SAMYN Y, CONAND C. 2023. Commercially important sea cucumbers of the world – second edition. FAO Species Catalogue for Fishery Purposes 6(1).

PURCELL SW. 2012. Principles and science of stocking marine areas with sea cucumbers. Asia-Pacific sea cucumber aquaculture. Canberra: Australian Center for International Agricultural Research. p. 92–103.

QUIMPO TJR, CABAITAN PC, OLAVIDES RDD, DUMALAGAN JR EE, MUNAR J, SIRINGAN FP. 2018. Preliminary observations of macrobenthic invertebrates and megafauna communities in the upper mesophotic coral ecosystems in Apo Reef Natural Park, the Philippines. Raffles Bulletin of Zoology 66: 1–11.

RAMOFAFIA C, BATTAGLENE S, BELL J, BYRNE M. 2000. Reproductive Biology of *Holothuria fuscogilva* (Echinodermata: Holothuroidea) in the Solomon Islands. Marine Biology 136: 1045–1056.

RAVAGO-GOTANCO R, KIM KM. 2019. Regional genetic structure of sandfish *Holothuria (Metriatyla) scabra* populations across the Philippine archipelago. Fisheries Research 209: 143–155.

RAYNAL JM, CRANDALL ED, BARBER PH, MAHARDIKA GN, LAGMAN MC, CARPENTER KE.

2014. Basin isolation and oceanographic features influencing lineage divergence in the humbug damselfish (*Dascyllus aruanus*) in the Coral Triangle. Bulletin of Marine Science 90(1): 513–532.

ROZAS J, FERRER-MATA A, SÁNCHEZ-DELBARRIO JC, GUIRAO-RICO S, LIBRADO P, RAMOS-ONSINS SE, SÁNCHEZ-GRACIA A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12): 3299–3302.

SANCIANGCO JC, CARPENTER KE, ETNOYER PJ, MORETZSOHN F. 2013. Habitat availability and heterogeneity and the Indo-Pacific warm pool as predictors of marine species richness in the tropical Indo-Pacific. PLOS One 8(2): e56245.

SHEDRAWI G, KINCH JP, HALFORD AR, BERTRAM I, MOLAI C, FRIEDMAN KJ. 2019. CITES listing of sea cucumber species provides opportunities to improve management of the beche-de-mer trade. SPC Fisheries Newsletter 159: 6–8.

SLATKIN M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics 16(1): 393–430.

SLATKIN M. 1987. Gene flow and the geographic structure of natural populations. Science 236(4803): 787–792.

SOLIMAN T, FERNANDEZ-SILVA I, REIMER JD. 2016. Genetic population structure and low genetic diversity in the over-exploited sea cucumber *Holothuria edulis* Lesson, 1830 (Echinodermata: Holothuroidea) in Okinawa Island. Conservation Genetics 17: 811–821.

SULARDIONO B, HARTOKO A, AINI AN, WULANDARI D, BUDIHARJO A. 2022. Genetic diversity of commercial sea cucumbers *Stichopus* (Echinoderm: Stichopodidae) based on DNA Barcoding in Karimunjawa, Indonesia. Biodiversitas Journal of Biological Diversity 23(2).

TAMURA K, STECHER G, KUMAR S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis, version 11. Molecular Biology and Evolution 38(7): 3022–3027.

TANITA I, NISHIHAMA S, HAYASHIBARA T. 2021. Identification of species of teatfish (Holothuroidea: Holothuriida) in Japan based on mitochondrial *cytochrome oxidase subunit I* (*COI*) sequences, morphology, and ossicles. Plankton and Benthos Research 16(3): 200–209.

UTHICKE S, BENZIE JAH. 2003. Gene flow and population history in high dispersal marine invertebrates: Mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. Molecular Ecology 12(10): 2635–2648.

UTHICKE S, BYRNE M, CONAND C. 2010. Genetic barcoding of commercial bêche-de-mer species (Echinodermata: Holothuroidea). Molecular Ecology Resources 10(4): 634–646.

UTHICKE S, O’HARA TD, BYRNE M. 2004. Species composition and molecular phylogeny of the Indo-Pacific teatfish (Echinodermata: Holothuroidea) bêche-de-mer fishery. Marine and Freshwater Research 55(8): 837–848.

VON DER HEYDEN S, BEGER M, TOONEN RJ, VAN HERWERDEN L, JUINIO-MEÑEZ MA, RAVAGO-GOTANCO R, FAUVELOT C, BERNARDI G. 2014. The application of genetics to marine management and conservation: examples from the Indo-Pacific. Bulletin of Marine Science 90(1): 123–158.

WEN J, HU C, ZHANG L, FAN S. 2011. Genetic identification of global commercial sea cucumber species on the basis of mitochondrial DNA sequences. Food Control 22(1): 72–77.

WILLIAMS ST, JARA J, GOMEZ E, KNOWLTON N. 2002. The marine Indo-West Pacific break: contrasting the resolving power of mitochondrial and nuclear genes. Integrative and Comparative Biology 42(5): 941–952.

WOLFE K, BYRNE M. 2022. Overview of the Great Barrier Reef sea cucumber fishery with focus on vulnerable and endangered species. Biological Conservation 266: 109451.