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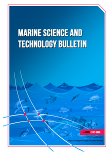

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RESEARCH ARTICLE

A novel frontier in the geographic distribution of the Japanese sea cucumber *Apostichopus japonicus* (Selenka, 1867) (Stichopodidae: Holothuroidea) in the world

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ABSTRACT

The natural distribution of the Japanese sea cucumber (*Apostichopus japonicus* Selenka, 1867), a sea cucumber species, is characterized by the Northwest Pacific. Long-term monitoring studies from 2020 to 2024 document a significant deviation from the known natural distribution of *A. japonicus* and its ability to extensively colonize the Gulf of Izmit, the easternmost part of the Sea of Marmara. This record is also the first documentation of the species from the Mediterranean Sea. Population data extracted from 67 samples allowed the determination of size and weight distribution, length-weight relationship, and condition factor. The study delves into the implications for fisheries management by evaluating the species' dispersion beyond its natural range and its adaptation, shedding light on potential threats from illegal hookah diving fisheries. While its presence offers potential economic benefits through fishing income, its emergence as an invasive species can pose critical ecological risks to the receiving ecosystem.

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Introduction

Sea cucumbers, scientifically known as holothuroids, constitute a diverse group of marine invertebrates, with approximately 1200 species distributed across the world's seas. They inhabit a variety of ecosystems, including coral reefs, shallow tropical coasts, and globally diverse marine environments (Aydın, 2016). Beyond their ecological importance, these organisms serve as integral components of aquatic ecosystems and stand as vital economic assets for fisheries in numerous coastal regions worldwide (Purcell et al., 2023). Sea cucumbers are deposit feeders, engaging in the consumption of diatoms and bacteria mixed with seafloor debris, thereby contributing significantly to the regulation of particulate organic matter covering benthic vegetation on hard reef surfaces (İşgören-Emiroğlu & Günay, 2007; Purcell et al., 2016). Notably rich in nutritional content (Çakli et al., 2004), sea cucumbers have been a preferred food source globally for centuries, particularly in Far East countries (Aydın et al., 2023). These creatures play a crucial role in aquatic ecosystems, serving as effective sources of income for fisheries worldwide (Dereli & Aydın, 2021).

Japanese sea cucumber (*Apostichopus japonicus* Selenka, 1867) is an echinoderm species belonging to the family Stichopodidae. Its distribution extends to the temperate waters of the Northwest Pacific, including Japan, China, the Korean Peninsula, and Far Eastern Russia, creating vital communities within shallow seawater habitats (Park et al., 2013; Purcell et al., 2023). Typically, this species inhabits from the intertidal zone to depths of 40 m. Substrate, salinity, water temperature, and habitat are factors that affect the distribution of the species (Lysenko et al., 2018; Purcell et al., 2023). The habitat of *A. japonicus* encompasses diverse coastal environments, where juveniles frequently inhabit areas characterized by the presence of algal and seagrass beds, along with oyster colonies (Minami et al., 2018). Within these habitats, *A. japonicus* plays a vital ecological role as a detritivore, consuming a wide array of organic matter, including diatoms, protozoa, bacteria, as well as animal and plant detritus. Furthermore, it efficiently re-utilizes residual food and fecal matter, contributing significantly to nutrient cycling processes within its ecosystem (Hamel & Mercier, 2013; Lysenko et al., 2015). The most economically important species, *A. japonicus*, is sold dried for $3000 per kilogram, at least twice as expensive as products from other holothurians (Purcell et al., 2018). This economic significance has led to substantial exploitation, resulting in declines in population abundance across its range. So, this species is listed as Endangered in the IUCN Red List of Threatened Species due to the decreasing population trend (Hamel & Mercier, 2013).

The sea cucumber assemblage comprises 37 species distributed among nine families in the Mediterranean Sea, nine of which are documented from Turkish seas (Aydın, 2016). This study presents the first documented occurrence of *A. japonicus* in the Sea of Marmara (SoM), in the easternmost part of the Mediterranean Sea, a significant deviation from its known natural geographical distribution. The research aims to understand the factors contributing to the species' presence and expansion in this distant region in the Mediterranean Sea by synthesizing existing literature. This study not only expands our understanding of the species' geographic range but also offers a crucial paradigm for comprehending marine ecosystem dynamics, influencing future research on species adaptability and conservation strategies in the broader context of the Mediterranean Sea.

Material and Method

The study area covers the coasts of the Gulf of Izmit located southeast of the SoM, which includes the Mediterranean General Fisheries Commission (GFCM) Geographical Sub-Area 28. The inception of the study transpired within the context of a biodiversity survey conducted on January 22, 2020, wherein researchers fortuitously encountered three *A. japonicus* samples in a specific geographical area. Extensive interviews were conducted with local fishing cooperatives and divers in the study area to confirm the recent presence of the species. This qualitative inquiry, coupled with insights from the fishing community, corroborated the existence of the sea cucumber species. As of this discovery in 2020, a longitudinal monitoring study was initiated to understand the dynamics of this newfound presence comprehensively. Several samples were also recorded during SCUBA surveys of another sea cucumber project carried out under the responsibility of the authors (TÜBİTAK project numbered 122Y004, covering the years 2022-2023). The monitoring studies incorporated a participatory approach involving citizen science. A total of 42 citizen reports covering the period 2021-2023 were diligently compiled through active engagement with individuals within the community. These reports served as vital inputs for expanding the understanding of the existence and distribution limits of the species. In conjunction with citizen participation, SCUBA diving was strategically carried out at regular intervals. These underwater surveys aimed to document and validate the extent of the *A. japonicus* systematically.

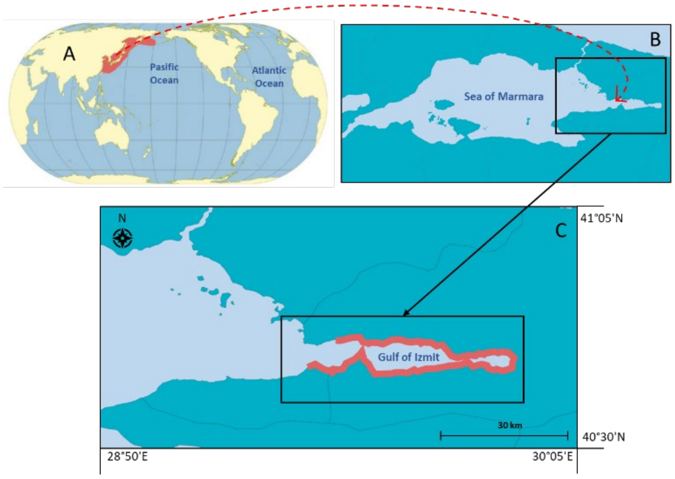

Figure 1. Map of the natural distribution area of *Apostichopus japonicus* (A - Drawn by Purcell et al., 2023) and its new distribution areas in the Gulf of Izmit (C) in the Sea of Marmara (B). The red solid line in C represents the distribution area for the first record in the Sea of Marmara.

Samples were identified according to the holothurian taxonomic identification key (Purcell et al., 2023), and their scientific name was checked from SeaLifeBase (Palomares & Pauly, 2023). The morphological characteristics of the identified specimens were systematically examined. The sea cucumber showcases three distinct ventral color variations: red, green, and black. These color types are accompanied by differing morphological characteristics (Choe, 1963). Dorsally, one may observe small dots ranging from brownish to greyish, while these dots tend to be more abundant on the ventral side. Its body shape is square in cross-section, tapering slightly towards both ends. Notably, large conical papillae are arranged in loose rows along the dorsal surface and in two rows at the lateral margins of the ventral surface. Ventral podia are arranged in three irregular longitudinal rows. The mouth is located ventrally and is surrounded by 20 tentacles. Lastly, the anus is situated terminally and lacks teeth (Purcell et al., 2023). This comprehensive morphological examination ensures accurate species identification and contributes to the broader understanding of the species' visual attributes (Figure 2).

For molecular and population studies, 67 samples were collected from depths of 3-20 m in long-term monitoring studies that lasted from the beginning of 2020 to the end of 2023. Collected samples were transported to the Ordu University Faculty of Marine Sciences Fisheries Laboratory in specialized tanks filled with seawater, providing optimal conditions for further examination. Given the dynamic nature of holothurians, where size and weight may vary due to morphometric measurements, particular attention was given to the potential impact of their ability to extract and expel internal organs or relax (González-Wangüemert et al., 2014). The samples were temporarily stored in plastic tanks filled with seawater during the transport. Subsequently, a meticulous procedure was employed for the extraction of internal organs. A longitudinal incision, approximately 3 cm in length, was made along the lateral surface of each specimen, allowing for the removal of the respiratory tree, gonads, and digestive tract (Aydın, 2019). A prompt measurement of the length from the mouth to the anal orifice of the sample was performed to mitigate potential size contraction post-gutting. Gutted length (GL in cm) measurement, crucial for accurate morphometric analysis, was executed using an ichthyometer with a precision of 0.1 cm. Simultaneously, the body weight (GW in g) was measured using a digital balance with a precision of 0.01 g.

The relationship between size and weight (LWR) was estimated employing Pauly's power equation: $W = aTL^b$, where W represents total gutted weight (GW in g), TL is the gutted length (GL in cm), and a and b denote regression parameters, representing the intercept (initial growth coefficient) and slope (growth coefficient), respectively. This equation was further utilized in its logarithmic form: $\ln(GW) = \ln(a) + b \cdot \ln(GL)$ (Ricker, 1975). Post-examination of curvilinear plots, outliers in GL and GW were excluded from the logarithmic analysis, as recommended by Froese (2006). Subsequently, 95% confidence limits (*CI*) for parameters a and b were calculated, and the coefficient of determination (r^2) was employed to assess the correlation between GW and GL. Pauly's t-test (Pauly, 1984) was employed to ascertain if the b-value coefficient significantly deviated from 3, determining whether species growth is isometric or allometric (Sokal & Rohlf, 1969). Additionally, Fulton's coefficient of condition factor (K) was calculated using the formula $K = 100[GW/(GL^3)]$, where GL is the gutted length, and GW is the gutted weight of the specimen (Froese, 2006). All statistical analyses were conducted using SPSS Ver. 26 software.

For genetic analysis, tissue samples were taken from the body wall of two specimens. DNA was extracted by using Genomic DNA Isolation Kit (AMBRD) according to the user's manual. Mitochondrial Cytochrome c oxidase subunit I (COI) sequences were partially amplified from the isolated DNA by PCR. PCR mixture included 10 ng of genomic DNA, 5 µl of 5X PCR Master Mix (AMBRD), 0.1 µM of each primer and final volume was 25 µl. PCR steps were as follows: initial denaturation at 95°C for 2 min; 30 cycles of denaturation at 95°C for 30 s; annealing at 50°C for 30 s; extension at 72°C for

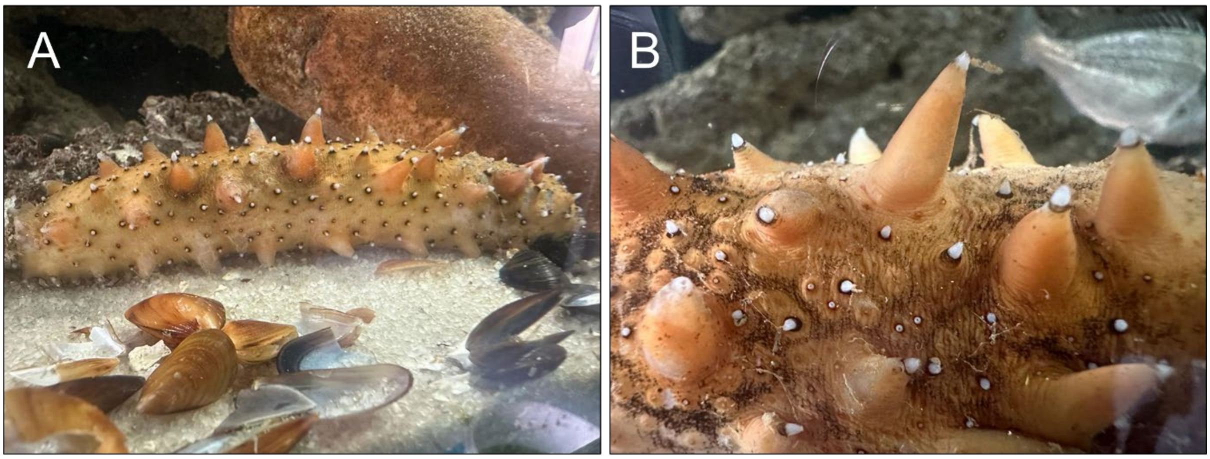

Figure 2. External appearance (A) and morphologically characterized papillae (B) of the *Apostichopus japonicus* (Photo: Dr. Aydın)

1 min. The PCR was completed with a final extension step at 72°C for 5 min. COIeF and COIeR primers were used to amplify 692 bp part of the COI gene (Arndt et al., 1996). Products were sequenced both ways (Macrogen Inc., Seoul, South Korea).

Chromatograms were manually checked and primer sequences were removed. The sequences were deposited in the NCBI GenBank (PP334764 and PP334765). Phylogenetic analysis was conducted on MEGA X platform (Kumar et al., 2018). With BLAST search highly similar sequences and related *Holothuroidea* spp. sequences obtained from NCBI GenBank were included for phylogenetic analysis. Sequences were aligned automatically and trimmed to minimize missing characters. The final data matrix consisted of 600 bases. The best-fit model of evolution was determined using the Akaike information criterion (Akaike, 1974) implemented in MEGA X. "GTR+G+I" was determined to be the best-fit model. Maximum Likelihood (ML) analysis was tested with 1000 bootstrap replicates. *Protelpidia murrayi* was used as outgroup.

Results and Discussion

The discovery of *A. japonicus* in the SoM is the first record for the Mediterranean Sea and marks a significant geographical range expansion worldwide. Previous records confined the presence of this species to specific areas along the coastal areas of northeast Asia (Choe, 1963; Takahashi, 2003; Hamel & Mercier, 2013; Purcell et al., 2023), making the current finding a noteworthy extension of its known distribution (Figure 1). The identification of *A. japonicus* in the SoM not only contributes valuable data to the understanding of its ecological preferences but also raises intriguing questions about the factors influencing its dispersion. The hypothesis implicating ballast water of ships as a key factor for the transportation of *A. japonicus* is indeed compelling. The SoM is a crucial maritime transportation route with international circulation. According to official records (Kocaeli Governorship, Port Area in the Gulf of Izmit Report), Kocaeli Port ranked first with a share of 19% in the regional distribution of the number of ships calling at Turkish ports in 2021. The vast majority (6,669 ships with 69.8%) of the ships entering the Gulf of Izmit were foreign-flagged ships. The prevalence of foreign-flagged ships entering the Gulf of Izmit underscores the potential role of maritime transport in facilitating the translocation of *A. japonicus* to SoM. Human activities such as alternative fishing sources, aquaculture, or deliberate releases for biocontrol purposes may contribute to the spread of the species beyond its natural range. Additionally, the impact of climate change on the distribution patterns of marine species must be considered. Environmental changes, including global warming, can alter the distribution patterns of sea cucumbers (Uthicke et al., 2009; Scannella et al., 2022). If *A. japonicus* is adaptable and able to thrive in a broader range of conditions, it could naturally expand its distribution. The SoM may have environmental conditions such as temperature, salinity, and substrate type that are suitable for *A. japonicus*.

The first *A. japonicus* samples were recorded in the Gulf of Izmit on January 22, 2020. Throughout the monitoring studies, samples were found to be irregularly limited to a few individuals until the end of 2022. By 2023, significant densities have been recorded commonly distributed along approximately 40 km of coastline, indicating successful colonization. According to the monitoring studies covering the period 2020-2024, the species is distributed at depths of 3-20 meters in the region but is densely distributed at depths of 10-11 meters. The average abundance is approximately 80-100 individuals per 100 m^2. By the second half of 2023, it was recorded that an average of 300-400 individuals were recorded during a one-hour dive in different regions of the Gulf of Izmit. Such a high abundance indicates the successful settlement and reproduction of the *A. japonicus* in the Gulf of Izmit. The adaptability and phenotypic plasticity exhibited by certain species can be instrumental in their successful colonization of new environments (Yuan et al., 2018). *A. japonicus* may have evolved mechanisms to tolerate a diverse range of environmental conditions within the SoM. Considering the adaptation and colonization of *A. japonicus* in the SoM over time shows that various environmental parameters, including the temperature range suitable for gametogenesis, larval development, and settlement, are compatible with the reproductive requirements of the species. The plasticity in physiological and behavioral traits could be advantageous, allowing the species to acclimate to varying substrate types, water temperatures, and salinities (Morgan, 2008; Sun et al., 2018). The establishment of symbiotic relationships with native organisms or the exploitation of vacant ecological niches represents another avenue for successful colonization (Azevedo e Silva et al., 2023). *A. japonicus* may have formed ecological partnerships or adapted its feeding strategies to exploit available resources efficiently. The absence of natural predators in the SoM may emerge as a pivotal factor contributing to the successful colonization of *A. japonicus*. This absence may confer a significant ecological advantage, allowing the sea cucumber to proliferate without the constraints of predation pressure. Due to the colonization of the SoM, *A. japonicus* may compete with native holothurian species and consequently constitute a potential danger for them. Over time, populations of *A. japonicus* in the SoM may have undergone evolutionary changes, potentially enhancing their adaptability to the local environment.

A total of 67 specimens of *A. japonicus* were collected in the study area. GL and GW of the population exhibited a broad range, spanning from 7.5 to 21.0 cm and 12.51 to 122.74 g, respectively. The histogram distribution curve revealed a right-skewed trend (Figure 3), indicating a predominance of juvenile individuals within the population. Considering that the length of first sexual maturity begins at approximately 20 cm size (Palomares & Pauly, 2023) or 110 g body weight (Purcell et al., 2023), it is noteworthy that only a small part of the population (4.5% in both size and body weight, n=3) has reproductive potential. The existence of adult individuals capable of reproduction creates the potential for the sustainability of the population over time. Comparisons with previous studies from the natural distribution areas reveal recorded diverse sizes, with the largest reported size of 33 cm documented by Minami et al. (2018) in Maizuru Bay in the Sea of Japan. The observed size distribution in the SoM may be attributed to various regional factors, with significant impacts documented in the literature. Notably, the size distribution of *A. japonicus* appears to be predominantly influenced by its adaptive capacity to cope with physical environmental stressors, as opposed to being driven by the biotic and chemical properties of the water and substrate (Chen et al., 2022; Huo et al., 2024). Diverse factors significantly shape the size distribution of holothurians, and this pattern is particularly evident in populations facing challenges such as illegal fishing (Purcell et al., 2010). Interviews with fishermen's associations in the Gulf of Izmit revealed that *A. japonicus* was frequently collected illegally by hookah or SCUBA divers. *A. japonicus* juveniles remain in the leaves of rocky reefs and later migrate to deeper, muddy silt areas as adults (Arakawa, 1990), making them more vulnerable to illegal fishing. Illegal fishing activities that result in the loss of large individuals reflect the trend in size distribution and have been identified as a vital risk factor to the sustainability of populations.

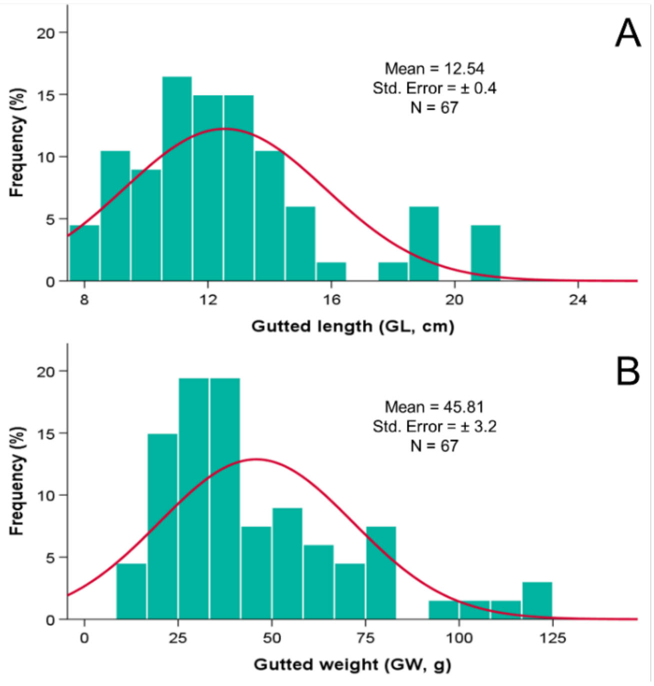

Figure 3. Histogram chart of gutted length (A) and gutted weight (B) of the *Apostichopus japonicus* in the Sea of Marmara. Red lines represent the normal curve of the population

Table 1 provides detailed information on the population structure and statistical data relevant to evaluating the LWR of *A. japonicus*. The calculated LWR equation was determined as $GW = 0.2669GL^{2.0033}$. The estimated b value was significantly lower than 3 (t–test, $t_{67} = 10.531$, $p < 0.05$), indicating a negative allometric growth pattern within the population. This finding aligns with limited previous studies (Dong & Zhou, 1984; Zhan et al., 2019) and suggests that as the specimens grow, their weight does not increase proportionally, reflecting potential ecological and environmental influences on growth. Various factors contribute to the observed negative allometric growth, including changes in environmental parameters, nutritional conditions, sex, reproductive status, seasonal variations, and the physiological state of the holothurians. Food competition, overfishing, and the trophic potential of marine ecosystems may be identified as potential drivers for this growth pattern (Jennings et al., 2001; Ahmed et al., 2018; Aydın, 2020). Additionally, recent algal blooms in the SoM (Karadurmuş & Sarı, 2022) and intense domestic and industrial pollution in the Gulf of Izmit (Ediger et al., 2016) may indicate poor water conditions, which could directly or indirectly impact the growth of the specimens. The strong correlation between GL and GW ($r^2 \geq 0.85$) suggested a consistent relationship between body size and weight, indicating that size may be a reliable indicator of the weight of *A. japonicus* (Froese, 2006).

Table 1. Estimated length-weight relationship parameters of *Apostichopus japonicus* from the Sea of Marmara (95% *Cl*: confidence intervals)

Population structure	
Sample size (*n*)	67
Length range (GL, cm)	7.5 – 21.0
Weight range (GW, g)	12.51 – 122.74
Regression parameters	
Intercept (*a*-value)	45.979
95% *Cl* of *a*	55.643 – 36.314
Slope (*b*-value)	7.318
95% *Cl* of *b*	6.572 – 8.064
***b* test**	
Coefficient (r^2)	0.85
Pauly's *t*-test	10.531
Growth	Negative allometry

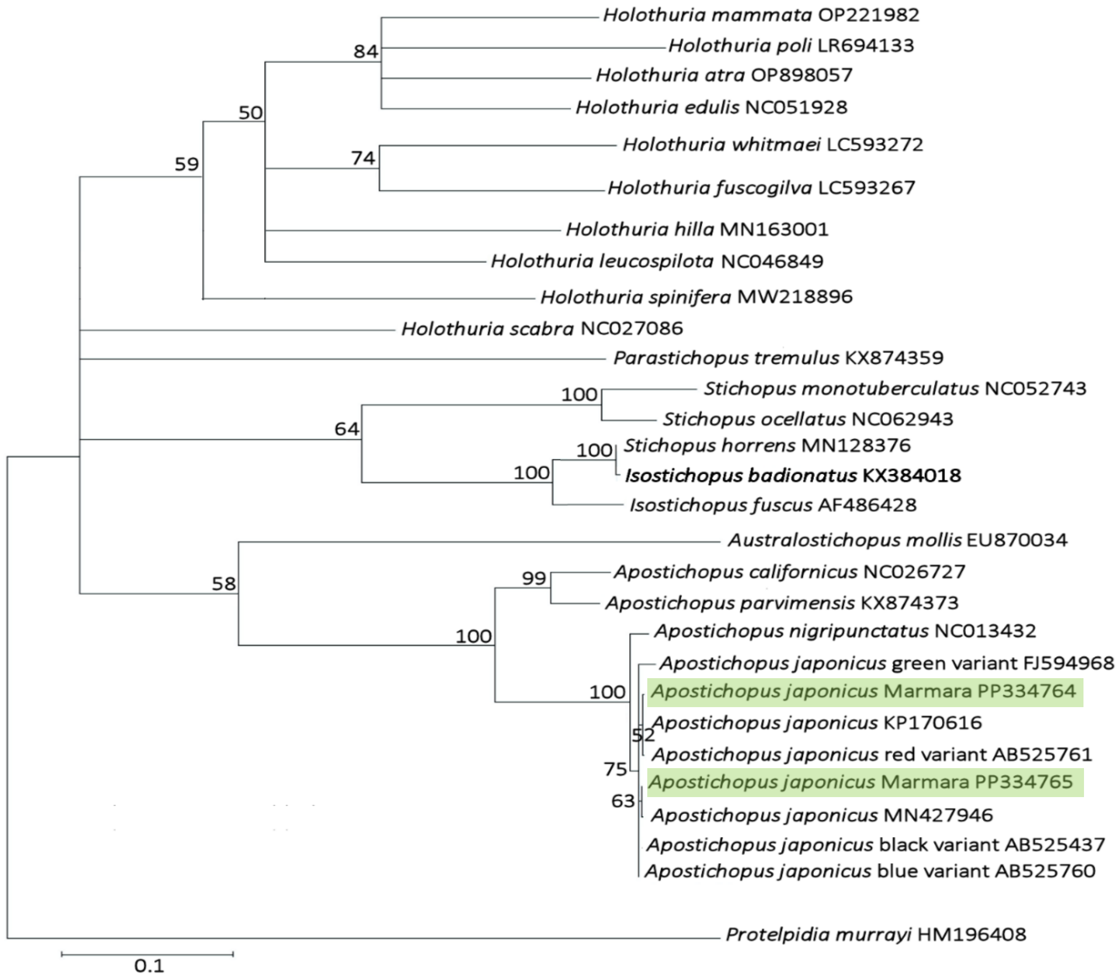

Figure 4. Maximum likelihood (ML) tree obtained by mitochondrial COI gene sequences of the samples from this study and related Holothuroidea spp. sequences deposited in NCBI GenBank. The sequences obtained in this study are painted in green. *Protelpidia murrayi* was used as outgroup. The condensed tree is shown with cut-off value at 50%. Branch support obtained after 1000 bootstrap replicates is indicated above lines. The scale bar is expected changes per site.

The average K value of the population was calculated as 2.32±0.08 (ranging from 0.94 to 4.62) and was significantly higher from optimal growth condition 1 (One-sample *t*-test; df = 66, $t = 15.526$, $p < 0.05$). The K value provides a holistic perspective of current ecological dynamics, and the higher the K value, the better the condition of the marine organism. Environmental conditions, including temperature, water quality, and habitat structure, may be potential determinants affecting the K value of the population (Froese, 2006). It also reflects the role of food availability, fishing pressure, predation, reproduction capacity, and the complex interaction of these factors (Nash et al., 2006; Aydın, 2020). A high K value of the population may indicate a resilient and thriving population in the SoM in the face of seemingly favorable environmental and social conditions.

The two genetically examined samples had different COI haplotypes, which showed the *A. japonicus* population inhabiting the Sea of Marmara may be genetically heterogenous, most probably due to multiple introductions. BLAST search revealed that the Marmara Sample 2675 (Accession no: PP334764) with a single mismatch showed 99.85% identity with *A. japonicus* from Vladivostok, Russia (Accession no: KP170616), whereas the Marmara Sample 2721 (Accession no: PP334765), again with a single mismatch, showed 99.85% identity with *A. japonicus* specimens from China (Accession no: MN427946) and Yamaguchi, Japan (Accession no: AB525437 and AB525760). The ML phylogenetic tree showed the genus *Apostichopus* is monophyletic and the species are genetically distinct (Figure 4). However, there is no significant genetic difference between the COI sequences of the color morphotypes of *A. japonicus*. These findings are in accordance with Jo et al. (2016).

Conclusion

The discovery of *A. japonicus* in the SoM constitutes a remarkable extension of its known distribution within the Mediterranean Sea, raising multifaceted ecological inquiries. Monitoring studies reveal a significant and successful colonization of *A. japonicus* in the Gulf of Izmit, with dense populations recorded at specific depths. The observed size distribution within the population, skewed towards juveniles, indicates ongoing reproduction and the potential for sustainability. They mature in a much shorter period of two years compared to other temperate climate species (Chen, 2003). The high reproductive capacity of *A. japonicus* (Kang et al., 2017; Zhang & Lai, 2024) guarantees the sustainability of stocks in the SoM. However, the sustainability of species in the SoM faces significant challenges, particularly from illegal hookah or SCUBA diving practices (Karadurmuş & Aydın, 2023). The species is frequently targeted and collected illegally, posing a substantial threat to the stability of stocks. Urgent measures need to be taken to prevent illegal harvesting of the species, especially during spawning periods that coincide with warm seasons (Park et al., 2007; Kang et al., 2017). In addition to the landing quota application, a regulation regarding the minimum landing size (MLS) is recommended to control the stock increase and to consider the sustainability of the species. The presence of this sea cucumber may have both positive and negative repercussions for cohabiting species. The presence of this alien species in the region may eventually lead to serious problems in terms of biodiversity and food competition in the Sea of Marmara. Further research and specific studies are needed to clarify these complex relationships.

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Compliance With Ethical Standards

Authors' Contributions

MA: Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Writing-Review and Editing, Data Curation, Visualization

UK: Conceptualization, Methodology, Validation, Resources, Writing - Original Draft, Writing-Review and Editing, Supervision

SÜK: Methodology, Writing-Review and Editing

MBY: Methodology, Formal Analysis, Writing - Original Draft, Writing-Review and Editing, Visualization

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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