Journal of *Marine Science and Engineering*

MDPI

Article

Long-Term Population Genetic Features of the *Rhopilema nomadica* Jellyfish from the Israeli Mediterranean Coasts

Jacob Douek [1,*], Giovanni Giallongo [1], Zoya Harbuzov [1], Bella S. Galil [2] and Baruch Rinkevich [1,*]

[1] National Institute of Oceanography, Israel Oceanographic & Limnological Research, Tel Shikmona, P.O. Box 2336, Haifa 3102201, Israel; giovanni.giallongo1994@gmail.com (G.G.); zoya.garbu@ocean.org.il (Z.H.)
[2] Steinhardt Museum of Natural History, Tel Aviv University, Tel Aviv 6997801, Israel; bgalil@tauex.tau.ac.il
* Correspondence: douek@ocean.org.il (J.D.); buki@ocean.org.il (B.R.); Tel.: +972-4-8565233 (J.D.)

Abstract: The rhizostomatid scyphozoan *Rhopilema nomadica* is one of the most notorious marine invasive species established in the eastern Mediterranean Sea. Using seven microsatellite loci, here, we examined the population genetic structures on 587 individual tissue samples collected from 21 sites along the Mediterranean coast of Israel over a period of 16 years. The results indicate unique microsatellite landscapes for all samples, which belong to a single unstructured population. The >20 alleles found in most loci, low fixation index (F) values (average 0.106), and high heterozygosity (average 0.667) suggest random or assortative mating. Additionally, the low overall differentiation (Fst) values (0.043) and pairwise Fst values between the samples collected in different years indicated gene flow and random mating over the years, potentially due to the long-lasting podocytes, scyphistomae, and adults causing a population overlap between the sampled months/years. Likewise, analyses were conducted between seasons, sites, and early/intermediate/late periods of collecting years. These results support the previous analyses performed on the mitochondrial gene cytochrome oxidase subunit I (COI) sequences, altogether indicating a highly polymorphic single unstructured *R. nomadica* population in the Levant, possibly backed by independent introductions. The results hint to the existence of highly functional connectivity with a genetically highly diverse source population.

Keywords: *Rhopilema nomadica*; Israel; microsatellite markers; genetic diversity; jellyfish; Mediterranean Sea

Citation: Douek, J.; Giallongo, G.; Harbuzov, Z.; Galil, B.S.; Rinkevich, B. Long-Term Population Genetic Features of the *Rhopilema nomadica* Jellyfish from the Israeli Mediterranean Coasts. *J. Mar. Sci. Eng.* **2024**, *12*, 171. https://doi.org/10.3390/jmse12010171

Academic Editor: Ka Hou Chu

Received: 14 December 2023
Revised: 11 January 2024
Accepted: 12 January 2024
Published: 16 January 2024

1. Introduction

The rhizostomatid scyphozoan *Rhopilema nomadica* Galil, 1990, is a notorious marine invasive species introduced into the Mediterranean through the Suez Canal and established along the Eastern and Central Mediterranean coasts. The first record of this scyphozoan in the Mediterranean Sea dates back to 1977 on Israel's coast (Figure 1) [1]. Promoted by the increase in Mediterranean seawater temperature [2,3], the species has subsequently extended its distribution across the Eastern and Central Mediterranean Sea sites, including Egypt, Italy (Pantelleria, Aegadian archipelago off Sicily), Lebanon, Syria, Turkey, Greece, Malta, and Tunisia, reaching the Western Mediterranean Sea in 2015 (Sardinia) [4–8]. With the remarkable ability for sexual and asexual reproduction and the competence to undergo second and even third strobilation events [9–11], each summer since the mid-1980s, and sporadically during winter months later on, *R. nomadica* appears to form massive swarms in the Levantine Sea (bordering the coasts of Egypt, Israel, and Turkey [4,12–15]), some up to 100 km long, with a 10 km width and 30 m depth, reaching 160,000 specimens km^2 and approximately 30 million specimens per swarm [5,16].

The pronounced invasiveness of *R. nomadica*, the substantial size of its summer and winter swarms [4–14], the overall economic impacts [17], the massive human health bearings, and this species' effective integration with native fauna [18] call for better knowledge of its population genetics patterns and characteristics in the Mediterranean Sea to be obtained. To achieve this objective, a recent study [19] has delved into the mitochondrial

COI haplotype diversity of the Levantine *R. nomadica* populations. This study, encompassing more than a decade-long specimen collection period along the Israeli Mediterranean coast, validated the morphological taxonomy, and then elucidated three major outcomes: (a) all Israeli *R. nomadica* populations, over time, form a single unstructured population; (b) the population displays a significantly high polymorphism at the COI locus, comprising at least 89 haplotypes, including 46 singletons (>50% of all haplotypes), with a distinct north-to-south haplotype gradient; and (c) the number of singletons has increased with time, and its Israeli population is rapidly expanding. The apparent extensive repertoire of COI haplotypes, further characterized by a high number of singletons, may hint to multiple and independent *R. nomadica* introductions through the existence of functional connectivity with a genetically highly diver source population. Thus, in contrast to other invasive species (e.g., [20–23]), the *R. nomadica* population in the Levant is not under high founder's effects or genetic bottleneck risks [19]. Accordingly, the prospect of numerous separate introductions through the Suez Canal corridor [24] cannot be ruled out for the Israeli *R. nomadica* population [1,25].

Figure 1. *Rhopilema nomadica* (photo by Hagai Nativ, Morris Kahn Marine Research Station, Israel).

In order to gain a deeper understanding of the invasion trajectories of *R. nomadica*, the evolving population structure, the spatial connectivity of jellyfish blooms, as well as alterations over both space and time, it is of prime importance to undertake a comprehensive population genetics study. Using the new developed panel of microsatellite alleles for *R. nomadica* [26], this long-term study explores the Israeli *R. nomadica* population characteristics in light of north to south sites' trajectories and seasonality and yearly changes throughout a period of 16 years, including 11 sampling years (2004 and the 2010–2019 period).

2. Materials and Methods

2.1. Samples Collection

From 2004 to 2019, a total of 1091 *Rhopilema nomadica* samples were obtained from 24 locations along the Israeli Mediterranean coasts (details in [19]; Table S1, Figure 2). Most jellyfish samples were collected from the shore or shallow waters, <1 m depth. Some of the samples from Hadera and Ashkelon were collected from the electric power station area and in front of Ashdod.

2.2. DNA Extractions and PCR Amplifications

Genomic DNA was isolated from jellyfish tissue using Phenol/Chloroform extraction (detailed protocols in [27]) and analyzed using 7 microsatellite markers (loci RN3, RN6, RN8, RN10, RN11, RN12, and RN18, as described in [26]). For each sample, 20 µL of reaction mixture containing 10 µL of 2X Taq PCR MasterMix (Tiangen, Beijing, China), 2 µL

of 1:50 diluted genomic DNA, and 0.5 µM of primer mix containing forward primer labeled with one of four florescent dyes (VIC, FAM6, NED, and PET), and fluorescence unlabeled reverse primer were used. The reaction conditions were as follows: 5 min at 95 °C, followed by 30 cycles of 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 1 min, and additional 10 min incubation at 72 °C. The PCR products were examined on 1.5% agarose gel.

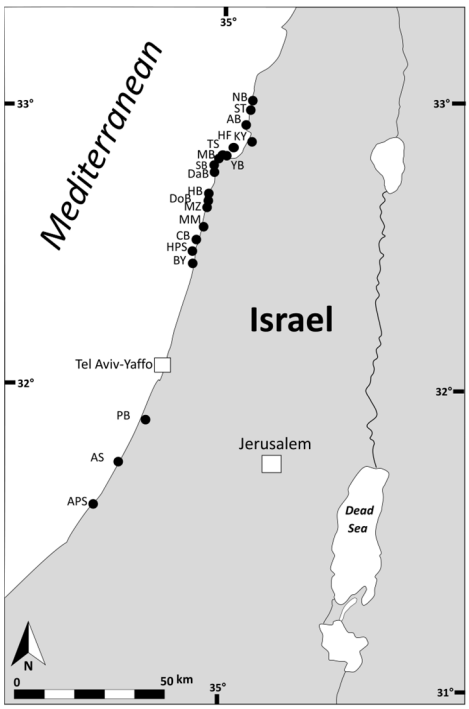

Figure 2. Map of Israel with the *Rhopilema nomadica* collecting sites.

Positive PCR products were analyzed in automated sequence analysis system (Applied Biosystems ABI PRISM 3100 Genetic Analyzer (Waltham, MA, USA); University of Cambridge, UK) as follows: 0.25 µL of each amplification product was mixed with 0.4 µL of LIZ size standard (MapMarker DY632, 50–1000 bp, BioVenture Inc., Murfreeboro, TN, USA) and 8.6 µL of HiFi Formamide (ThermoFisher, Waltham, MA, USA).

2.3. Microsatellite and Statistical Analysis

The fluorescent amplification products were scored using the genotyping software GeneMepper version 4.0 and Peak Scanner Version 1.0 Software (Applied Biosystems). The raw data generated by the genotyping process were analyzed and binned using an Excel Macro, AutoBin 0.9, written by Franck Salin (INRA Pierroton-UMR BIOGECO). GenAlex6.5 [28] was used for calculating observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F), allele numbers and frequencies, pairwise Fst, and hierarchical analysis of molecular variance (AMOVA). Polymorphic information content (PIC) by locus was estimated using Cervus version 3.0.7 [29]. BAPS 6 [30] and STRUCTURE 2.2.3 [31] software were used to analyze population structures [32]. For BAPS, mixture analyses and clustering were performed as individuals or groups of individuals, while the input maximum number of populations ranged between 1 and 30 with at least 5 repeats. For STRUCTURE, admixture analyses were performed, where each run consisted of 100,000 iterations with a burn-in of 100,000 for each value of K (from 1 to 10). For each K, the run was repeated five times. STURCTURE results were analyzed using "structure Harvester" [33] for the most likely K.

For the statistical analyses, the microsatellite data were arranged and grouped according to the following parameters: collecting sites, the collecting years, seasons and months

over all years, and haplotype sequences of the mitochondrial gene cytochrome oxidase subunit I (COI) they share.

3. Results

Out of 1091 tissue samples (details in [19]), 912 DNA samples were used for PCR amplification, with 7 sets of primers for each one. Not all PCR amplifications were successful. In about one third of the cases, while positive bands of the PCR products were seen on the agarose gel, no high-quality products were obtained following the repeated PCR amplifications and reiterated sequences. As a result, we obtained 587 samples with good PCR products on at least three microsatellite loci per sample from 20 collecting sites (Figure 2, Table S1).

3.1. The Levantine R. nomadica Samples

Each of the 587 *R. nomadica* specimens collected over 11 years (2004 and the 2010–2019 period) from 21 different sites showed a unique allelic pattern on the microsatellite loci (no asexual reproduction). The allele numbers per microsatellite locus ranged between 7 alleles (Locus RN8) and 57 alleles (Locus RN10), while 6/7 loci possessed > 20 alleles per locus (Table 1). The observed heterozygosity (Ho) value ranged from 0.315 (RN8) to 0.819 (RN6), and the expected heterozygosity (He) value fluctuated from 0.342 (RN8) to 0.886 (RN6); the fixation index (F) varied between 0.058 (RN10) and 0.273 (RN11), and the polymorphic information content (PIC) ranged from 0.322 (NR8) to 0.874 (NR6) (Table 1). The BAPS cluster analysis of the whole jellyfish individuals (the samples' order was arranged according to cascading collecting years and then months or collecting sites) revealed 25–29 clusters for either parameter (years, months, or seasons), collecting sites, or COI haplotypes (Figure 3a). The STRUCTURE analyses followed by analyses with "Harvester" revealed an optimal K value (k = 2) with an admixture of all samples (Figure 3b).

Table 1. Indices of population genetics for the jellyfish samples. n = no. of alleles per locus; N = no. of samples per locus; HObs = observed heterozygotes; HExp = expected heterozygotes; PIC = polymorphic information content; F = fixation index; HW = deviation from Hardy–Weinberg equilibrium.

Locus	n	N	HObs	HExp	PIC	F	HW
Locus RN10	57	493	0.751	0.797	0.790	0.058	NS
Locus RN11	24	507	0.515	0.708	0.670	0.273	***
Locus RN12	26	458	0.784	0.834	0.816	0.060	NS
Locus RN18	22	473	0.778	0.866	0.852	0.102	NS
Locus RN3	34	523	0.707	0.779	0.752	0.092	**
Locus RN6	22	464	0.819	0.886	0.874	0.076	NS
Locus RN8	8	400	0.315	0.342	0.322	0.079	NS
Average	27.6	474	0.667	0.745	0.725	0.106	-

Significance (with Bonferroni correction), ** $p > 0.01$, *** $p > 0.001$, NS = not significant.

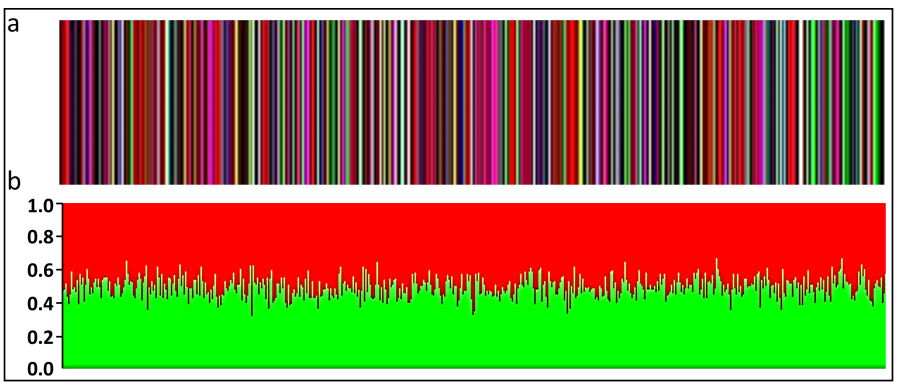

Figure 3. Population structure analysis of all samples treated as individuals (**a**). Cluster analysis using BAPS software (each color represents a cluster, with 26 clusters in total) (**b**). Cluster analysis was carried out using "STRUCTURE" software (optimal k = 2).

3.2. Annual Analyses

By employing molecular variance (AMOVA) to organize and categorize the gathered samples according to their respective collection years, it was found that the primary diversity (96%) resides within the samples collected in each specific year (within populations), with only 4% of the observed divergence identified among the years (among populations) (0.043 (p = 0.001)) (Figure S1a). The analyses of the population genetic structures using the BAPS and STRUCTURE software unveiled a single cluster with BAPS and two genetic clusters (k = 2) with the STRUCTURE software when considering all of the jellyfish samples collected over the years (Figure S1b,c). The observed heterozygosity (Ho) value varied from 0.505 (2019) to 0.708 (2015), and the expected heterozygosity (He) value ranged from 0.520 (2010) to 0.761 (2018). The fixation index (F) ranged between −0.326 (2010) and 0.250 (2019) (Table S2a). The pairwise Fst between the years varied from 0.003 (between 2012 and 2017) to 0.173 (between 2004 and 2010, Table S2b). Analyzing the variation in allele frequencies of the predominant alleles at each locus over the years revealed that the fluctuations are primarily influenced by the number of samples collected in the respective analyzed year (Figure S2).

We then compared all samples collected in the early years (2004–2011) that were assembled into a single group with the late years (2017–2019) group as well as the combined intermediate years group (2012–2016). An analysis of molecular variance (AMOVA) showed that the entire diversity (100%) is contained within the population, indicating that there is no discernible divergence between the early years and the late years. The overall differentiation (Fst) value among all of the groups was 0.002 (p = 0.001) (Figure S3a). The pairwise Fst between the years varied from 0.002 (early to intermediate year groups) to 0.004 (early to late year groups, Table S3). The population structure analyses, conducted through BAPS and STRUCTURE, revealed the presence of a single cluster with BAPS and identified two genetic clusters (k = 2) using the STRUCTURE software (Figure S3) for all of the samples.

3.3. Seasonality Analyses

Examining all samples organized and grouped based on their collecting seasons did not reveal any difference among the four seasons. The molecular variance analysis revealed that the entire variance (100%) was within the seasons, with no variance observed between seasons, and the overall differentiation (Fst) value among all seasons was Fst = 0.001 (p = 0.223) (Figure S4a). The BAPS analysis revealed a single cluster for all populations, and the STRUCTURE analysis pointed to two genetic clusters (K = 2) for all seasons (Figure S4b,c). The observed heterozygosity (Ho) value ranged between 0.658 (Summer) and 0.703 (Autumn), and the expected heterozygosity (He) value varied from 0.736 (Spring) to 0.753 (Autumn). The fixation index (F) ranged between 0.049 (Autumn) and 0.116 (Summer) (Table S4a). The pairwise Fst between the seasons varied from 0.001 (spring vs. summer) to 0.007 (autumn vs. all other seasons, Table S4b).

3.4. Monthly Analyses

The assessments conducted on the collection months revealed that the *R. nomadica* samples were collected consistently over 10 months during the whole period, as due to their absence, there were no samples collected in September and October. A limited number of samples were obtained during May and August (three samples each, year 2016) and December (10 samples, year 2018). The observed heterozygosity (Ho) value ranged between 0.571 (May and August) and 0.709 (April), and the expected heterozygosity (He) value varied from 0.512 (August) to 0.753 (November). The fixation index (F) ranged between −0.155 (August) and 0.122 (July) (Table S5a). The pairwise Fst between the months varied from 0.002 (between June and July) to 0.127 (between August and December, Table S5b). An analysis of molecular variance (AMOVA) revealed that the entire molecular variance (100%) was confined within the months, with no variance observed among the months (Figure S5a). The overall differentiation (Fst) value among all months was 0.004 (p = 0.026)

(Figure S5a). A population structure analysis using BAPS consistently identified a single cluster for all populations and two genetic clusters (k = 2) in the STRUCTURE analysis for all samples across every collected month (Figure S5b,c).

3.5. Site Analyses

The 20 collecting sites were grouped into five regions spanning from the south to the north: area no. 1 is referred to as "Ashdod", encompassing Ashdod beach, Ashkelon power station, and Palmahim beach; area no. 2 was named "Mikhmoret", including the sites of Mikhmoret, Hadera power station, and the Caesarea and Beit Yanay beaches; zone no. 3 was labeled as "Habonim" and includes the sites of Dor, Maayan Tzvi, and Habonim beaches; area no. 4 was designated as "Haifa" for the samples collected in Shikmona beach, Yotvata beach, and the Dado, Maridian, and Student beaches (Haifa south beaches 1, 2, and 3); while region no. 5 was named "Nahariya" for the Nahariya, Shavei Tzion, Acre, and Kiryat Haim beaches.

An analysis of molecular variance (AMOVA) indicated that 100% of the molecular variance resided within the collection sites, and the overall differentiation (Fst) value among all collecting regions was Fst = 0.001 (p = 0.011, Figure S6a). The BAPS analysis identified a single cluster encompassing all populations and two distinct clusters for all collecting locations in the STRUCTURE analysis, with an optimal K value of 2 (Figure S6b,c). The observed heterozygosity (Ho) value ranged between 0.645 (Nahariya) and 0.676 (Habonim), and the expected heterozygosity (He) value varied from 0.727 (Mikhmoret) to 0.751 (Ashdod). The fixation index (F) ranged between 0.064 (Habonim) and 0.137 (Ashdod, Table S6a). The pairwise Fst between the collecting sites varied from 0.002 (between Mikhmoret and Haifa) to 0.013 (between Habonim and Nahariya, (Table S6b).

3.6. COI Haplotypes Analyses

While the entire 1091 jellyfish DNA samples included 89 distinct *R. nomadica* COI haplotypes [19], the current 587 analyzed DNA samples possessed 64 of these COI haplotypes. The samples were organized and grouped based on their COI haplotypes. While performing the AMOVA, populations comprising fewer than two samples were excluded from the analysis, in accordance with the program's limitations, resulting in 559 samples and 36 haplotypes. The AMOVA for the microsatellite data revealed that the majority of molecular variance is within the populations (99%), and only 1% is among the populations. The overall differentiation (Fst) value among all haplotype groups was 0.011 (p = 0.001, Figure S7a). Clustering the jellyfish samples as individuals, based on their COI haplotypes, yielded 26 BAPS clusters (probability of 0.95396), and notably, there was no correlation observed with the COI haplotype (Figure S7b). Utilizing BAPS to determine the population structure based on groups of individuals revealed a single cluster encompassing all haplotypes, and for the STRUCTURE analysis, two genetic clusters (k = 2) were elucidated for the different haplotypes (Figure S7c,d). The pairwise Fst values were calculated among the larger groups of samples (>5 samples in a group, totaling 20 groups/haplotypes), and the results varied from 0.004 (between haplotypes 8 and 13) to 0.094 (haplotypes 19 and 26, Table S7).

An additional analysis was conducted on the subset of microsatellite data associated with COI haplotype 8, which comprised the largest number of samples (154 samples with microsatellite results). Within this haplotype, the samples were arranged and analyzed based on certain criteria, including collecting seasons (Figure S8), years, months, and sites, while mirroring the approach used for the entire dataset. The findings consistently followed the broader population trend, as illustrated in Figure S8. When subjected to a population structure analysis based on collecting seasons, the individual analyses revealed 16 clusters. However, when considered collectively as a group of individuals, the analysis resulted in a singular cluster.

4. Discussion

By employing seven microsatellite loci as genetic markers, the present study analyzed the population genetic structure of rhizostomatid scyphozoan jellyfish *R. nomadica* collected from 21 sites over 11 collecting years throughout an extended period of 16 years (2004 and the 2010–2019 period), spanning between months and seasons. This set of samples was previously analyzed for the mitochondrial gene cytochrome oxidase subunit I (COI) sequences [19]. The analyses conducted on the 587 DNA samples revealed that all jellyfish samples were unique in terms of their microsatellite landscapes (no asexual reproduction was elucidated) and belonged to a single unstructured population, with a high number of alleles in most microsatellite loci (>20 alleles). The low fixation index F values (averaged 0.106) and the high heterozygosity (averaged 0.667) indicated random or assortative mating. Further, the low overall differentiation (Fst) value (0.043), as revealed in the AMOVA and the pairwise Fst between the samples collected in different years along the whole research period, suggests a gene flow and random mating over the years, which is a possible result of the long survival of the *R. nomadica* developing podocytes (>18 months [11]), the scyphistomae, and the long-lived adults, which cause population overlapping between sampled months/years. Yet, the pairwise Fst analyses revealed more gene flow between the early collecting years (2004, 2010–2011) and the following intermediate period (the 2012–2016 period, Fst = 0.002), and then from the intermediate period to the latest collection period (the 2017–2019 period, Fst = 0.004). This latest collection period showed high gene flow with the intermediate period (Fst = 0.002).

The above results are further supported by the low overall differentiation (Fst) value (0.001) and the pairwise Fst results between seasons, which suggest connectivity and gene flow between the four sampling seasons. In contrast, the heterozygosity in the summer months was lower compared to the other seasons, and the inbreeding index (F) was higher. It should be noted that the summer months are highlighted by the peaks in jellyfish numbers, sizes (the summer swarm), and the expression of sexual reproduction [5,11].

Most of the jellyfish collections were conducted throughout the months when they were spotted along the shores or in the very shallow waters, facilitating convenient gathering. The majority of the jellyfish samples were collected during June and July (Summer) as well as in February (winter), March, and April (Spring), and no collections were recorded in September and October, while only three samples were found in May and August. The overall differentiation (Fst) value of 0.004, along with the low pairwise Fst values between the collecting months, indicates connectivity and gene flow throughout the year. Yet, exceptions are noted for May, August, and December, which show a higher pairwise Fst, which is likely attributable to a lower number of samples during these months.

Both the summer and the winter swarms proceed from south to north along the Israeli coast [5]. The samples from these swarms were carried ashore and then collected from the different locations throughout the years, during various months and seasons. The AMOVA analysis revealed a minimal overall differentiation (Fst) value (0.001), signifying that all molecular variance is confined within the populations collected at distinct sites (100%), with no discernible variance among them. When assessing the pairwise Fst between the collecting areas, a notable trend emerged, indicating greater connectivity and gene flow in the southern collection sites compared to those in the north (Nahariya). This pattern aligns with the findings of the COI analysis [19], where the samples from all collecting sites consistently belonged to the same genetic cluster with no exceptions.

The 587 tissue samples that were analyzed revealed 64 COI haplotypes, of which 36 haplotypes that consisted of more than a single sample revealed just a 1% variance in the microsatellite alleles among the haplotypes, compared with 99% of the variance within the haplotypes. The low overall differentiation (Fst) value (0.011) and the pairwise Fst values between the haplotypes suggest gene flow and connectivity among them. In the cluster analysis of the microsatellite data, the individual sample analysis did not align with the COI haplotypes, grouping them as one, with two genetic clusters being found for all samples when they were analyzed as groups (haplotypes) of individuals. This suggests a lack of

connection between microsatellite genetic identity and the COI haplotype identity. The absence of any genetic background information (nor demographic data) for the *R. nomadica* source population/s is yet to be considered.

The population genetic outcomes of the present study (using microsatellite loci) and the former study (employed COI haplotypes [19]) admit the possibility of additional independent introductions of *R. nomadica* through a Suez Canal corridor into the Levantine waters [24]. This population is characterized by a high number of alleles on most of the studied microsatellites (mirroring multi-specimen introductions), which is notably attributed not only to the increase in Mediterranean seawater temperatures [2,3], but primarily to human activities, which have reduced physical barriers to the transport of invasive organisms throughout the Suez Canal [34,35], further facilitating the continuous *R. nomadica* introduction into the Levant. The aforementioned conclusion is strengthened by the overall extended time frame of the present DNA sampling (16 years), which deviates from the majority of short-term studies on marine invasive species [19]. Unlike those studies, which often concentrate on short time scales with temporal sampling protocols limited to a single event or a few years, our approach provides a more comprehensive perspective.

The 2022 report by the Israeli State Comptroller's Office [36] assessed the economic impact of *R. nomadica* along the Israeli coast. The report estimated the annual financial loss due to diminished beach use (3–10%) at NIS 21.8 million, and estimated the losses to coastal fishers at over NIS 5 million. These losses pale compared to the costs borne by Israel's coastal power plants when swarms block intake seawater pipes used for cooling. The annual cost of removing the jellyfish could amount to about NIS 688,000. This report further assessed that the multi-annual loss to desalination facilities could amount to NIS 32 million. In view of the extensive losses caused by *R. nomadica* and the ongoing westward spread of its populations, it is crucially important to continue long-term DNA sampling activities across the Central Mediterranean. The aim is to gain deeper insights into its populations, changes in invasion trajectories and their following emerging population structures, and the spatial connectivity webs within and between the jellyfish blooms. Additionally, tracking source populations in the Red Sea would be valuable to confirm the characteristics of the Suez Canal corridor, assess the proposition of multiple independent introductions, and verify the robustness of an open genetic corridor between the tropical Red Sea and the Mediterranean—an issue of grave importance to the conservation of native biota in a warming sea.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/jmse12010171/s1. Figure S1: Between-year comparisons. Figure S2: Abundance frequencies. Figure S3: Comparisons between the early collecting period and the late collecting period. Figure S4: Between-season comparisons. Figure S5 Between-month comparisons. Figure S6: Comparisons between collecting regions. Figure S7: Comparisons between COI haplotypes. Figure S8: Population structure analyses of samples sharing COI haplotype 8. Between-season comparisons. Table S1: List of sampling sites, coordinates, years of sampling, and number of collected specimens per site. Table S2: Statistical analysis for the sampled jellyfish populations, organized based on their collecting year. Table S3: Pairwise population Fst values between collecting periods (representing three groups of years). Table S4: Summary of statistical analyses for the jellyfish populations, organized based on their collecting seasons. Table S5: Summary of the statistical analysis conducted on the jellyfish samples categorized by the month of collection. Table S6: Summary of statistical analyses for jellyfish populations, organized based on their collecting regions. Table S7: Pairwise population Fst values between haplotypes (for >5 samples in each haplotype).

Author Contributions: B.R., J.D. and B.S.G. conceived the research idea; J.D. and Z.H. collected and sampled *Rhopilema nomadica* specimens; J.D., Z.H. and G.G., performed the molecular study including DNA isolation and PCR and sequence analyses; G.G., J.D. and B.R. analyzed the results; G.G. and B.R. wrote the first draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data is reported in the supplenemtary material.

Acknowledgments: We gratefully thank Guy Paz for creating the figures and tables and Hagai Nativ, Morris Kahn Marine Research Station, Israel for providing the *R nomadica* photo. All lab members of the National Institute of Oceanography and Israel Oceanographic & Limnological Research are acknowledged for their help in collecting the jellyfish.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Galil, B.; Spanier, E.; Ferguson, W. The Scyphomedusae of the Mediterranen Coast of Israel, Including Two Lessepsian Migrants New to the Mediterranean. *Zool. Meded.* **1990**, *64*, 95–105.
2. Pastor, F.; Valiente, J.A.; Palau, J.L. Sea Surface Temperature in the Mediterranean: Trends and Spatial Patterns (1982–2016). *Pure Appl. Geophys.* **2018**, *175*, 4017–4029. [CrossRef]
3. Bindoff, N.L.; Cheung, W.W.; Kairo, J.G.; Arstegui, J.; Guinder, V.A.; Hallberg, R.; Hilmi, N.; Jiao, N.; Karim, M.S.; Levin, L.; et al. Changing ocean, marine ecosystems, and dependent communities. In *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate*; Pörtner, H.O., Roberts, D.C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., Mintenbeck, K., Alegría, A., Nicolai, M., Okem, A., et al., Eds.; IPCC: Geneva, Switzerland, 2019; Chapter 5.
4. Madkour, F.F.; Hanafy, M.H.; Zaghloul, W.S. Record of Aggregation of Alien Tropical Schyphozoan *Rhopilema Nomadica* Galil, 1990 in the Mediterranean Coast of Egypt. *Int. Mar. Sci. J.* **2019**, *2*, 1–11. [CrossRef]
5. Edelist, D.; Guy-Haim, T.; Kuplik, Z.; Zuckerman, N.; Nemoy, P.; Angel, D.L. Phenological Shift in Swarming Patterns of *Rhopilema Nomadica* in the Eastern Mediterranean Sea. *J. Plankton Res.* **2020**, *42*, 211–219. [CrossRef]
6. Deidun, A.; Arrigo, S.; Piraino, S. The Westernmost Record of *Rhopilema Nomadica* (Galil, 1990) in the Mediterranean–off the Maltese Islands. *AI* **2011**, *6*, S99–S103. [CrossRef]
7. Yahia, M.N.D.; Yahia, O.K.D.; Gueroun, S.K.M.; Aissi, M.; Deidun, A.; Fuentes, V.; Piraino, S. The Invasive Tropical Scyphozoan *Rhopilema Nomadica* Galil, 1990 Reaches the Tunisian Coast of the Mediterranean Sea. *BioInvasions Rec.* **2013**, *2*, 319–323. [CrossRef]
8. Balistreri, P.; Spiga, A.; Deidun, A.; Gueroun, S.K.; Daly Yahia, M.N. Further Spread of the Venomous Jellyfish *Rhopilema Nomadica* Galil, Spannier & Ferguson, 1990 (Rhizostomeae, Rhizostomatidae) in the Western Mediterranean. *BioInvasions Rec.* **2017**, *6*, 19–24. [CrossRef]
9. Lotan, A.; Ben-Hillel, R.; Loya, Y. Life Cycle of *Rhopilema Nomadica*: A New Immigrant Scyphomedusan in the Mediterranean. *Mar. Biol.* **1992**, *112*, 237–242. [CrossRef]
10. Lotan, A.; Fine, M.; Ben-Hillel, R. Synchronization of the Life Cycle and Dispersal Pattern of the Tropical Invader Scyphomedusan *Rhopilema Nomadica* Is Temperature Dependent. *Mar. Ecol. Prog. Ser.* **1994**, *109*, 59. [CrossRef]
11. Dror, H.; Angel, D. Rising Seawater Temperatures Affect the Fitness of *Rhopilema Nomadica* Polyps and Podocysts and the Expansion of This Medusa into the Western Mediterranean. *Mar. Ecol. Prog. Ser.* **2023**, SHIFTav2. [CrossRef]
12. Deval, M.C.; Olguner, M.T. The Spring Outbreak of the Invasive Scyphomedusa *Rhopilema Nomadica* Galil, Spannier & Ferguson, 1990 in the Antalya Bay, the Eastern Mediterranean. *Nat. Eng. Sci.* **2019**, *4*, 247–252. [CrossRef]
13. Galil, B.S. Seeing Red: Alien Species along the Mediterranean Coast of Israel. *Aquat. Invasions* **2007**, *2*, 281–312. [CrossRef]
14. El-Regal, M.A.A.; Temraz, T.A. Blooming of nomad jelly fish *Rhopilema nomadica* along the Egyptian Mediterranean coasts. In Proceedings of the 5th International Jellyfish Bloom Symposium, Barcelona, Spain, 30 May–3 June 2016; Volume 41, pp. 489–490.
15. Öztürk, B.; İşinibilir, M. An alien jellyfish *Rhopilema nomadica* and its impacts to the Eastern Mediterranean part of Turkey. *J. Black Sea/Mediterr. Environ.* **2010**, *16*, 149–156.
16. Galil, B.S.; Zenetos, A. A Sea Change—Exotics in the Eastern Mediterranean Sea. In *Invasive Aquatic Species of Europe. Distribution, Impacts and Management*; Springer: Dordrecht, The Netherlands, 2002; pp. 325–336. [CrossRef]
17. Tsirintanis, K.; Azzurro, E.; Crocetta, F.; Dimiza, M.; Froglia, C.; Gerovasileiou, V.; Langeneck, J.; Mancinelli, G.; Rosso, A.; Stern, N.; et al. Bioinvasion Impacts on Biodiversity, Ecosystem Services, and Human Health in the Mediterranean Sea. *AI* **2022**, *17*, 308–352. [CrossRef]
18. Zeidler, W.; Douek, J.; Rinkevich, B.; Gevili, R.; Goren, M.; Galil, B.S. Validation and Redescription of the Hyperiidean Amphipod *Brachyscelus rapacoides* Stephensen, 1925 (Crustacea: Amphipoda: Hyperiidea: Brachyscelidae), a New Record of Association with the Scyphozoan Jellyfish *Rhopilema Nomadica* Galil, 1990 (Scyphozoa: Rhiz). *Zootaxa* **2018**, *4471*, 523–534. [CrossRef] [PubMed]
19. Giallongo, G.; Douek, J.; Harbuzov, Z.; Galil, B.S.; Rinkevich, B. Long-Term Changes in Population Genetic Features of a Rapidly Expanding Marine Invader: Implication for Invasion Success. *Biol. Invasions* **2021**, *23*, 2541–2552. [CrossRef]
20. Darling, J.A.; Bagley, M.J.; Roman, J.; Tepolt, C.K.; Geller, J.B. Genetic Patterns across Multiple Introductions of the Globally Invasive Crab Genus *Carcinus*. *Mol. Ecol.* **2008**, *17*, 4992–5007. [CrossRef] [PubMed]
21. Bai, C.; Ke, Z.; Consuegra, S.; Liu, X.; Li, Y. The Role of Founder Effects on the Genetic Structure of the Invasive Bullfrog (*Lithobates Catesbeianaus*) in China. *Biol. Invasions* **2012**, *14*, 1785–1796. [CrossRef]
22. Teem, J.L.; Alphey, L.; Descamps, S.; Edgington, M.P.; Edwards, O.; Gemmell, N.; Harvey-Samuel, T.; Melnick, R.L.; Oh, K.P.; Piaggio, A.J.; et al. Genetic Biocontrol for Invasive Species. *Front. Bioeng. Biotechnol.* **2020**, *8*, 452. [CrossRef]

23. Blakeslee, A.M.H.; Manousaki, T.; Vasileiadou, K.; Tepolt, C.K. An Evolutionary Perspective on Marine Invasions. *Evol. Appl.* **2020**, *13*, 479–485. [CrossRef]
24. Galil, B.S. The Marine Caravan–The Suez Canal and the Erythrean Invasion. In *Bridging Divides*; Gollasch, S., Galil, B.S., Cohen, A.N., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 207–300. ISBN 978-1-4020-5046-6.
25. Stiasny, G. *Die Scyphomedusen des Roten Meeres. Noord-Hollandsche Uitg. Maatsch*; Verhandelingen der Koninklijke Nederlandsche Akademie van wetenschappen te Amsterdam; Natuurkunde 37, l–35; Royal Netherlands Academy of Arts and Sciences: Amsterdam, The Netherlands, 1938.
26. Douek, J.; Harbuzov, Z.; Galil, B.S.; Rinkevich, B. Developing Novel Microsatellite Markers by NGS Technology for *Rhopilema nomadica*, an Invasive Jellyfish. *Mol. Biol. Rep.* **2020**, *47*, 4821–4825. [CrossRef] [PubMed]
27. Douek, J.; Barki, Y.; Gateño, D.; Rinkevich, B. Possible Cryptic Speciation within the Sea Anemone *Actinia equina* Complex Detected by AFLP Markers. *Zool. J. Linn. Soc.* **2002**, *136*, 315–320. [CrossRef]
28. Peakall, R.; Smouse, P.E. GenALEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research-an Update. *Bioinformatics* **2012**, *28*, 2537–2539. [CrossRef]
29. Kalinowski, S.T.; Taper, M.L.; Marshall, T.C. Revising How the Computer Program CERVUS Accommodates Genotyping Error Increases Success in Paternity Assignment. *Mol. Ecol.* **2007**, *16*, 1099–1106. [CrossRef] [PubMed]
30. Corander, J.; Waldmann, P.; Marttinen, P.; Sillanpää, M.J. BAPS 2: Enhanced Possibilities for the Analysis of Genetic Population Structure. *Bioinformatics* **2004**, *20*, 2363–2369. [CrossRef]
31. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
32. Putman, A.I.; Carbone, I. Challenges in Analysis and Interpretation of Microsatellite Data for Population Genetic Studies. *Ecol. Evol.* **2014**, *4*, 4399–4428. [CrossRef]
33. Earl, D.A.; vonHoldt, B.M. STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
34. Golani, D.; Azzurro, E.; Corsini-Foka, M.; Falautano, M.; Andaloro, F.; Bernardi, G. Genetic Bottlenecks and Successful Biological Invasions: The Case of a Recent Lessepsian Migrant. *Biol. Lett.* **2007**, *3*, 541–545. [CrossRef]
35. Lutmar, C.; Rubinovitz, Z. (Eds.) The Suez Canal: Past Lessons and Future Challenges. In *Palgrave Studies in Maritime Politics and Security*; Springer International Publishing: Cham, Switzerland, 2023; ISBN 978-3-031-15669-4.
36. The 2022 Report by the Israeli State Comptrollers' Office. Available online: https://www.mevaker.gov.il/sites/DigitalLibrary/Pages/Reports/7456-20.aspx (accessed on 11 January 2024).