

Article

# Biometry, Distribution and Genetic Characterization of Blue Jack Mackerel *Trachurus picturatus* (Bowdich, 1825), a Rare Pelagic Fish Species in the Adriatic Sea

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**Abstract:** The blue jack mackerel *Trachurus picturatus* (Bowdich, 1825) specimens (N = 155) were collected during the MEDITS survey, done along the eastern side, precisely, of the Croatian fishing ground in July 2018. Biometrical analysis of ten morphometric and five meristic characters, as well as genetic analysis proved that the collected specimens were blue jack mackerel. The total length (TL) and weight (W) of all observed specimens ranged from 9.2 to 33.7 cm ( $12.15 \pm 2.95$  cm) and from 5.79 to 384.94 g ( $17.64 \pm 39.42$  g), respectively. All calculated length–length relationships were linear ( $r > 0.923$ ). Sex was determined only on two larger specimens (28 cm < TL < 32.8 cm), which were females. In the length–weight relationship, positive allometry was established ( $b = 3.1789$ ). Based on 37 partial *cytochrome b* sequences, the overall haplotype diversity (h) of  $0.812 \pm 0.048$  and nucleotide diversity ( $\pi$ ) of  $0.0064 \pm 0.0007$  indicated high levels of haplotype and low nucleotide diversity. The obtained sequences were compared to previously published research within the Northeast Atlantic Ocean and the Mediterranean Sea, confirming the absence of genetic structure among these populations.

**Keywords:** central Mediterranean; mitochondrial DNA; morphometry; pelagic fish; phylogeography; *Trachurus*

## 1. Introduction

Over the years, environmental variables have changed, mostly due to increasing globalisation processes [1]. The modification of those variables directly influenced whole marine ecosystems. Consequently, fish assemblage has either accommodated or transformed. Appearance and increased abundance of new and/or rare species surely affected the structure and functioning of species communities; therefore, systematic monitoring is compulsory.

The blue jack mackerel *Trachurus picturatus* (Bowdich, 1825) is a benthopelagic fish species widely distributed and exploited in the area of East Atlantic (southern Bay of Biscay to south Morocco, including the Macaronesian archipelagos, Tristan de Cunha and Gough Islands). In the area of the Mediterranean Sea and its adjacent seas, this species can be found in its western part and the area of the Black Sea [2]. Within the distribution area of this species, its economic value is the lowest in the Mediterranean. In general, its specimens appear over the continental shelf and around offshore islands down to 400–500 m sea depth [3]. As with other pelagic fish species, it forms schools and tends to migrate from open sea to coastal areas [4]. In the area of the Adriatic, this species is rare and its specimens, in the past, were sporadically caught and noted [5–10]. Until now, only Bolognini et al. [11] reported the minor catch (N = 4) of blue jack mackerel in the Adriatic Sea.

Recently, during the scientific surveys, a Croatian scientist noticed a higher amount of not so common *Trachurus* species in the catches. As geographical expansion of this rare species might have an impact on Adriatic biodiversity, its monitoring is crucial. It is believed that ocean warming is among the main driving forces of species abundance and distribution changes and, consequently, of changes in species biodiversity and marine ecosystem functioning [12,13]. Žužul et al. [14] offered ocean warming as one of the explanations behind the increase in the number of wild gilthead seabream noted in the coastal parts of the Adriatic Sea [15]. The authors further argued that it is possible that wild gilthead seabream, as a species that thrives in warmer areas, is becoming more numerous at its northern distribution areas [16], as a consequence of increased larval survival and recruitment success [14].

Hence, to better understand what could be expected from the possible emergence of a blue jack mackerel population in the Adriatic Sea, the aim of this study was to investigate the biometrical analysis, the length–weight relationship and genetic diversity of the blue jack mackerel caught in the Adriatic Sea and compare the results to previously published research within the Northeast Atlantic Ocean and the Mediterranean Sea. The present paper provides phenotypic and genetic characteristics of this rare fish species in the Adriatic Sea, which might help us define this population that has obviously entered the Adriatic over the last decade.

## 2. Materials and Methods

### 2.1. Sample Processing and Biometric Analysis

Specimens presumed to be blue jack mackerel (N = 155) and Mediterranean horse mackerel (N = 2) were collected by bottom trawl during the “MEDITS” research survey in July 2018 performed along the whole Croatian fishing ground. The tows were carried out over the continental shelf, between 40 to 800 m for a duration of 30 to 60 min, depending on the depth, and the mean trawl speed was 3 knots. Studied specimens were caught in 13 out of 63 hauls (Figure 1). Collected specimens were immediately frozen and by the end of the survey transported to the laboratory for detailed analysis.

In the laboratory, fourteen morphometric variables were measured: total length (TL), standard length (SL), fork length (FL), preanal (PAL) distance, head length (LH), eye diameter (O), maximum (H) body height, length of first (LD1) and second dorsal fin (LD2), length of pectoral fin (LP), as well as five meristic characters: number of rays in first (D1) and second dorsal fin (D2), in pectoral fin (P), in ventral fin (V) and in anal fin (A), according to Jardas [6].

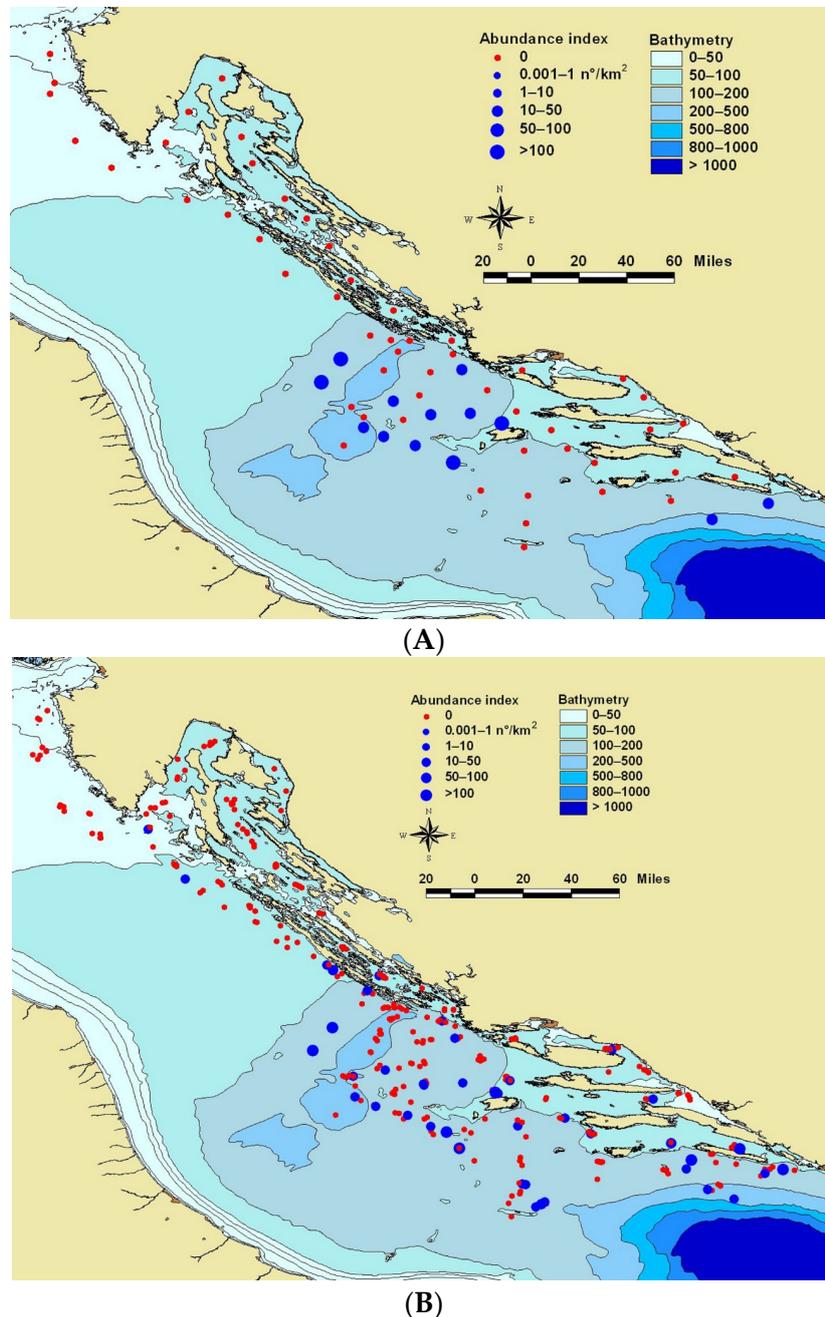
Each body length ( $\pm 0.1$  mm) and total body weight (W) ( $\pm 0.01$  g) was measured. Morphometric characters were expressed as % of TL, with the exception of eye diameter (O), which was expressed as % of LH.

Length–length relationships were determined by the method of least squares to fit a simple linear regression model. Length conversion equations were derived for total length (TL) and the head length (LH). The length–weight relationship was calculated by applying the exponential regression  $W = aTL^b$ , where W = total fish weight in g; TL = total length in cm; a = proportionality constant; and b = allometric growth parameter. The hypotheses of isometric growth were tested using Student’s *t*-test ( $p < 0.05$ ).

### 2.2. Genetic Analysis

For genetic analysis, the sub-samples of muscle tissue were collected from 40 individuals and stored in absolute ethanol until genomic DNA (gDNA) extraction. Total genomic DNA (gDNA) was isolated using standard proteinase K digestion and phenol–chloroform extraction protocols [17] and stored in TE buffer at  $-20$  °C. Quantity and quality of isolated gDNA were assessed using Nanophotometer (IMPLEN), at 260 and 280 nm. Samples with a 260/280 ratio between 1.7 and 1.9 were used as templates in a polymerase chain reaction (PCR). The fragment of *cytochrome b* locus (*cytb*) was amplified by PCR using the universal primers L14841 and H15149, described by Kocher et al. [18]. PCR was run in 25  $\mu$ L reactions combining 0.125  $\mu$ L of HotStarTaq DNA Polymerase (5 u/ $\mu$ L), 2.5  $\mu$ L of

10xPCR buffer, 1  $\mu\text{L}$  of  $\text{MgCl}_2$  (25 mM), 0.5  $\mu\text{L}$  of dNTP (0.25 mM each), 0.5  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ) and DNase/RNase free PCR water to a volume of 25  $\mu\text{L}$ . PCR conditions were as follows: 15 min at 95  $^\circ\text{C}$ , 35 cycles of 94  $^\circ\text{C}$  for 45 s, 51  $^\circ\text{C}$  for 45 s and 72  $^\circ\text{C}$  for 1 min, with a final extension at 72  $^\circ\text{C}$  for 10 min. PCR products were visualized on 1% agarose gel under UV transilluminator. Purification and Sanger sequencing was performed commercially by Macrogen Europe (Amsterdam, The Netherlands).



**Figure 1.** Map of the Adriatic Sea showing the sampling stations of the survey MEDITS done in July 2018 (A) and MEDITS surveys done over the last six summers (2013–2018) (B). The red (negative stations) and blue (positive stations) dots indicate the sampling stations, where blue dot dimensions varied depending on the number of the blue jack mackerel *Trachurus picturatus* collected.

The obtained sequences were analysed for similarity with other known vertebrate sequences using Blast Local Alignment Search Tool (BLAST) [19]. The obtained sequences (~350 bp) were compiled along all publicly available partial *cytb* sequences of *T. picturatus*, as well as selected sequences of

related Carangidae species, *T. trachurus* and *T. mediterraneus* in MEGA 7 [20] and aligned by ClustalW incorporated in the software, using default parameters. The alignment had to be shortened in order to overlap all publicly available *cytb* sequences of *T. picturatus* with those sequenced within this research. This resulted in 296 partial *cytb* sequences of 200 bp. Since the resulting fragment was sufficiently variable, it was deemed more beneficial to compare the new data to all the data collected thus far, than to use full length of the new sequences and not be able to compare them with a large part of the compiled dataset.

Bayesian inference (BI) [21] analysis was performed on 296 partial *cytb* sequences in MrBayes v3.2.7 [22] using a HKY evolutionary model of nucleotide substitution [23], previously selected in MEGA 7 [20] by AICc (Akaike Information Criterion, corrected) value. Four incrementally heated Markov chains were carried through 4,000,000 generations. Sampling frequency was set at 1000 generations, applying default discard of the first 25% samples from the cold chain. Markov chain Monte Carlo (MCMC) parameters were set at default. A 50% majority rule consensus tree produced in the BI analysis was illustrated using FigTree v1.4.4 [24]. The tree was rooted using *Selene dorsalis* as an outgroup, as *S. dorsalis* belongs to the same family as the genus *Trachurus* (Carangidae). GenBank accession numbers of all compiled sequences are incorporated in their IDs, for easier access and traceability.

DnaSP 5.19 [25] and Arlequin 3.5.2 [26] were used to calculate genetic diversity indices. The demographic history for *T. picturatus* populations in Mediterranean Sea and Atlantic Ocean were tested using Tajima's D [27] and Fu's  $F_S$  [28], where negative values indicated population expansion or historical bottleneck [29]. 10,000 simulated samples were used to test the significance under a model of constant population size in Arlequin 3.5.2 [26]. A hierarchical spatial analysis of molecular variance (AMOVA; 10,000 permutations) was performed on two groups: Mediterranean (Adriatic Sea, Central and Eastern Mediterranean Sea) vs. Atlantic Ocean (mainland Portugal, Madeira, Canary Islands and Azores).

Network was constructed in PopART using the integer neighbour-joining (IntNJ) method and sampling localities were used to depict geographical distribution of the sequenced haplotypes [30]. The IntNJ method computes a distance matrix from which a neighbour-joining tree is inferred, while the integer edge lengths are chosen using integer linear programming [30]. Two sequences were excluded from Arlequin analysis and network construction, as they were the only two belonging to Western Mediterranean locality (N = 251 partial *cytb* sequences). The figures were prepared for publication in Inkscape version 1.0.

### 3. Results

All collected specimens were mainly caught above the continental shelf of the open sea area of the middle eastern Adriatic, precisely from 50–200 m of the sea depth (Figure 1A.). Total length of all *T. picturatus* analysed specimens (N = 155) varied from 9.2 to 33.7 cm (mean  $\pm$  SD: 12.15  $\pm$  2.95 cm), while their total body weight ranged from 5.79 to 384.94 g (mean  $\pm$  SD: 17.64  $\pm$  39.42 g). Length frequency distribution was unimodal with very few specimens whose total length was above 14 cm. The results of relative morphometric relationships of measured body proportions expressed in percentages, as well as five meristic characteristics, are given in Table 1. Obtained coefficients of variation (CV) values were relatively low (CV < 10%) for all phenotypic characteristics (Table 1). Obtained relative morphometric relationships were also analysed as function of total body length (TL) and head length (LH). Those analyses pointed out that, with the increase in blue jack mackerel total body length (TL) morphometric relationships of FL/TL (FL/TL = 92.44 – 0.095TL;  $p < 0.05$ ) and PAL/TL (PAL/TL = 61.35 – 0.499TL;  $p < 0.05$ ) significantly decrease, while the morphometric relationship of LP/TL (LP/TL = 16.74 + 0.238TL;  $p < 0.05$ ) significantly increases. According to obtained data, blue jack mackerel has the following set of meristic characters: D1: VI–IX, D2: I + 25–35, A: II + 24–32, P: 17–23, V: 5.

All calculated length–length relationships and their coefficient of determination ( $r^2$ ) are presented in Table 2. Overall, obtained length–length regressions were statistically significant ( $p < 0.05$ ) with a high value of the coefficient of correlation ( $r > 0.923$ ).

**Table 1.** Morphometric (relative relationships of measured body proportions) and meristic characteristics of blue jack mackerel *Trachurus picturatus* collected during the MEDITS survey in July 2018, eastern Adriatic Sea.

Morphometric					
Relation	N	Range (%)	$\bar{x} \pm SD$ (%)	$\bar{x} \pm SE$ (%)	CV (%)
FL/TL	155	79.9–98.3	91.3 ± 2.0	91.3 ± 0.1	1.42
SL/TL	155	75.0–95.7	86.8 ± 2.5	86.8 ± 0.1	1.59
PAL/TL	155	40.3–63.7	55.3 ± 17.7	55.3 ± 0.3	4.21
LH/TL	155	15.3–32.8	24.1 ± 2.8	24.1 ± 0.1	1.67
O/LH	155	17.9–38.9	24.8 ± 5.9	24.8 ± 0.2	2.43
H/TL	155	13.9–23.5	17.8 ± 1.4	17.8 ± 0.1	1.16
LD1/TL	155	11.8–20.0	14.3 ± 1.3	14.3 ± 0.1	1.14
LD2/TL	155	29.2–41.5	35.5 ± 2.4	35.5 ± 0.1	1.56
LP/TL	155	16.5–26.4	19.6 ± 2.0	19.6 ± 0.1	1.43
Meristic					
Characteristic	N	Range	$\bar{x} \pm SD$	$\bar{x} \pm SE$	CV
1st dorsal fin rays (D1)	59	6–9	7.8 ± 0.58	7.8 ± 0.07	7.39
2nd dorsal fin rays (D2)	59	26–36	32.0 ± 2.33	32.0 ± 0.30	7.31
Pectoral fin rays (P)	59	17–23	19.9 ± 1.50	19.9 ± 0.19	7.53
Anal fin rays (A)	59	26–34	30.9 ± 1.78	30.9 ± 0.23	5.78
Ventral fin rays (V)	59	5	5 ± 0.00	5.0 ± 0.00	0

$\bar{x}$ —mean value; SD—standard deviation; SE—standard error of a mean value; CV—coefficient of variation.

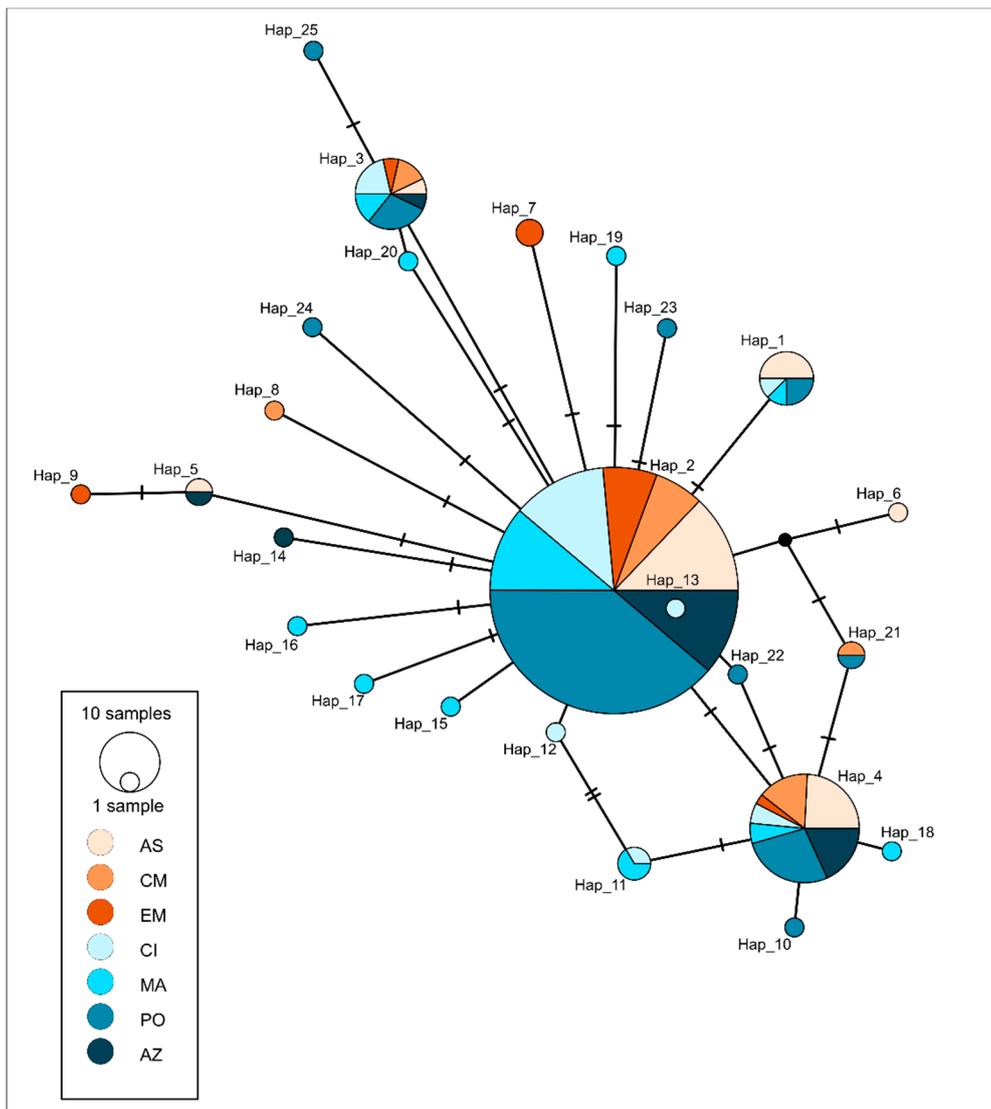
**Table 2.** Length–length regression parameters (a,b) and determination coefficient ( $r^2$ ) of blue jack mackerel *Trachurus picturatus* (N = 155) collected during the MEDITS survey in July 2018, eastern Adriatic Sea.

Equation	a	b	$r^2$
FL = a + bTL	0.29	0.89	0.995
SL = a + bTL	−0.05	0.87	0.994
PAL = a + bTL	1.8	0.4	0.852
LH = a + bTL	−0.11	0.25	0.934
O = a + bLH	−0.02	0.25	0.883
H = a + bTL	−0.22	0.2	0.943
LD1 = a + bTL	0.05	0.14	0.877
LD2 = a + bTL	−0.10	0.36	0.967
LP = a + bTL	−0.85	0.27	0.957

Length–weight relationships of investigated species pointed out that blue jack mackerel has positive allometric growth ( $W = 0.0048TL^{3.1789}$ ,  $r^2 = 0.9801$ ), since its allometric coefficient (b) was statistically significant different ( $p < 0.05$ ) from isometric value ( $b = 3$ ).

Sequencing of the fragment of the *cytochrome b* locus revealed that specimens belonged to the *Trachurus picturatus* (Bowdich, 1825) (N = 37, length of the sequence 319 bp, GenBank accession numbers MN412596 to MN412632). Two specimens caught in the same batch were confirmed as *T. mediterraneus* (Steindachner, 1868) (GenBank accession numbers MN412633 and MN412634). Among 11 polymorphic sites observed in *cytb* fragment of *T. picturatus*, 4 were singleton variable sites and 7 parsimony informative sites, giving total of 10 haplotypes. Overall haplotype diversity (h) of  $0.812 \pm 0.048$  and nucleotide diversity ( $\pi$ ) of  $0.0064 \pm 0.0007$  indicated high levels of haplotype diversity and low nucleotide diversity. When the sequences obtained within this research were aligned with all publicly available data (N = 251) on *T. picturatus cytb* mitochondrial gene, the overall h was  $0.521 \pm 0.036$  and  $\pi$  was  $0.0033 \pm 0.0003$ . Descriptive statistics of genetic diversity and demographic history of *T. picturatus* for each sampling locality, based on collected *cytb* sequence data, is summarised in Table 3.





**Figure 3.** Integer neighbour-joining (IntNJ) network depicting mutational relationships between haplotypes of blue jack mackerel *Trachurus picturatus* (represented by circles in the lower left corner and coloured according to sampling locality (shades of orange represent Mediterranean Sea: AS—Adriatic Sea, CM—Central Mediterranean and EM—Eastern Mediterranean, while shades of blue represent Atlantic Ocean: CI—Canary Islands, MA—Madeira, PO—Portugal mainland and AZ—Azores). Samples collected from the Adriatic Sea were sequenced within this research, while the rest of the sequences were publicly available from GenBank. The integer edge lengths are chosen using integer linear programming by default, when IntNJ is selected in PopART [30].

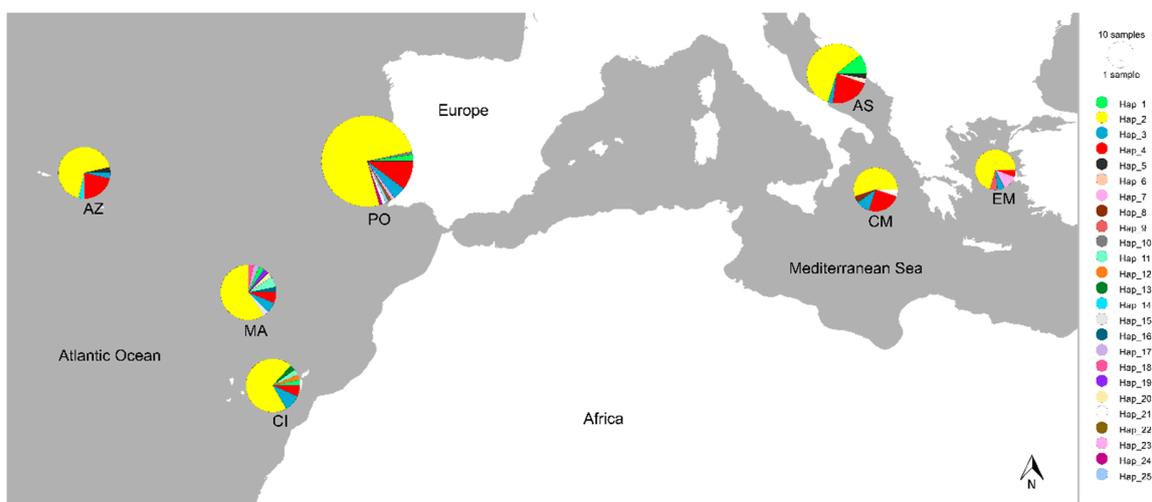
**Table 3.** Descriptive statistics of genetic diversity and demographic history of *Trachurus picturatus*, based on *cytb* sequence data. Sequences from the Adriatic Sea were obtained within this study, while the rest of the sequences were collected from GenBank, for comparison purposes.

Sampling Locality	N	h (±SD)	π (±SD)	S	Tajima's D (p = 0.10)	Fu's F <sub>S</sub> (p = 0.06)
Adriatic Sea	37	0.602 ± 0.075	0.0038 ± 0.0007	6	-1.274 (p = 0.10)	-2.217 (p = 0.06)
Eastern Mediterranean Sea	17	0.945 ± 0.027	0.0085 ± 0.0016	5	-1.719 (p = 0.02)	-2.308 (p = 0.01)
Central Mediterranean Sea	20	0.652 ± 0.093	0.0042 ± 0.0009	4	-0.760 (p = 0.26)	-1.592 (p = 0.08)

Table 3. Cont.

Sampling Locality	N	h ( $\pm$ SD)	$\pi$ ( $\pm$ SD)	S	Tajima's D	Fu's $F_S$
Portugal mainland	87	0.415 $\pm$ 0.065	0.0027 $\pm$ 0.0005	9	-1.788 ( $p = 0.01$ )	-8.135 ( $p < 0.001$ )
Canary Islands	30	0.508 $\pm$ 0.108	0.0032 $\pm$ 0.0008	5	-1.352 ( $p = 0.07$ )	-4.511 ( $p < 0.001$ )
Madeira	32	0.649 $\pm$ 0.096	0.0047 $\pm$ 0.0010	9	-1.764 ( $p = 0.02$ )	-8.764 ( $p < 0.001$ )
Azores	28	0.131 $\pm$ 0.082	0.0004 $\pm$ 0.0003	4	-1.182 ( $p = 0.11$ )	-2.239 ( $p = 0.04$ )

N is the number of analysed sequences, h is haplotype diversity ( $\pm$ SD),  $\pi$  is nucleotide diversity ( $\pm$ SD) and S is the number of segregating sites. Tajima's D and Fu's  $F_S$  are the two neutrality tests applied.



**Figure 4.** Sampling localities of sequenced blue jack mackerels *Trachurus picturatus* were placed on a map using PopART and they were named in the same manner as on the IntNJ network (Mediterranean Sea: AS—Adriatic Sea, CM—Central Mediterranean and EM—Eastern Mediterranean; Atlantic Ocean: CI—Canary Islands, MA—Madeira, PO—Portugal mainland and AZ—Azores). Colours indicate different mitochondrial haplotypes (N = 25), and the size of the circles reflects the observed haplotype frequency. Note the presence of the most frequent haplotype Hap\_2 on each sampling locality. Samples collected from the Adriatic Sea were sequenced within this research, while the rest of the sequences were publicly available from GenBank. GPS of the localities was approximated according to the description from published research papers/supplementary data or sequence description on GenBank.

#### 4. Discussion

This is the first time that specimens of blue jack mackerel were collected in sufficient quantity that allowed for the analysis of its phenotypic and genetic characteristics and the area of its distribution in the Adriatic Sea. Specimens of blue jack mackerel occurred over the continental shelf (depth range: 50–200 m, Figure 1) of the middle-Eastern Adriatic, characterised mostly by the muddy sea bottom [31] during the investigated period. According to the available literature, this species was also found in the areas of the shallow Northern Adriatic [11], which coincided with the distribution pattern obtained for this species over the last six MEDITS surveys (2013–2018; Figure 1B). The area of blue jack mackerel appearance in the Adriatic was in line with the studies done in the area of the Macaronesian archipelagos, where this species is common and represents an important fishery resource [3,32,33]. Due to the length frequency distribution, it was obvious that, throughout this survey, the majority of collected specimens were smaller (TL < 14.0 cm) and immature, which was in accordance with reported length at first maturity for this species [34]. The possible confirmation

of blue jack mackerel spawning in the Adriatic Sea was through the genetic identification of blue jack mackerel larva collected (42°56'21" N, 15°10'5" E; near Jabuka/Pomo pit) during the scientific survey, performed along the Eastern Adriatic (Croatia) in June–July 2019 (GenBank accession number MT795964). A few larger specimens (TL > 28.0 cm) had gonads and were determined as females. Blue jack mackerel morphological values obtained within this study were in line with those provided by Bolognini et al. [11], for the same species inhabiting the same geographical area, as well as the ones reported via [www.fishbase.org](http://www.fishbase.org). Meristic characters of studied species collected in the Adriatic Sea fit within the ranges of the same ones provided through determination keys of [2] and Jardas [6]. The only meristic difference between this study and Jardas [6] was observed in ventral fin, as Jardas [6] noted the presence of one spine in mentioned fin.

All length–length relationships were linear and coincide with the previously reported length–length relationship in the Adriatic [11] and other areas. Concerning the length–weight relationship, blue jack mackerel specimens observed within this study had positive allometric growth ( $b = 3.1789$ ) which was in line with all the previous studies analysing this relationship along the blue jack mackerel area of distribution [35–37].

The genetic identification and phylogenetic reconstruction in this study was expanded and put in a wider context of the Mediterranean Sea and the Northeast Atlantic Ocean. These results offered an additional confirmation of the genetic distances of the three European species of the genus *Trachurus* (*T. trachurus*, *T. mediterraneus* and *T. picturatus*). Furthermore, recurring evidence of high levels of haplotype diversity and low levels of nucleotide diversity across the blue jack mackerel's distribution [38–41], now including the Adriatic Sea results, suggested no established genetic structure. This observed lack of significant variation between populations [39,41] indicated considerable gene flow and the existence of a single panmictic population. In contrast, other approaches, such as using geometric morphometrics and otolith phenotypic variability in the Northeast Atlantic have established the existence of a complex population structure [42] or indicated towards distinguishable populations [43]. Nevertheless, the most recent assessment of the population genetic structure of *T. picturatus* using microsatellites, a much more sensitive tool to detect recent changes than mitochondrial genes, often applied thus far, revealed a lack of genetic structure in the Northeast Atlantic and Mediterranean Sea [44]. The authors concluded that the most probable explanation is the faster evolutionary rate of environmentally dependent phenotypic traits (for example Tanner et al. [45]). Although at this point in time there lacks genetic evidence of separate stocks, it is possible a more comprehensive study design and greater number of analysed blue jack mackerels would have revealed these subtle genetic changes in population structures or they will follow the divergence of environmentally-dependent phenotypic traits. What can be claimed with certainty is that the local spawning aggregations do exist. They have been confirmed in the Azores [3] and Canary Islands [42] and now it seems the spawning might have occurred in the Adriatic Sea as well.

## 5. Conclusions

Bearing in mind that, over the last few decades, climate regimes in the Adriatic [46] have changed, the occurrence of new species or increased abundance of very rare species is not surprising. Hence, awareness of the role of this within an ecosystem and the possible effect that it has on local populations should be monitored and well documented.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-2818/12/12/463/s1>, Table S1: Number of *cytb* haplotypes obtained in each population of *Trachurus picturatus*, Table S2: List of *cytb* haplotypes obtained from *Trachurus picturatus* and the sequences that belong to each haplotype, Table S3: Hierarchical analysis of molecular variation (AMOVA) for *Trachurus picturatus* populations, based on *cytb* sequences data.

**Author Contributions:** B.Z. and N.V. designed the concept of the study, V.Č.K. and I.I. analysed the biometric data, V.V. performed field work and measurements, I.L.P. and I.R. isolated D.N.A. and edited sequence data, I.B. analysed genetic data, B.Z. and I.B. wrote the majority of the manuscript, while all authors reviewed the text and

contributed to writing within their area of specialty. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Ethical Statement:** The sampling within this research was conducted during “MEDITS” research survey, supported by the European Commission (Directorate for Fisheries) jointly with the contribution of the partner countries. It complies with the Marine Fisheries Act in Croatia (NN 62/17; NN 130/17; NN 14/19). The animals collected through this scientific survey, and subsequently used in the research presented here, were not exposed to any unnecessary pain, injuries or suffering. Obtaining specialised permission was not necessary for the type of research conducted.

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