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The black goby *Gobius niger* Linnaeus, 1758 in the Marchica Lagoon (Alboran Sea, Morocco): spatio-temporal distribution, its environmental drivers, and the site-related footprint

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Abstract

Gobies (Gobiidae) are well represented in the Marchica Lagoon on the Moroccan Mediterranean coast, both in terms of species richness and in terms of abundance, with the black goby, *Gobius niger* Linnaeus, 1758, being the dominant species. The present study aims to examine (1) the spatial and temporal distribution of *G. niger* and its environmental drivers in the lagoon and (2) the potential lagoon-related footprint using morphometric, genetic, and parasitological proxies. Systematic monthly sampling covering the whole lagoon basin performed between October 2015 and September 2016 revealed a year-long presence of *G. niger* throughout the lagoon with significantly low densities in winter. The highest abundances were recorded in the shallow bottoms of the lagoon's inner margins on a variety of substrates (mud, muddy-sand, sandy-mud, and fine sand) mostly covered by macroalgae and/or seagrass meadows. Depth was revealed to be the most important predictor of the distribution of the black goby in the lagoon. It should, however, be regarded as a variable that acts synergistically with other factors, such as temperature, vegetation cover, and the sediment's mean grain size. Comparison of black goby populations from the lagoon with their conspecifics from the adjacent Mediterranean coast of Morocco revealed that specimens caught at sea are larger than those of the lagoon population. Of the 180 gobies investigated, not a single one hosted the parasites we targeted in the parasitological approach, monogenean flatworms. The absence of population structuring, low genetic diversity, and the presence of common haplotypes indicate no apparent restriction in the gene flow between the two populations. Therefore, the observed morphometric differences seem to be due to external environmental conditions rather than genetic differences.

Keywords: Transitional waters; Mediterranean Sea; abundance; Gobiidae; morphometry; parasites; genetics.

Introduction

Coastal lagoons are aquatic ecosystems at the terrestrial and marine interface, occupying approximately 13% of the world's coastline (Kjerfve, 1994). Due to the multiple ecosystem services they provide (Levin *et al.*, 2001) (e.g. shoreline protection, fishery resources, and nursery area), lagoons are considered one of the most valuable coastal habitats on the planet (Pérez-Ruzafa *et al.*, 2019). However, combined natural and man-made stressors make them among the most heavily exploited and threatened natural systems worldwide (Eisenreich, 2005; Newton *et al.*, 2018).

Fish play a fundamental role in ecological processes, through trophic relationships with other biotic components (Stein *et al.*, 1995; Vanni, 2002), and are essential for the functioning and resilience of lagoon ecosystems (Koutrakis *et al.*, 2005; Franco *et al.*, 2006; Aliaume *et al.*, 2007). They are highly valuable for the local human population as a food supply and for providing livelihood

(Holmlund & Hammer, 1999; Lopes & Videira, 2013; Newton *et al.*, 2014). Moreover, fish are relevant biotic indicators of the biodiversity and ecological status of ecosystems (Whitfield & Elliott, 2002; Breine *et al.*, 2010). Therefore, reliable scientific data on the fish fauna and abiotic components of lagoon ecosystems are of particular importance for effective management (Vasconcelos & Galyean, 2007), ensuring the sustainability of ecosystem functions and services.

With 2,949 currently recognized species, Gobiidae is the most species-rich family of fishes (Fricke *et al.*, 2023; Renoult *et al.*, 2022). Gobies are found in both marine and freshwater environments (Renoult *et al.*, 2022). They show the highest species richness in warm temperate and tropical seas, on the continental shelf, mostly in the shallow part. Typically, gobies are small and have short lifespans, with the majority residing within the substrate or concealed within various hidden spaces. Among them are epibenthic, hyperbenthic, and cryptobenthic species, while some gobiid species are nektonic (Kovačić & Patzner, 2011). Gobies have a crucial trophic function by linking benthic invertebrates to larger predatory fish (Casabianca & Kiener, 1969; Miller, 1988; Raffaelli *et al.*, 1989; Tiralongo *et al*., 2021). Despite their low commercial value, gobies play an important role as food resources for many commercially important species.

In the Mediterranean basin, 78 species of Gobiidae are currently known to occur (Kovačić *et al.*, 2022; Mavruk *et al.*, 2022). In the Mediterranean, 249 fish species were listed as inhabiting estuaries and/or lagoons, including both resident fishes (euryhaline species, which spend their entire life cycle inside lagoons and estuaries) and migratory fishes (species that, after spending some time in lagoons, are obliged to return to their marine or river environment to complete their life cycle) (Kara & Quignard, 2019). Gobiidae (at least 11 species) constitutes the most represented family of resident fish in Mediterranean lagoons (Kara & Quignard, 2019).

The Marchica Lagoon (35.16° / -2.85°), situated on the Moroccan Mediterranean coast, is known to host a diverse fish fauna supporting important fishing activities (Selfati, 2020). Since the first inventory in 1911 (Oden, 1914), Gobiidae has been found to be well represented in the Marchica Lagoon, with the black goby, *Gobius niger* Linnaeus, 1758, as the dominant species in the fish fauna (Selfati *et al.*, 2020). However, very little information exists on the ecological characteristics of this species, in particular its spatio-temporal structure in relation to environmental factors.

Resident animals, such as some gobies, are the most suitable ones to be used as indicators of lagoon environmental conditions (Bortone *et al.*, 2005). Their presence and abundance may provide important indications of the status of coastal lagoon habitats (Facca *et al.*, 2020). Assuming that the black goby *G. niger,* the most common species of goby in the Marchica Lagoon, is a resident fish in coastal lagoons (Franco *et al.*, 2008a, 2008b, 2012; Kara & Quignard, 2019; Selfati *et al.*, 2019), our study aims (1) to analyse the spatial and temporal distribution of *G. niger* and its environmental drivers using systematic sampling covering the whole lagoon basin and (2) to examine the potential lagoon-related footprint, i.e. whether the supposed resident population of the black goby in the Marchica Lagoon differs from black gobies from the adjacent Mediterranean coast of Morocco by considering morphometric, genetic, and parasitological proxies.

Materials and Methods

Study area

The Marchica Lagoon (Fig. 1) (35.16° / -2.85°), also called the lagoon of Nador, is one of the largest coast-

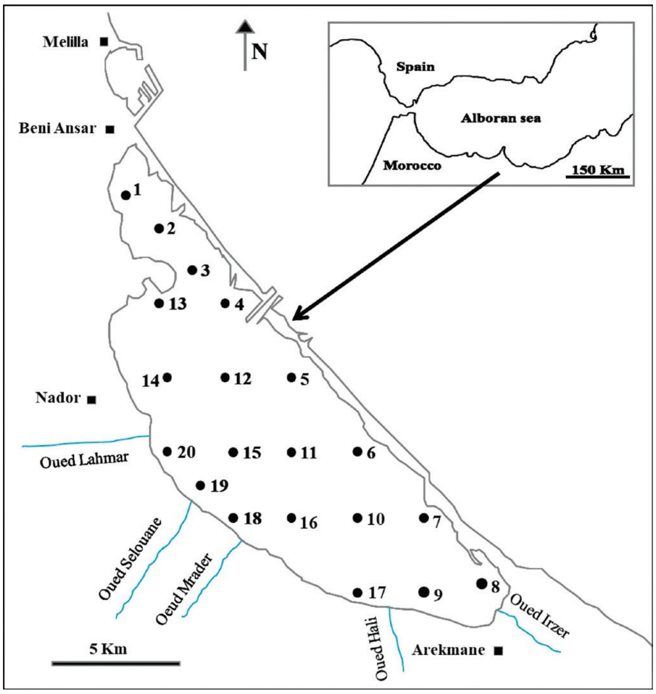

Fig. 1: Map showing the geographical localization of the Marchica Lagoon and the sampling stations of *Gobius niger*.

al lagoons in the Mediterranean (115 km², 25 km long, and 7.5 km wide) and the only one on the Mediterranean coast of Morocco (Selfati, 2020). The maximum depth is approximately 8 m, and the lagoon is separated from the sea by a 25-km-long sandbar (Lido), with one artificial opening (300 m wide and 6 m deep) that allows water exchange. Despite its ecological value (Site of Biological and Ecological Interest since 1996; RAMSAR site since 2005) and socio-economic value (mainly artisanal fisheries), the lagoon is under pressure from a complex mixture of human-mediated stressors (urbanization, pollution, overfishing, tourism, and wastewater, among others) (Selfati *et al.*, 2017; El Kamcha *et al.*, 2020).

Sampling design and environmental data

To examine the spatial and temporal variations in species abundance of *G. niger* and its environmental drivers in the Marchica Lagoon, data on monthly abundances were obtained by monitoring the fish fauna in the lagoon between October 2015 and September 2016. The monitoring was carried out according to an optimized network of 20 stations (S1–S20) covering the whole lagoon (Fig. 1). The collecting gear was a purse seine of about 110 m in length and 11 m in height, with a mesh size of 6 mm. The catches were expressed per unit area. The seine described a circle on the surface of the water with an estimated radius of 16 m, so the encircled surface was 800 m², which was regarded as the unit area (UA). Environmental parameters were measured at each station. Water temperature (T), salinity (S), and conductivity (Cond, mS/cm) were measured *in situ* using a conductivity meter (Cond 315i/SET), and depth (D) was recorded using an LCD Digital Sounder (HONDEX PS-7). The water pH was measured in the laboratory from water samples collected in the field using a pH meter (IONOMETER-EUTECHINSTRUMENTS-CYBERSCAN-PH-510). The measurement of suspended matter (SM, mg/l) in water samples was performed in the laboratory by the 0.45-µm membrane filtration method. The percentage of vegetation cover (VC: combined seagrass and macroalgae) and the nature of the substratum expressed as mean grain size (MGS, µm) were derived from the literature (Najih *et al.*, 2016, 2017). Data were obtained by overlapping the fish sampling points on the vegetation cover map and on the distribution map of surface sediment facies.

To examine the potential lagoon-related footprint, i.e. whether the supposed resident population of the black goby in the Marchica Lagoon differs from black gobies from the adjacent Mediterranean coast of Morocco considering morphometric, genetic, and parasitological proxies, 120 black gobies from the Marchica Lagoon and 60 black gobies from the adjacent Mediterranean coast of Morocco were collected as bycatch of fishing boats in July 2020. All the specimens were stored in separate plastic bags, transported in a portable freezer (Engel MT45), and then stored in the laboratory in a freezer (-20 °C) for further study. After thawing, the fish were numbered, labelled, and photographed. A piece of the pectoral fin of each fish was taken and kept in an Eppendorf tube filled with 96% ethanol for molecular characterization.

Species identification

Many ichthyologists agree that species-level identification of members of Gobiidae is challenging (Kovačić, 2008). Given the small size and the very high species richness in Gobiidae, we have applied two protocols to identify our species in the present work. Firstly, we have used a simpler determination inspired by the determination protocols provided by Brownell & Collignon (1978), Bauchot & Pras (1980), and Bauchot (1987) to determine the genus, and the species was identified by a minimum combination of characters that positively identify the specimens of *G. niger* among species of Gobiidae in the Mediterranean (Kovačić, 2020): (1) suborbital sensory papillae of the head lateral line system without suborbital row *a*, (2) all three head canals of the head lateral line system present, (3) anterior dorsal row *g* of sensory papillae ending behind or on the lateral end of row *o*, (4) six suborbital transverse rows *c* of sensory papillae, (5) anterior oculoscapular head canal with pore *α* at the rear of the orbit, (6) oculoscapular row x^1 of sensory papillae ending forward behind pore *β*, (7) longitudinal scale count < 50, (8) predorsal area scaled, and (9) suborbital row *d* of sensory papillae continuous.

Spatial and temporal distribution of G. niger in the Marchica Lagoon and environmental drivers

Spatial and temporal variations in abundance of *G. niger* (expressed as densities per 800 m²) were illustrated on maps using 11 classes of abundance based on the Sturges rule (Sturges, 1926). The spatial pattern of abundances (expressed as abundance per month and per station) of *G. niger* in the Marchica Lagoon was explored to identify affinity groups of stations. To achieve this, a hierarchical cluster analysis associated with a similarity profile test (SIMPROF) was used. The analysis was conducted on a transformed fourth-root abundance similarity matrix, aiming to mitigate the impact of highly abundant occurrences on the final results (Clarke, 1993). The similarity matrix was based on the Bray–Curtis similarity measure, which is robust in cases of concurrent absences (Field *et al.*, 1982). Differences between sampling stations and between seasons (winter: December, January, and February; spring: March, April, and May; summer: June, July, and August; autumn: September, October, and November) were tested with a two-way crossed PERMANOVA design, followed by post hoc pairwise tests.

Distance-based linear modelling (DISTLM) was performed to identify the key environmental drivers of the black goby's distribution pattern in the Marchica Lagoon. The best overall model was selected using the BEST selection procedure with the Akaike information criterion (AIC) in order to reveal the significant variables influencing the observed patterns in spatial abundance (Akaike, 1973; Anderson *et al.*, 2008). A distance-based redundancy analysis (dbRDA; Legendre & Anderson, 1999; McArdle & Anderson, 2001) was used to illustrate the results graphically. The draftsman plots, and the associated correlation matrix between all pairs of variables, were examined for evidence of collinearity (Clarke *et al.*, 2014). All the multivariate analyses were performed using the PRIMER 6 software (Clarke & Gorley, 2006).

Characterization and comparison of goby populations from the Marchica Lagoon and adjacent Mediterranean Sea

Morphometric and meristic analysis

Based on the literature (Gaamour *et al*., 2001), six morphometric characters were measured on each specimen with a 1-mm resolution using a Vernier calliper: total length (TL), standard length (SL), head length (HL), snout length (SnL), body height (BH), and eye diameter (ED). Moreover, five meristic characters were considered: number of rays in the first dorsal fin (DF1), number of rays in the second dorsal fin (DF2), number of rays in the anal fin (AF), number of rays in the pectoral fin (PF), and number of rays in the ventral fin (VF) (Gaamour *et al*., 2001) (Fig. 2).

In order to characterize specimens of *G. niger* collected from Marchica Lagoon and the Mediterranean Sea and to describe the relative variations in the different parts of their body, the length–length relationship was estimated using the following expression: Log y = a Log x + Log b, between the standard length (SL) and the total length (TL), the head length (HL), and the body height (BH), as well as between the head length (HL) and the eye diameter (ED) and the snout length (SnL). All the parameters of the length–length relationship were estimated by linear regression analysis using STATISTICA software.

Statistical analyses (Student's t-test) were performed to detect differences in terms of size between specimens from two locations (Marchica Lagoon and adjacent sea). In order to explore whether there is a clustering based on the origin of *G. niger*, a principal component analysis (PCA) was performed using morphometric and meristic characters, after a transformation ($\mathrm{Log}_{10}X + 1$) of the raw measurements in order to linearize the allometries (Huxley, 1932) and to roughly equalize the variances (Jolicoeur, 1963). All morphometric and meristic analyses were performed in the PAST v4.03 software (Hammer *et al*., 2001).

Genetic differentiation

DNA extraction of 120 specimens (60 from the Marchica Lagoon and 60 from the adjacent Mediterranean coast) was performed according to the protocol of Aljanabi & Martinez (1997). Approximately 50 µg of a pectoral fin fragment was digested at 55 °C overnight with 20 µl of proteinase *K* (20 mg/ml) and 180 µl of extraction buffer (0.4 M NaCl, 1 M Tris, 2 mM EDTA, and 40 µl of 20% SDS). The extracted DNA was suspended in 150 µl of sterile double-distilled water and stored at -20 °C until amplification by PCR. We targeted a fragment of the 16S rRNA gene, since mitochondrial ribosomal sequences are well represented in the genetic literature on Mediterranean and European gobies. As they capture interspecific and intraspecific diversity in these fishes, they can be considered potential barcoding markers (Vanhove *et al.,* 2012, 2016, 2022, and references therein). Amplification of the 16S rRNA gene was performed in a final volume of 20 µl, containing 4 µl buffer (10X Standard *Taq* Reaction Buffer), 1 µl of 10 mM dNTPs, 0.8 µl of 10 µM forward primer 16SH (5'-CGCCTGTTTATCAAAAACAT-3'), 0.8 µl of 10 µM reverse primer 16SL 5'-CGCCTGTTTATCAAAAACAT-3') (Palumbi *et al.*, 1991), 0.4 µl (2 units) of *Taq* polymerase, 1 µl of genomic DNA, and 12 µl of nuclease-free water. Amplification reactions were performed in a thermal gradient PCR according to the following program: initial denaturation at 94 °C for 3 min, followed by 40 cycles each with denaturation for 30 sec at 94 °C; a hybridization step for 30 sec at 55 °C and an elongation phase for 1 min at 72 °C; and, at the end, a final elongation phase for 10 min at 72 °C. The PCR products were checked on a 1% agarose gel and sent to the National Center for Scientific and Technical Re-

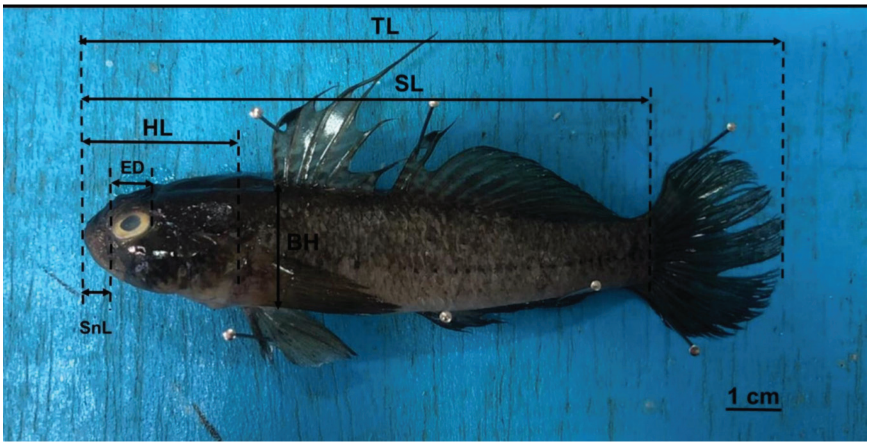

Fig. 2: Picture of *Gobius niger* from the Marchica Lagoon showing the main measurements taken: total length (TL), standard length (SL), head length (LT), snout length (SnL), body height (BH), and eye diameter (ED).

search (CNRST) in Rabat, where they were sequenced by a Genomix sequencer (MGX) using the same forward and reverse primers as for the PCR.

Each DNA sequence obtained in both directions was cleaned and checked in MEGA X (Kumar *et al.*, 2018) to assemble the corresponding consensus sequence. The obtained sequences were aligned with the CLUSTAL W algorithm (Thompson *et al.,* 1994), and then each sequence was blasted (Altschul *et al.*, 1990) with the 16S rDNA sequences of *G. niger* available in NCBI GenBank to check for possible matches.

To make sure that all of our 16S sequences, both the newly obtained Moroccan sequences and those we included from other studies, cluster monophyletically without representatives of other species, a phylogenetic tree was built including all other species of *Gobius* that are represented in the GenBank by the targeted fragment of the 16S rRNA gene (see Supplementary Material Table S1). The phylogenetic tree was constructed with MEGA X using the neighbour-joining algorithm and the Kimura 2-Parameter substitution model (Kimura, 1980) with 1000 bootstrap replicates.

All sequences were confirmed to belong to *G. niger* (Supplementary Material Figure S1) and hence were included in a maximum parsimony analysis in the software package FITCHI (Matschiner, 2016) in order to visualize the genealogical relationships across the geographical range of *G. niger*.

DNASP v6.12.03 (Rozas *et al.*, 2017) was used to calculate molecular diversity indices: number of segregating sites (K), number of haplotypes (H), haplotype diversity (h), and nucleotide diversity (π). Moreover, Fu and Li's F and Tajima's neutrality tests were performed to check for possible selection or change in population demography. The pairwise fixation index (F_{ST}) was calculated in ARLEQUIN v3.5 (Excoffier & Lischer, 2010) using 100 permutations to express the degree of genetic differentiation among sets of individuals.

A matrix correlation analysis (Mantel test; Mantel, 1967), permuting a morphological distance matrix against a genetic distance matrix, was performed using the R v3.5 software package ape (Paradis *et al.*, 2004), running 1000 permutations.

Parasitological screening

Each specimen was placed in plastic bags with tap water, and then the bags were vigorously shaken to detach the parasites from the skin. After that, the external surface of the skin, the fins, and the holding water were examined. Gill arches on the right side of each specimen were removed through ventral and dorsal sections, placed in a petri dish, and rinsed with a rinsing bottle filled with tap water. Then, the water and gill arches were examined under a stereoscope (Wild M8).

Results

Spatial and temporal distribution of* G. niger *in the Marchica Lagoon and its environmental drivers

Cluster analysis based on abundances of *Gobius niger* in the 20 sampled stations in the Marchica Lagoon, supported by a SIMPROF test, separated the samples into two large groups, labelled as G1 and G2, in addition to three atypical stations (S4, S5, and S12), located near the pass (Fig. 3). The G1 group, in which abundances were high (mean = 87.3 ± 166 ind/UA), concerns the north-west (S1 and S2) and south-east extremities of the lagoon (S7, S8, and S9), as well as the west, inner coast around Oued Selouane (S17, S18, S19, and S20). Stations S1 and S17, corresponding to the north-west and south-east extremities, stand out as a subgroup within G1. The G2 group corresponds roughly to the centre of the lagoon, where the goby was overall less abundant (mean = 10 ± 18.5 ind/UA).

The DISTLM marginal tests assessed the importance of each variable separately. They indicated that depth, temperature, and substratum had a significant effect ($p < 0.05$), explaining respectively 49.6%, 22.5%, and 21.3% of the spatial variations of *G. niger* in the Marchica Lagoon (Table 1). Salinity and vegetation cover were close to the limit of significance, and each explained about 13% of the total variance. The remaining variables, pH and suspended matter, did not show any significant impact, and each accounted for less than 1% of the variability. The best model was obtained by a combination of depth, salinity, and suspended matter, which accounted for 58.89% of the variability in the data. In the dbRDA performed on the selected model, the first two axes accounted for 57.9% of the total variability (dbRDA1 = 56.6%; dbRDA2 = 1.3%) (Fig. 4). Depth was the main contributor to the first axis (loading dbRDA = 0.91), while the main contributors to the second factor were salinity and suspended matter (loading dbRDA = 0.57 and -0.64, respectively). Furthermore, depth was negatively correlated with all parameters considered in the present study, with significance for temperature (Spearman's rho; $r = -0.65$, $p < 0.05$) and substrate (Spearman's rho; $r = -0.52$, $p > 0.05$).

The black goby was present in the Marchica Lagoon throughout the sampling year, both over space and time but with variable abundance (Fig. 5). Overall, the monthly abundance in all stations fluctuated between zero and a maximum of 643 individuals. The mean abundance (± SD) was 44.13 (± 88) individuals per station (Table S2). The PERMANOVA results showed significant differences in total abundance between seasons and stations (Table 2). Interactions between the two factors were not significant (Pseudo F = 1.072, Pperm > 0.05). A posteriori pairwise comparison revealed that the black goby was significantly less abundant in winter than in the other seasons (Table 3). Regarding the 'station' factor, most of the significant differences concern the combinations formed by the peripheral and central stations (Table S3).

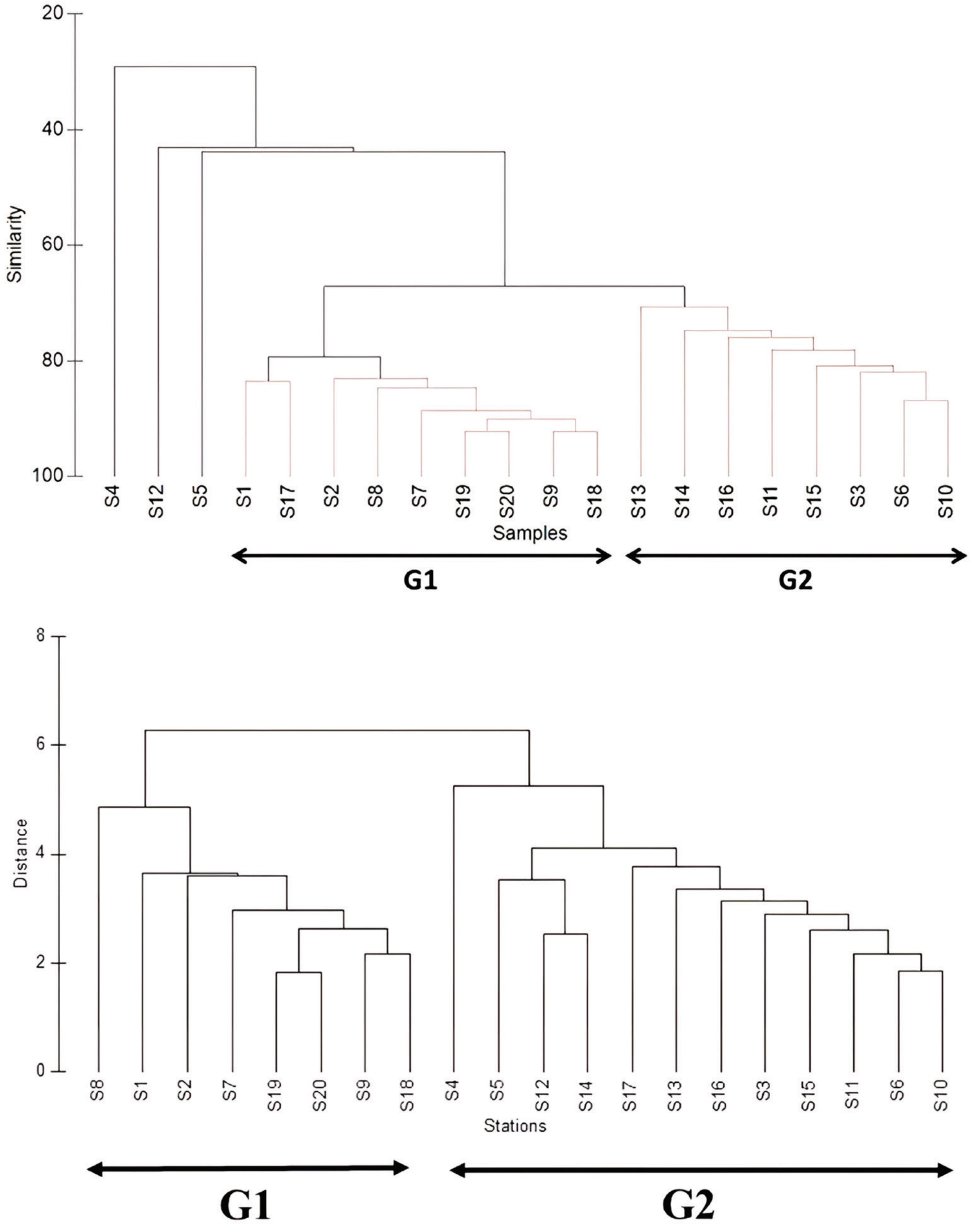

Fig. 3: Cluster analysis associated with the similarity profile test (SIMPROF), based on abundances of *Gobius niger*, reveals reciprocal relations among the 20 sampled stations in the Marchica Lagoon using the Bray–Curtis distance.

Table 1. DISTLM marginal test results: significance (p-value) of the relationship between abiotic factors and monthly abundances of *Gobius niger*. Prop. (%): relative contribution of each environmental variable to the variation in spatial structure.

Variable	SS (trace)	Pseudo-F	p-value	Prop. (%)
Temperature	63.356	5.2469	0.01*	0.2257
Depth	139.49	17.781	0.001*	0.49694
Salinity	38.621	2.8717	0.073	0.13759
pH	25.637	1.8092	0.138	9.13E-02
SM (suspended matter)	14	0.94485	0.348	4.99E-02
VC (vegetation cover)	36.035	2.651	0.072	0.12837
MGS (sediment mean grain size)	60.034	4.8969	0.012*	0.21387

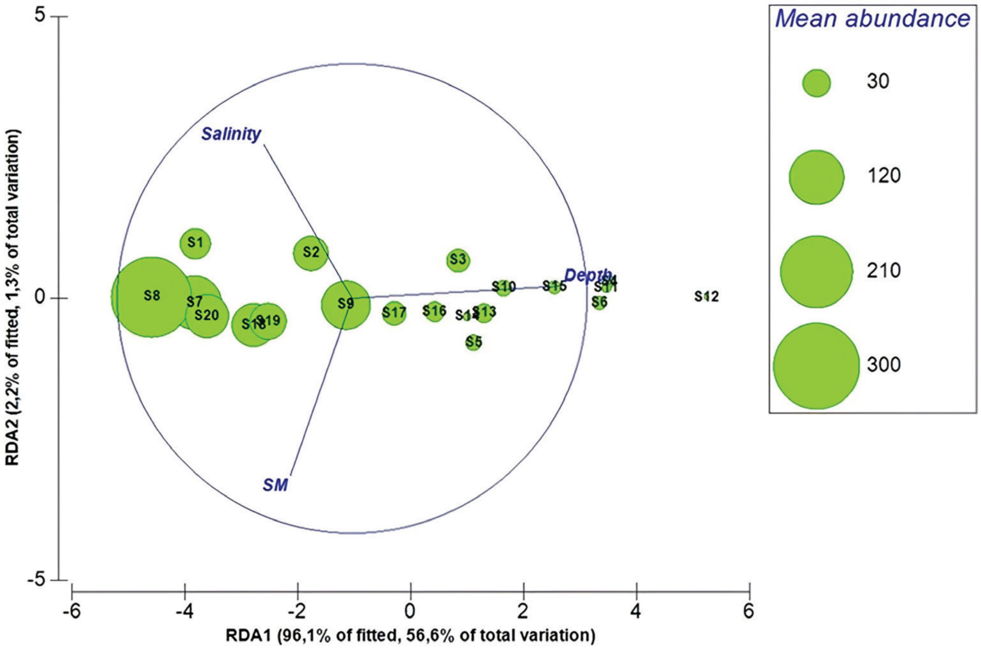

Fig. 4: Two-dimensional redundancy analysis (RDA) ordination representing the spatial distribution of *Gobius niger* related to the predictor variables selected through the best linear models based on distance (DISTLM). SM: suspended matter.

Characterization and comparison of goby populations from the Marchica Lagoon and adjacent Mediterranean Sea

Morphometric and meristic analysis

Data from meristic and morphometric characters of *G. niger* sampled at sea and in the Marchica Lagoon are reported in Tables S4 to S7, respectively. Overall, specimens caught in the sea (110.2 ± 11.2 mm) differ significantly ($t = -12.46$; $p < 0.05$) from the ones caught in the lagoon (84.8 ± 11.1 mm).

The length–length relationships (Log Y = a LogX + Logb) between the standard length (SL) and the total length (TL), the head length (HL), and the body height (BH), as well as between the head length (HL) and the eye diameter (ED) and the snout length (SnL), are summarized in Tables S8 and S9. The results of the morphological analysis indicate significant differences between morphological characteristics (SL and TL, HL, BH; HL and ED, SnL) in the two sites.

The first PCA (PC1 vs. PC2) performed on the morphometric and meristic data allowed the identification of a distinct stratification based on the origin of the individuals. The first two axes are the most informative, representing respectively 78.67% and 9.75% of the total inertia, thus 88.42% of the total variation (Fig. 6). PC1 was strongly correlated with SL (Pearson correlation 0.962) (Fig. 7). PC2 and PC3 were not correlated with SL (Pearson correlation -0.022 and -0.004, respectively), indicating that among-group differences on this axis are independent of specimen size and are directly interpretable as differences in shape. The first axis was correlated with morphometric characteristics, while the second axis was correlated with meristic characteristics.

Genetic differentiation

From the 120 individuals of *G. niger* from Morocco, 88 sequences were obtained. The alignment of the portion of the 16S rRNA gene was 547 base pairs long and contained 26 polymorphic sites; a total of 31 haplotypes were found.

Diversity measures, calculated for the two groups of *G. niger* (Marchica Lagoon and the adjacent sea), are reported in Table 4.

The pairwise F_{ST} comparisons show a low value; the F_{ST} p-value is not significant. This implies that there is not a considerable degree of genetic differentiation between the different groups of *G. niger* from Marchica Lagoon and the adjacent Mediterranean coast of Morocco.

The maximum parsimony network shows three ancestral haplotypes (numbers 22, 29, and 41 in Fig. 8). There is no visible geographical segregation except for the sequences from Turkey and Greece whose haplotypes were not shared with the other individuals. The Western and Central Mediterranean and the Atlantic Ocean populations share haplotypes, while the Eastern Mediterranean population does not.

The result of the Mantel test indicates no correlation between the genetic and morphological distance matrices ($r = -0.11$; $p = 0.97$), suggesting no isolation by distance and no limitation of gene flow between the lagoon and the sea (Fig. 9).

Monogenean parasites from G. niger

Of the 180 specimens of *G. niger* examined, no fish was parasitized by monogenean flatworms, either in the sea or in the Marchica Lagoon.

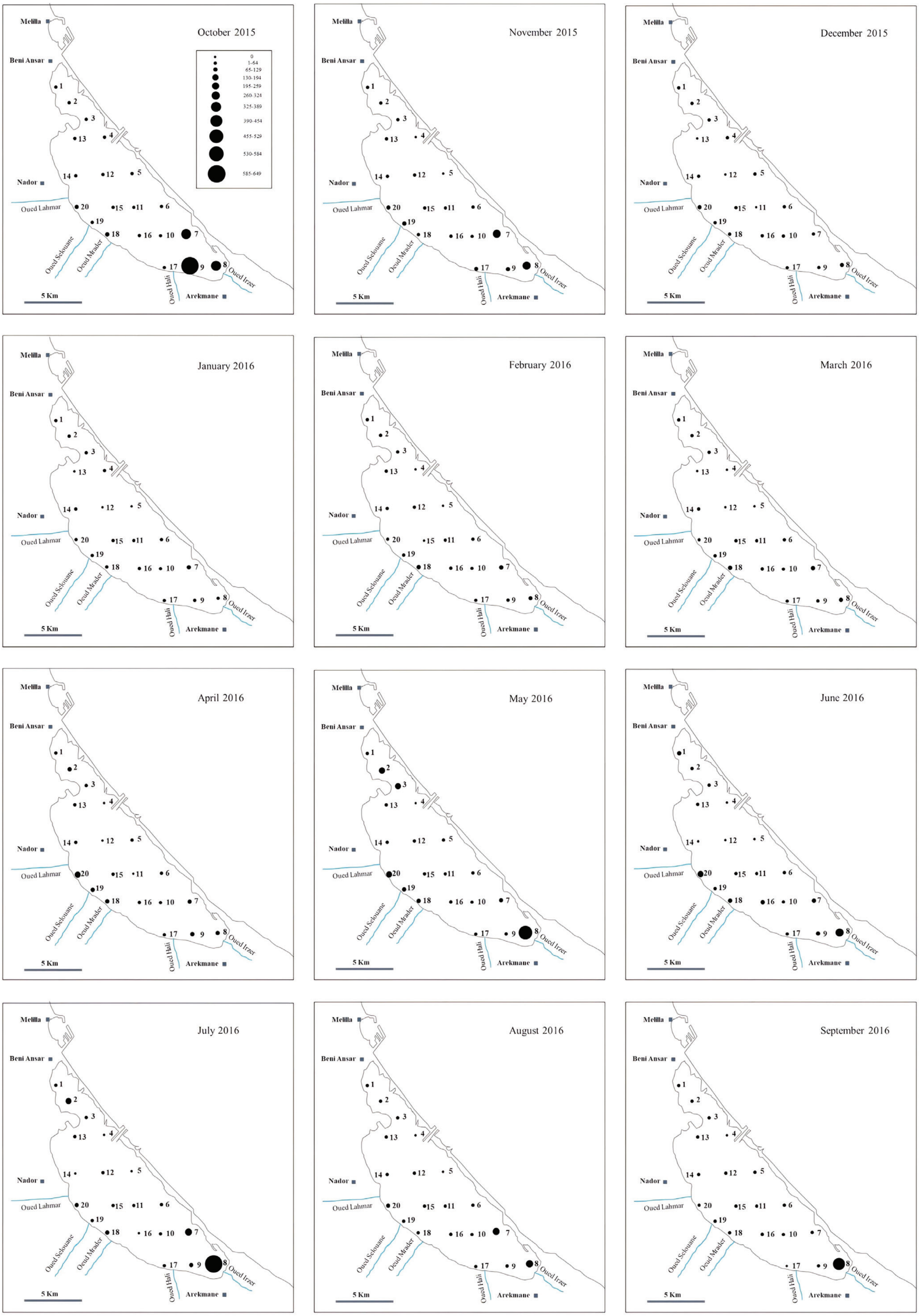

Fig. 5: Spatial and temporal distribution of *Gobius niger* in the Marchica Lagoon.

Table 2. Results of the multivariate permutational analysis (PERMANOVA) of *Gobius niger* considering season and station. Df: degrees of freedom; Pperm: level of significance.

	Sum sq	Df	Mean sq	F value	Pperm
Season	49908	3	16636	3.905	0.011*
Station	860873	19	45309	10.636	0.001*
Season : Station	260307	57	4567	1.072	0.302
Residuals	681627	160	4260		

Table 3. Seasonal mean abundance of *Gobius niger* with pairwise comparisons. The compact letter display ‘a’ and ‘b’ indicates significant mean abundance differences.

	Autumn	Summer	Winter	Spring
Abundance (mean ± SD)	57.1 ± 117^a	53.8 ± 102^a	20.3 ± 27.1^b	45.3 ± 75.1^a

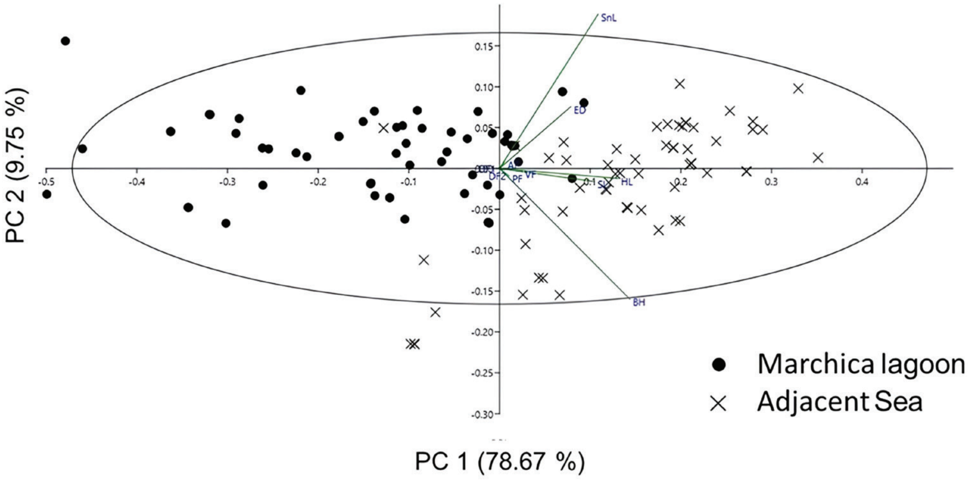

Fig. 6: PCA of the morphological variables of *Gobius niger* (standard length, SL; body height, BH; head length, HL; snout length, SnL; eye diameter, ED; first dorsal fin, DF1; second dorsal fin, DF2; anal fin, AF; pectoral fin, PF; ventral fin, VF) with projection of phenotypic groups. PC1 vs. PC2 and PC2 vs. PC3. The percentage of variation explained by each PC axis is given within parentheses.

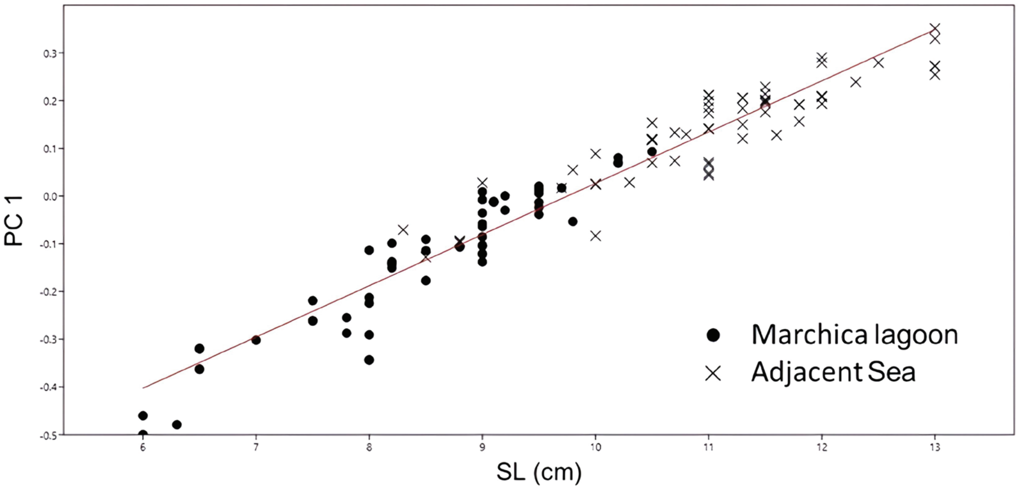

Fig. 7: Linear regression of the principal component score axis (PC1) from morphometric measurements on the log standard length of *Gobius niger* with projection of phenotypic groups.

Table 4. Results of the haplotype diversity analysis of the two groups of *Gobius niger*. N: number of sequences; S: number of segregating sites; Hn: number of haplotypes; Hd: haplotype diversity; π: nucleotide diversity. Significant values at α ≤ 0.05.

Groups	N	S	Hn	Hd	π	F_{ST}	p-value	Fu & Li's F	Tajima's D
Sea	50	20	21	0.85878	0.00413	0.01286	0.09180 ± 0.0088	-2.53253	-1.70437
Marchica Lagoon	38	16	17	0.93741	0.00462				

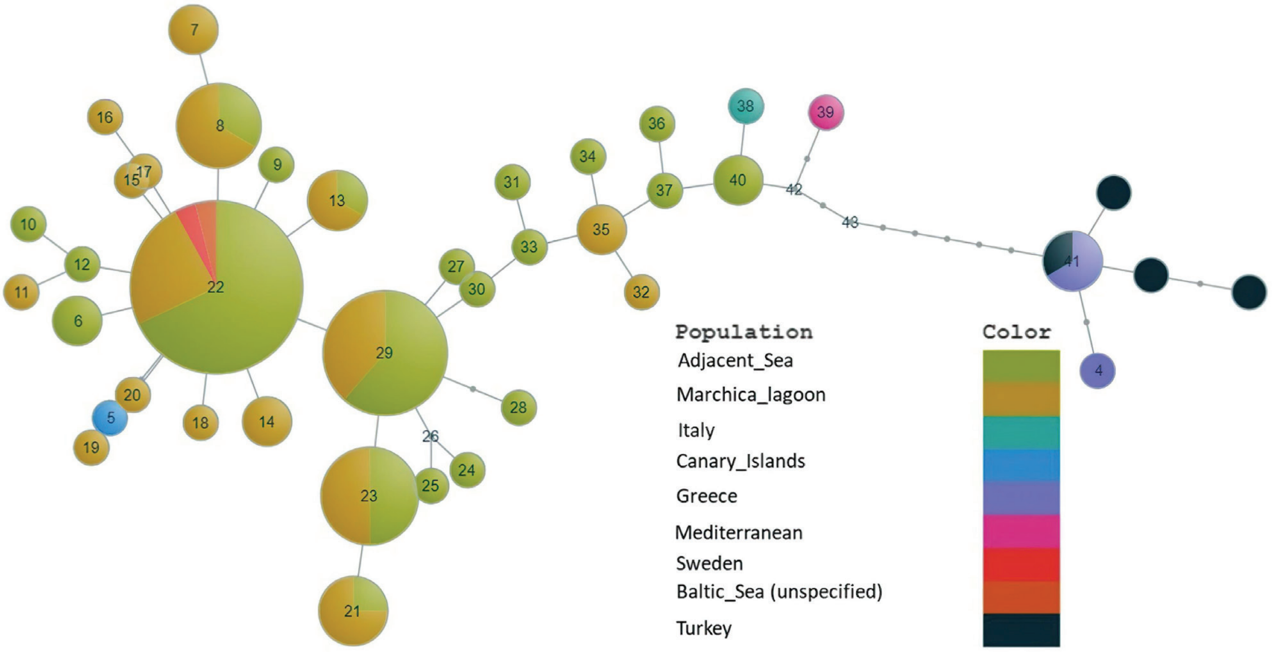

Fig. 8: Haplotype network constructed from 16S rDNA sequences of *Gobius niger*. The size of a particular circle reflects the haplotype frequency. The numbers indicate the nodes.

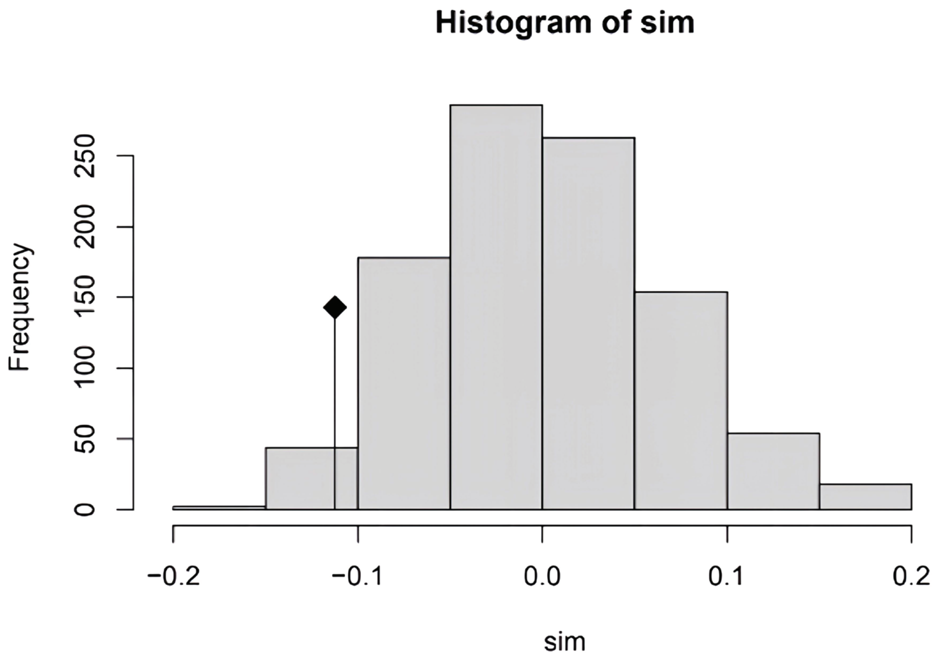

Fig. 9: Histogram of the Mantel test assessing the relationship between genetic and morphologic distance for *Gobius niger*. Sim: simulations; Frequency: frequency values of the correlation between the genetic and morphologic distances. The dot represents the original value of the correlation between the distance matrices.

Discussion

Spatial and temporal distribution of **G. niger** *in the Marchica Lagoon and environmental drivers*

The spatial and temporal distribution of *G. niger* evidenced that the species is permanently present in the Marchica Lagoon, with higher abundances in the peripheral shallow bottoms of the lagoon on a variety of substrates (mud, muddy-sand, sandy-mud, and fine sand) mostly covered by macroalgae and/or seagrass meadows (Najih *et al*., 2016, 2017). This is consistent with the ecology of the species in other Mediterranean coastal lagoons, where it prefers shallow habitats (depth < 1.5 m) on sandy and sandy-muddy vegetated beds (Kara & Quignard, 2019). Moreover, its permanent and abundant presence in the Marchica Lagoon supports its status as resident (Franco *et al*., 2008a, 2008b, 2012; Kara & Quignard, 2019; Selfati *et al*., 2019) and a very frequent fish in Mediterranean coastal lagoons (Kara & Quignard, 2019). *Gobius niger* plays a key eco-trophic role by providing a link between benthic invertebrates and large predators (Casabianca & Kiener, 1969; Miller, 1988; Raffaelli *et al*., 1989). Therefore, the shallow beds of the lagoon, where the species is abundant, are key habitats in the Marchica Lagoon and need to be considered in all management plans aiming at the conservation of biodiversity and ecological processes. Moreover, being a benthic and resident fish, *G. niger* is a suitable candidate for monitoring the ecological status of the Marchica Lagoon, especially in its most disturbed peripheral areas (Ben Hassine *et al*., 1999). Indeed, *G. niger* has been used in many pollution monitoring studies (Fossi *et al*., 1989; Katalay & Parlak, 2002; Migliarini *et al*., 2005; Maradonna *et al*., 2004; Barucca *et al*., 2006; Maradonna & Carnevali, 2007; Ramšak *et al*., 2007).

Overall, depth seems to be the most important predictor variable explaining the distribution of *G. niger* in the lagoon. This is in accordance with the distribution patterns of fish assemblages in the Marchica Lagoon, where a spatial gradient in the bentho-demersal component of the fish assemblage structure was observed, with more marine species occurring near the sea inlet and more resident species in the inner margins of the lagoon (Selfati *et al*., 2019). Moreover, depth is a variable that acts synergistically with other factors, such as vegetation cover, pH, temperature, dissolved oxygen, salinity, and suspended matter (Selfati *et al*., 2019). Furthermore, depth is negatively correlated with all parameters considered in the present study, with significance for temperature (Spearman's rho; $r = -0.65$, $p = 0.001$) and type of substrate (Spearman's rho; $r = -0.52$, $p = 0.01$). This indicates that the shallower areas of the lagoon present different conditions in terms of temperature, illumination, and other factors that support primary productivity. This is in line with the ecological requirements of the black goby, particularly in terms of reproduction, feeding, and shelter from predation. The black goby requires coarse substrates that act as shelters for nest construction (Vaas *et al.,* 1975; Wiederholm, 1987; Mistri *et al.,* 2000; Leatemia *et al.,* 2017). The vegetated habitats are important for their abundance of marine macroinvertebrates, which are the main prey resource for the black goby (Hajji *et al.,* 2013; Matern *et al.,* 2021). Vegetation cover also limits predation by increasing the complexity of the habitats (Heck & Orth, 1980; Kulczycki *et al.,* 1981). The significant decrease in *G. niger* during winter could be related to the low temperatures during this season, leading to migration of the species to deeper marine waters (Nash, 1984). Another explanation proposed by Arruda *et al*. (1993) in the Ria de Aveiro Lagoon (Portugal) is the massive mortality following the spawning period.

Characterization and comparison of goby populations from the Marchica Lagoon and adjacent Mediterranean Sea

Morphometric and meristic patterns

In the Moroccan Mediterranean, the black goby has previously been reported to occur both in the lagoon and in the open sea (Aloncle, 1961; Selfati *et al*., 2019). The work of Böhlke & Robins (1968), Hoese (1971, 1983), Bath (1973), Akihito (1986), Gill *et al.* (1992), and, more recently, Bouchereau *et al.* (2000) and Kovačić & Golani (2007) has shown that the standard length (SL) and head length (HL) are very useful in the classification of genera and species of Gobiidae. The phenotypic traits are under the influence of natural selection and the composite effects of environmental factors, such as temperature, depth strata preference, salinity, and trophic groups, in addition to genetic and ontogenetic factors (Bakhshalizadeh *et al*., 2022). The difference in size between individuals from the lagoon and their marine conspecifics is probably due to the depth difference between the two sites. Indeed, the majority of fishes show a size positively correlated with depth (Harvey & Stewart, 1991). Fish are exposed to the risk of predation; to reduce it, they choose deeper habitats and therefore will have a longer life span, which should be reflected to some extent in a larger body size (Harvey & Stewart, 1991). This difference in size can also be related to temperature. According to the gill oxygen limitation theory (GOLT), there is a negative correlation between the size of fish and the temperature of their habitat; smaller fish may have an advantage in warmer waters because they can extract more oxygen from the water per unit of body mass (Pauly, 2021).

Genetic structure: lagoon and open sea

The genetic structure of marine fish populations reflects the historical and contemporary interaction between a complex set of ecological, demographic, behavioral, genetic, oceanographic, climatic, and tectonic processes. The combined effect of these mechanisms, acting on a range of spatial and temporal scales, determines the rates and patterns of the dispersal of gametes, zygotes, larvae, and adults (Giovannotti *et al*., 2009).

Our study revealed the presence of common haplotypes shared by two groups from different habitats. According to Bortone *et al.* (2005), *G. niger* is considered a resident species in the Marchica Lagoon (Selfati, 2020). However, the results of our study suggest recurrent migrations between the groups of the sea and lagoon. Indeed, the haplotype network reconstruction revealed no apparent population subdivision and no geographical segregation between the lagoon and the sea. F_{ST} values are used to determine the level of gene flow (Chanthran *et al.*, 2020). According to Wright (1965), populations with F_{ST} values of 0–0.05 show a small differentiation, values of 0.05–0.15 a moderate differentiation, values of 0.15–0.25 an important differentiation, and values greater than 0.25 a very important differentiation. The occurrence of common haplotypes between the groups and a small to moderate differentiation based on the F_{ST} results suggests unrestricted gene flow with no relation to habitat.

Parasites of G. niger

Monogenean parasites are one of the largest groups of Platyhelminthes characterized by high species diversity and high host specificity (Gusev, 1995; Kearn, 1994; Poulin, 1998). The most abundant genus of monogeneans in gobies as well as in many other fishes is *Gyrodactylus* von Nordmann, 1832. According to Huyse & Volckaert (2005), species of *Gyrodactylus* parasitize on 19 orders of fresh and marine bony fishes. To date, three *Gyrodactylus* spp. parasitizing on *G. niger* have been recorded: *G. niger* sp. (*sensu* Huyse *et al.* 2003) from the North Sea, *Gyrodactylus flesi* Malmberg, 1957, and *G. proterorhini* Ergens, 1967 (Huyse *et al.*, 2003; Harris *et al.*, 2004).

The absence of monogenean parasites on the gills and the skin of *G. niger* from the Marchica Lagoon and sea may be related to environmental conditions. Another explanation for this absence is the immune system of fishes. Indeed, Zander *et al.* (1993) and Zander *et al.* (1999) showed that in the Baltic Sea, *Podocotyle atomon* (Rudolphi, 1802) is present in high abundances; however, in *G. niger*, this species was often absent, which was explained by its immune system efficiency.

Conclusion

Gobius niger was revealed to be present year-round in the Marchica Lagoon with higher abundances recorded in the shallow bottom of the lagoon's inner margins on a variety of mostly vegetated substrates and with a general decrease in abundances during winter. Depth is a key factor for understanding the spatial patterns of *G. niger* in the Marchica Lagoon, but it should be regarded as a variable that acts synergistically with other factors, such as vegetation cover, type of substrate, and temperature. Thus, the shallow beds of the lagoon, where the species is abundant, are key habitats in the Marchica Lagoon and need to be considered in all management plans aiming at the conservation of biodiversity and ecological processes. Comparison of black goby populations from the Marchica Lagoon with their conspecifics from the adjacent Mediterranean coast of Morocco revealed that specimens caught in the sea are significantly bigger than those from the lagoon. Moreover, the absence of population structuring and common haplotypes between the two populations indicates no apparent restriction in the gene flow between the two populations.

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Supplementary Material

The following supplementary material is available for this article:

Fig. S1: A neighbour-joining tree including all the species of *Gobius* that are represented in the GenBank by a targeted fragment of the 16S rRNA gene. The tree was created in MEGA X using 1000 bootstrap replicas and the Kimura 2-P substitution model. The scale bar represents the number of expected substitutions per site.

Table S1. Summary of species included in the phylogenetic tree, with accession numbers and locations of each species.

Table S2. Mean abundance of *Gobius niger* at different stations.

Table S3. Pairwise comparisons of *Gobius niger* abundance in terms of factor station.

Table S4. Meristic characters of *Gobius niger* from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S5. Meristic characters of *Gobius niger* from the Marchica Lagoon. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S6. Morphometric characters of *Gobius niger* from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S7. Morphometric characters of *Gobius niger* from the Marchica Lagoon. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S8. Length–length relationships between various morphometric characters measured in *Gobius niger* from the Mediterranean Sea. TL: total length; SL: standard length; HL: head length; BH: body height; ED: eye diameter; SnL: snout length; N: number of specimens; a: intercept of relationship; b: slope of relationship; R^2: coefficient of determination.

Table S9. Length–length relationships between various morphometric characters measured in *Gobius niger* from the Marchica Lagoon. TL: total length; SL: standard length; HL: head length; BH: body height; ED: eye diameter; SnL: snout length; N: number of specimens; a: intercept of relationship; b: slope of relationship; R^2: coefficient of determination.