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The black goby *Gobius niger* Linnaeus, 1758 in the Marchica lagoon (Alboran Sea, Morocco): Ecological traits and site-related footprint

Amal Lamkhalkhal [(1)(*)], Mohamed Selfati [(2)], Imane Rahmouni [(1)], Nassir Kaddouri [(1)], Bouabid Badaoui [(1)], Antoine Pariselle [(1,3)], Abdelaziz Benhoussa [(1)], Nikol Kmentová [(4)], Maarten P.M. Vanhove [(4)], Hocein Bazairi [(1,5)]

[(1)] Mohammed V University in Rabat, Faculty of Sciences, Laboratory of Biodiversity, Ecology and Genome, 4Avenue Ibn Battouta, B.P. 1014 RP, Rabat, Morocco
[(2)]) Laboratoire Santé Et Environnement, Faculté des Sciences Aïn Chock, Hassan II University of Casablanca, B.P 5366 Maarif, 20100 Casablanca, Morocco
[(3)] ISEM, CNRS, Université de Montpellier, IRD, Montpellier (France)
[(4)] Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity and Toxicology, Agoralaan Gebouw D - B-3590 Diepenbeek, Belgium
[(5)] Natural Sciences and Environment Research Hub, University of Gibraltar, Europa Point Campus, Gibraltar GX11 1AA

[(*)] Corresponding author: amal.lamkhalkhal@gmail.com

Abstract

Fish belonging to Gobiidae are well represented in the Marchica lagoon on the Moroccan Mediterranean coast, both in terms of species richness and abundance, with the black goby (*Gobius niger* Linnaeus, 1758) being the dominant species. The present study aims to examine (1) the ecological traits of *Gobius niger* and its environmental drivers in the lagoon and (2) the potential lagoon-related footprint using morphometric, genetic and parasitological proxies.

Systematic sampling covering the whole lagoon basin performed between October 2015 and November 2016 revealed year-long presence of *G. niger* throughout the lagoon with significantly low densities in winter. The higher abundances were recorded in the shallow bottoms of the lagoon inner margins on a variety of substrates (mud, muddy-sand, sandy-mud and fine sand) mostly covered by macroalgae and/or seagrass meadows. Multivariate analysis evidenced that depth and temperature were the important predictor variables explaining the spatial distribution of *G. niger* in the lagoon, with depth being the best model explaining about 33% of the total variability.

Comparison of black goby populations from the Marchica lagoon with their counterparts from the adjacent Mediterranean coast of Morocco revealed that specimens caught at the sea are of a bigger size compared to the ones from the lagoon without any effect of the colour morphs observed (dark vs clear). 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 presence of common haplotypes indicate no apparent restriction in the gene flow between the two populations. Moreover, the morphometric differences and colour morphs observed seems to be due to the external environment rather than genetic differences.

Gobius niger plays a key eco-trophic role by providing a link between benthic invertebrates and large predators. Therefore, ultimately, 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.

Key words: Coastal lagoon, Mediterranean Sea, Gobiidae, Morphometry, Parasites, Genetics

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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, fisheries resources, nursery area, etc.), lagoons are considered as 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 are highly valuable for their fundamental and demand-derived ecosystem services in coastal lagoons such as food supply and job creation (Holmlund & Hammer, 1999; Lopes & Videira, 2013; Newton *et al.*, 2014). In addition, they play a fundamental role in the 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). Moreover, fish are relevant biotic indicators to survey biodiversity and ecological status (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 2,042 currently recognized species, Gobiidae forms the most species-rich family of fish, (Fricke *et al.*, 2021; Renoult *et al.*, 2022) and are found both in marine and freshwater environments (Renoult *et al.*, 2022). Generally, they are small and short-lived, and they live discreetly on the substrate or hidden in various types of cavities. Among them are benthic, hyperbenthic, cryptobenthic and nektonic species (Kovačić & Patzner, 2011). Despite their low commercial value, gobies play a crucial role as food resources for many commercially important species. They also have a crucial trophic function by linking benthic invertebrates to larger predatory fish (Casabianca & Kiener, 1969; Miller, 1979; Raffaelli *et al.*, 1989).

In the Mediterranean basin, 73 species of Gobiidae are known to occur currently (Kovačić, 2020, Goren & Stern, 2021; Iglésias *et al.*, 2021, Kovačić *et al.*, 2021). In the Mediterranean, 249 fish species were inventoried inhabiting estuaries and/or lagoons including both sedentary (euryvalent species, that live out their entire lifecycle inside lagoons and estuaries) and migratory (species that, after spending time in lagoons, are obliged to return to their native marine or river environment to complete their life cycle) fishes (Kara & Quignard, 2019). Besides, Gobiidae (at least 11 species) constitute with Syngnathidae (at least ten species), the most represented families of sedentary fish in Mediterranean lagoons (Kara & Quignard, 2019).

The Marchica lagoon (35.156944° / -2.845278°), 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 remains well represented in the Marchica lagoon, both qualitatively and quantitatively, with the black goby (*Gobius niger* Linnaeus, 1758) being 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 the environmental factors.

Sedentary organisms, such as gobies, are the most suitable to be used as an indicator of lagoon environmental conditions (Bortone *et al.*, 2005). Their presence and abundance may provide important indications on the conservation status of coastal lagoon habitats (Facca *et al.*, 2020).

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Assuming that the black goby *G. niger,* the most representative species of gobies in the Marchica lagoon, is considered as sedentary fish in coastal lagoons (Franco *et al.*, 2008a, 2008b, 2012; Kara & Quignard, 2019; Selfati *et al.*, 2019) and that the species has its counterparts along the adjacent Mediterranean coast of Morocco, our study aims to (1) establish the spatial and temporal distribution of *G. niger* and its environmental drivers using a systematic sampling covering the whole lagoon basin, and (2) examine the potential lagoon-related footprint *i.e.* whether the supposed sedentary 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.

Materials and Methods

Study area

The Marchica lagoon (Fig.1) (35.156944° / -2.845278°), also called the lagoon of Nador, is one of the largest coastal 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 8m and the lagoon is separated from the Mediterranean 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 (Site of Biological and Ecological Interest since 1996; RAMSAR site since 2005) and socio-economic (mainly artisanal fisheries) values, 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 ecological traits of *G. niger* and their environmental drivers in the lagoon of Marchica, data on monthly abundances was extracted from a scientific monitoring of the fish fauna in the lagoon between October 2015 and November 2016. The monitoring was carried out according to an optimized network of 20 stations (S1-S20) covering the whole lagoon (Fig. 1). The fishing 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 are expressed by units of effort corresponding to the surface sampled, equivalent to 800 m². Environmental parameters were measured at each station. Water temperature (T) and salinity (S) 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’’. Other environmental variables were obtained from recently published literature (Mostarih *et al.*, 2016): dissolved oxygen (DO, as mg/l and percentage of saturation % O), suspended matter (SM, mg/l), suspended particulate inorganic matter (PIM, mg/l), and particulate organic matter (POM, mg/l).

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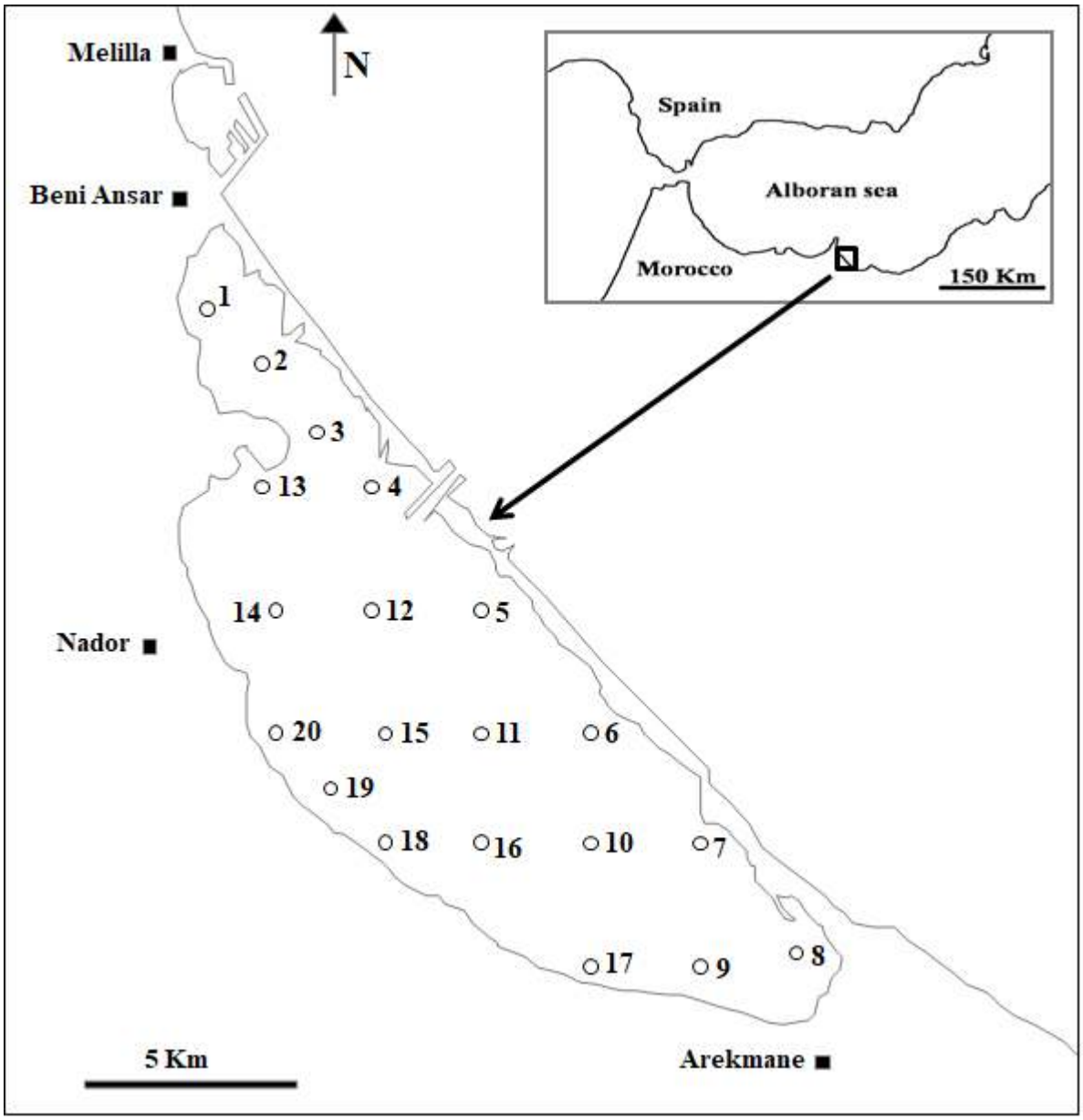

Fig. 1: Map showing the geographical localization of the Marchica lagoon and the sampling stations of *Gobius niger*.

In addition to the sampling described above, 120 black gobies from the Marchica lagoon and 60 ones from the adjacent Mediterranean coast of Morocco were collected as bycatch by fishing boats in July 2020. All the specimens were stored in separated plastic bags and were transported in a portable freezer (Engel MT45), 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.

Based on a colour pattern (clear vs dark), specimens of *G. niger* were separated, in the following analyses, into four groups: 'Lagoon clear' (specimens from the lagoon with clear colour), 'Lagoon dark' (specimens from the lagoon with dark colour), 'Sea clear' (specimens from the sea with clear colour) and 'Sea dark' specimens from the sea with dark colour).

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Species identification

Identification of fishes was performed based on a simple determination inspired by those provided by Brownell & Collignon (1978), Bauchot & Pras (1980) and Bauchot (1987) as well as the study of the lateral system. This is based on the positioning and arrangement of the mucous ducts and pores and the sensory papillae presented in two basic groupings of longitudinal and transverse series.

This species is characterized morphologically by: an elongated body; a slightly depressed head; the fourth ray of the first dorsal fin being more prolonged; very variable marbled coloration; a line of black dots often grouped by three, superimposed on a row of dark spots; a dark spot at the beginning of the first dorsal fin.

The number of rays at the pectoral fin varies from 15 to 18 rays, six rays (rarely five or seven) in the first dorsal and 11 to 15 soft rays on the second dorsal. The number of rays at the ventral fin varies between 9 and 11 rays; the number of rays at the anal fin varies from 11 to 13 rays.

Data collection and processing

Ecological traits 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, using a hierarchical cluster analysis, conducted on a transformed (four root) abundances similarity matrix based on the Euclidean distances. Then, the Analysis of similarities (ANOSIM) non-parametric test was performed to assess the level of significance of the groups of stations identified. Differences between sampling stations and 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.

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 graphically the results. 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, 2005).

Characterisation and comparison of goby populations

Morphometric analysis

Based on literature (Gaamour et al, 2001), six morphometric characters were measured on each specimen to millimetre using a Vernier calliper: total length (TL), standard length (SL), head

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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).

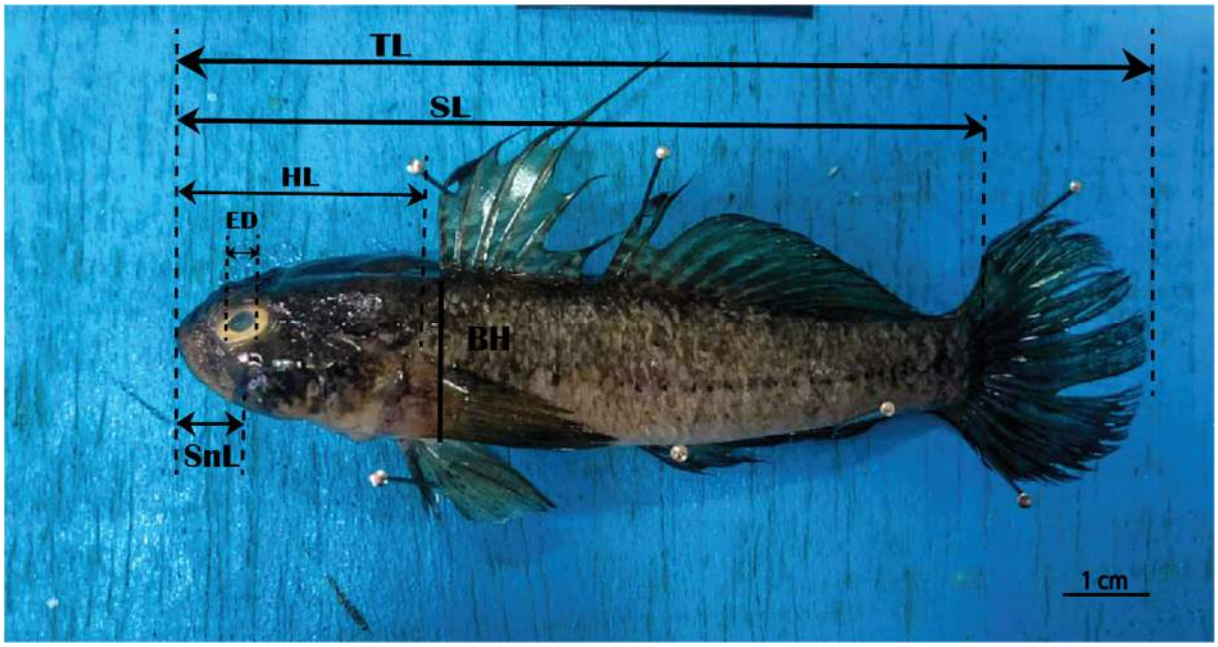

Fig. 2: Picture of a *Gobius niger* showing the main measurements taken: total length (TL), standard length (SL), head length (LT), snout length (SnL), Body height (BH) and eye diameter (ED).

In order to see if there is a stratification based on origin and colour of *G. niger*, a first Principal Component Analysis (PCA) analysis was performed using morphometric characters, after a transformation ($Log_{10}X+1$) of the raw measurements in order to linearize the allometries (Huxley, 1932) and to roughly equalize the variances (Jolicoeur, 1963).

An allometric correction was necessary to compare morphometries of series of specimens that differed in age and/or size structure. For this purpose, a linear regression was performed using standard length and principal components. A second PCA based on the residuals of this correlation was done to see if there is a correlation with size and a separation related to the geographic origin of the *G. niger* specimens.

All analyses were performed in the PAST v4.03 software (Hammer, Harper & Ryan, 2001).

Genetic differentiation

DNA extraction of 120 specimens (30 Sea clear, 30 Sea dark, 30 Lagoon clear, 30 Lagoon dark) was performed according to the protocol of Aljanabi & Martinez (1997): approximately 50 µg of pectoral fin fragment was digested at 55 °C overnight with 20 µl of proteinase *K* (20 mg/ml) and180 µl of extraction buffer (0.4M NaCl, 1M Tris, 2 mM EDTA and 40 µl of 20 % SDS). The extracted DNA was suspended in 150µl 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 rDNA gene was performed in a final volume of

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20 µl containing: 1µl of extracted DNA; 4 µl buffer (10× Standard Taq Reaction Buffer), 1 µl of 10 mM dNTPs, 0.8 µl of 10µM forward primers 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 of nuclease free water. Amplification reactions were performed in a gradient thermal cycler according to the following program: initial denaturation at 94°C for 3min followed by 40 cycles each with denaturation for 30 sec at 94°C; a hybridization for 30 secs at 55°C and an elongation phase for 1 min at 72°C and at the end a final elongation for 10 min at 72°C. The PCR products were checked on 1% agarose gel and sent to the National Center for Scientific and Technical Research (CNRST) in Rabat; then 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 (Molecular Evolutionary Genetics Analysis) (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 sequences available in NCBI GenBank to check for possible matches.

To make sure that all of our 16S sequences, both the recently Moroccan sequences, and those we include from other studies, cluster monophyletically, without representatives of other species, a phylogenetic tree was built including all other species of *Gobius* that are represented on GenBank by a targeted a fragment of the 16S rRNA gene (see Supplementary material Table S1). The phylogenetic tree was constructed with MEGA X using the Neighbor Joining algorithm and the Kimura 2-P substitution model 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, Li's F and Tajima's neutrality tests were performed to check for possible selection or change in population demography. Pairwise F_{ST} values were calculated in ARLEQUIN v3.5 (Excoffier & Lischer, 2010) using 100 permutations to express the degree of genetic differentiation between individuals.

A matrix correlation analysis (Mantel test; Mantel, 1967) permuting a morphological distance matrix against a genetic distance matrix was performed using 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, then the external surface of skin, fins and the holding water were examined. Gill arches on the right side of each specimen were removed through ventral and dorsal section, placed in a petri dish, and rinsed with a rinsing bottle filled with tap water. Then water and gill arches were examined under a stereoscope (Wild M8).

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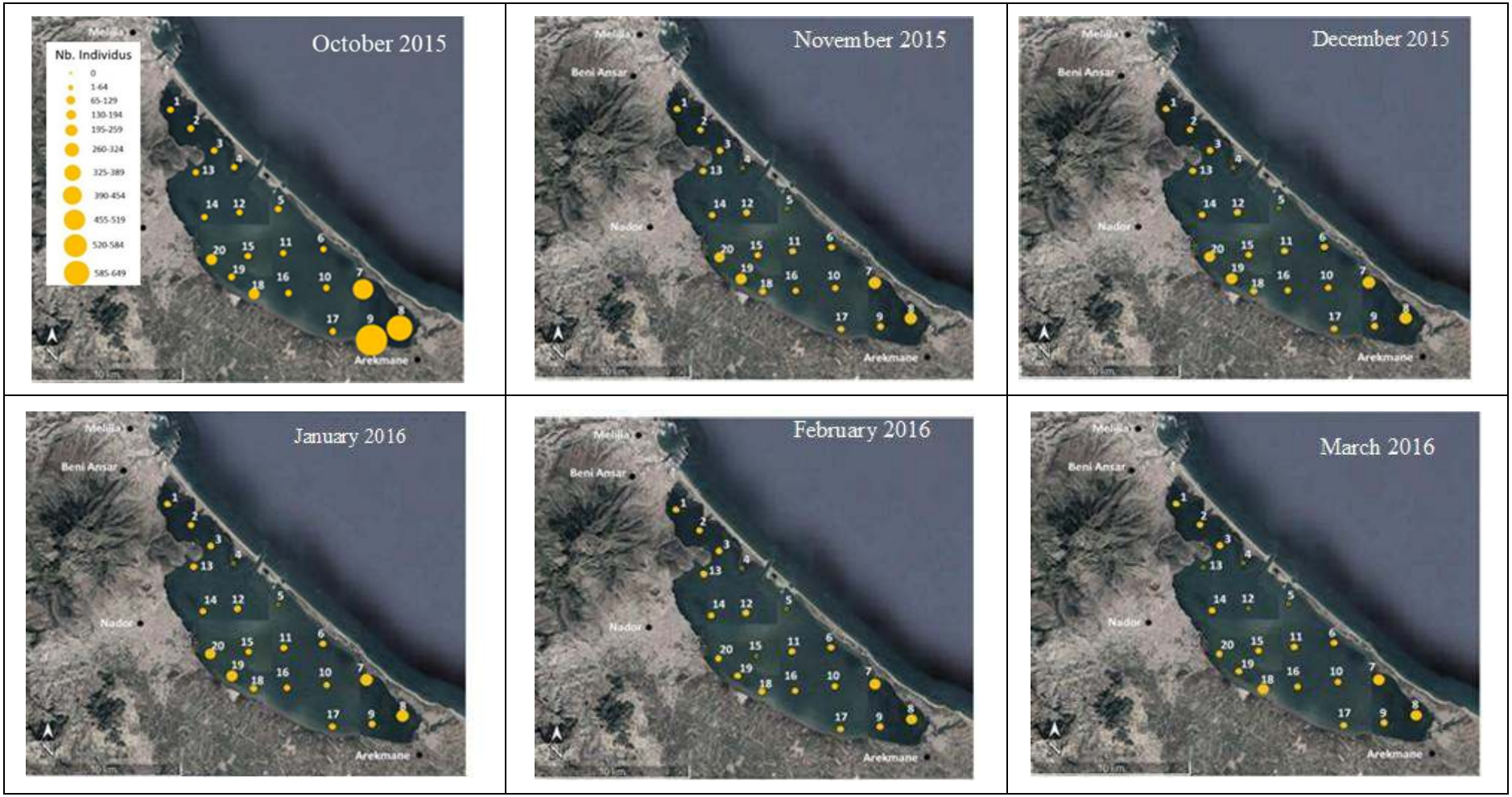
Results

Ecological traits of Gobius niger *in the Marchica lagoon and environmental drivers*

The black goby was permanently present in the Marchica lagoon, both over space and time but with differential abundances (Fig. 3). Overall, the monthly abundance fluctuated between no individuals and a maximum of 643 individuals. The mean abundance (± SD) was 44.13 individuals per station (± 88). PERMANOVA results showed significant differences in total abundance between seasons and stations. Interactions between the two factors were not significant (Pseudo F= 1.072, p (perm) < 0.05). A posteriori pairwise comparison revealed that the black goby was significantly less abundant in winter than in other seasons. Regarding the second factor, most of the significant differences concern the combinations formed by the peripheral and central stations.



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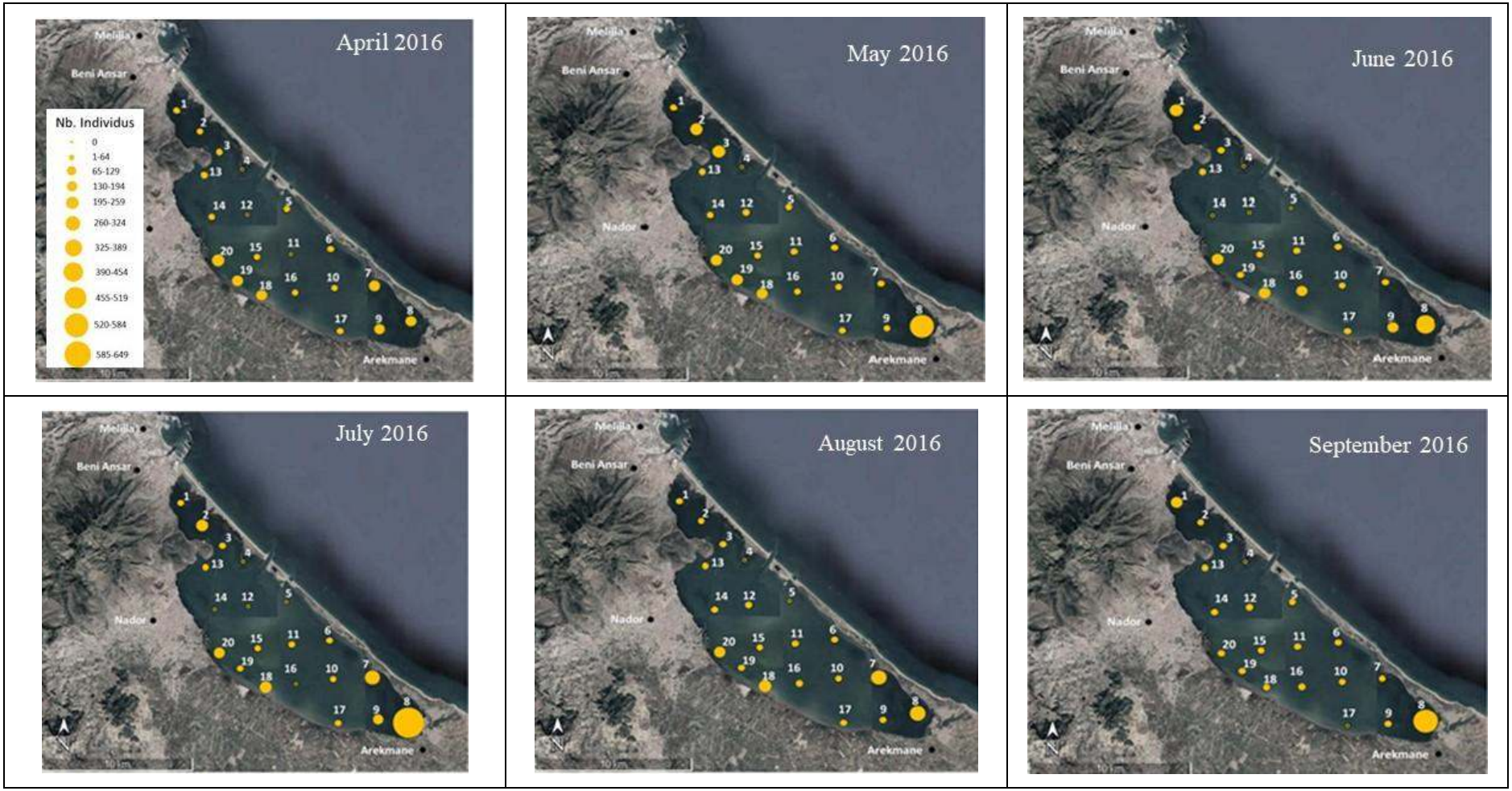

Fig. 3: Spatial and temporal distribution of *Gobius niger* in the Marchica lagoon.

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Cluster analysis of *G. niger* abundances in the 20 sampled stations in the Marchica lagoon separated the samples in two significant groups (ANOSIM, $P < 0.05$), labelled as G1 and G2 (Fig. 4). The Group G1, where abundances were high, concerns the NW and the SE extremities of the lagoon and the continental edge located on either side of Oued Selouane where a variety of substrates (mud, muddy-sand, sandy-mud and fine sand) occur, mostly covered by macroalgae and also by seagrass. The group G2 corresponds roughly to the center of the lagoon where the goby was overall less abundant.

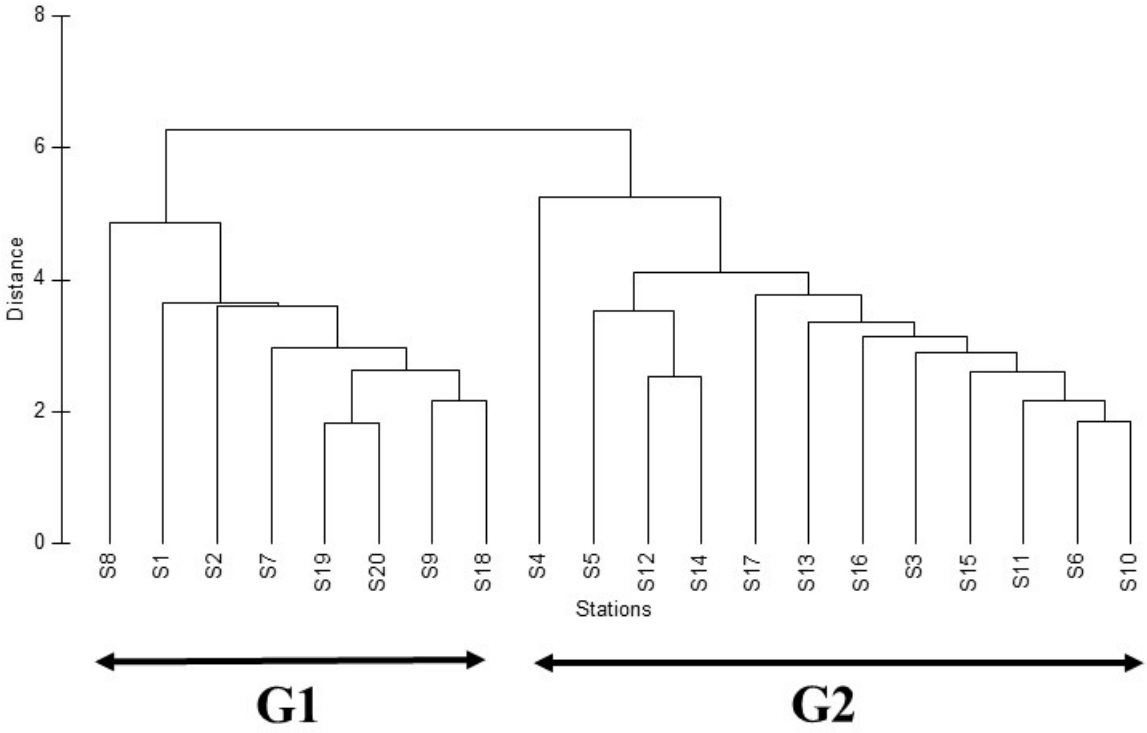

Fig. 4: Cluster analysis based on *Gobius niger* abundances showing reciprocal relations between the 20 sampled stations in the Marchica lagoon according to Euclidean distance similarities.

The DISTLM analysis revealed that depth and temperature had a significant effect ($p<0.01$), explaining respectively 33% and 19% of the spatial variations of *G. niger* in the Marchica lagoon (Table 1). The remaining variables, salinity, pH and suspended matter, did not show any significant impact and accounted for less than 1% of the variability for each. The first two axes of the dbRDA analysis capture 91.1% of the variability in this fitted model and 46% of the total observed variability (Fig. 5). Depth alone is the best model (lowest AIC value) explaining about 33% of the total variability.

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

Variable	SS(trace)	Pseudo-F	p	Prop. (%)
Temperature (Temp)	5757.8	4.3282	0.004*	0.19384
Depth	9853.8	8.9357	0.001*	0.33174
Salinity	2516.3	1.666	0.127	8.47E-02
pH	2893.1	1.9424	0.091	9.74E-02
Suspended Matter (SM)	1230.2	0.77768	0.56	4.14E-02

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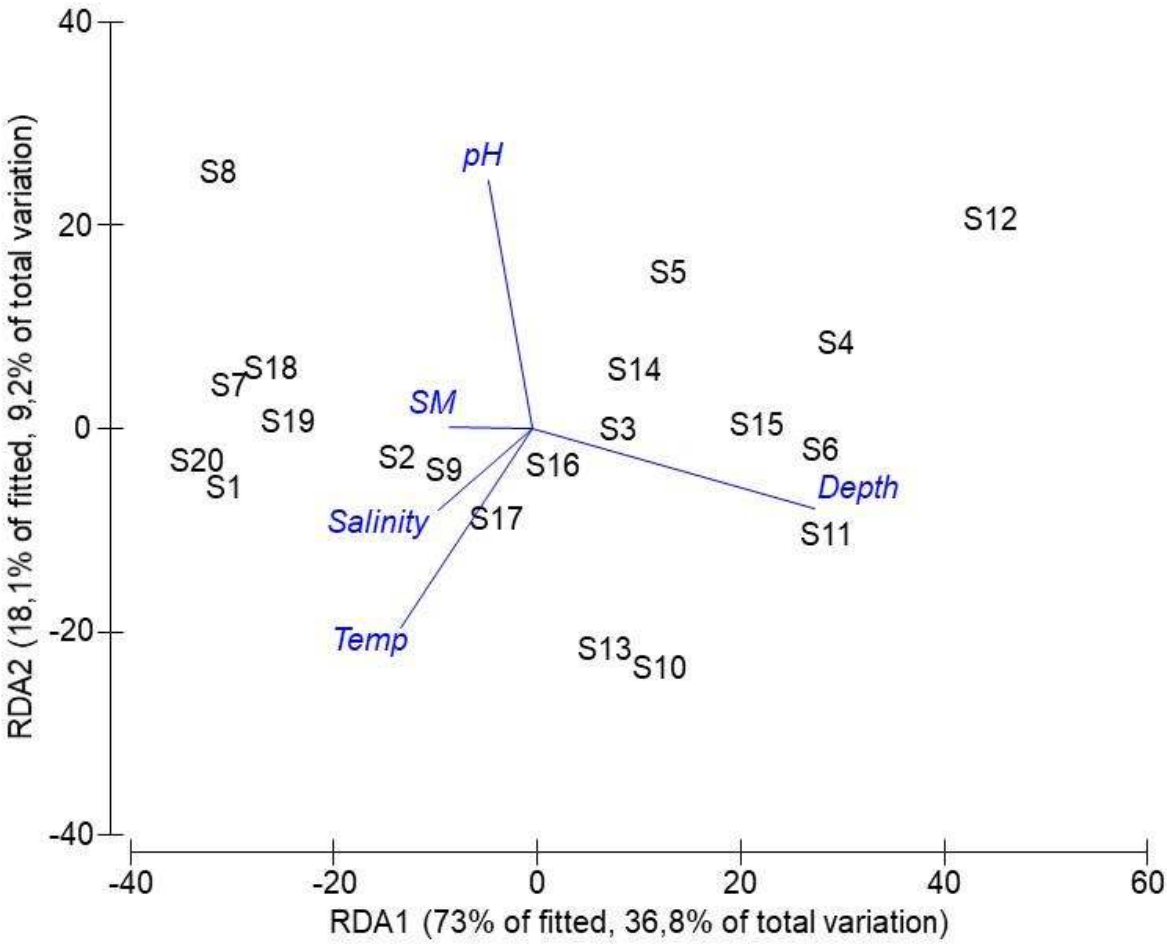

Fig. 5: Two-dimensional redundancy analysis (RDA) ordination representing the model of spatial variation in spatial distribution of *Gobius niger* related to the predictor variables selected through the best linear models based on distance (DISTLM). SM: Suspended matter; Temp: Temperature.

Site-related footprint

Morphometric analysis

Data from meristic characters of the four groups of *Gobius niger* sampled at sea and in the Marchica lagoon are reported in Tables S2 to S5, respectively. Overall, specimens caught at the sea are of a bigger size (SL from 80 mm to 135 mm) compared to the ones from the lagoon (SL from 54 mm to 105 mm).

The allometric relationships (Log Y = a LogX = Logb) between the standard length (SL) and the total length (TL), the head length (HL) and the body height (BC), and on the other hand, between the head length (HL) and the eye diameter (ED) and the snout length (SnL) are summarized in Tables S6 and S7 (*G. niger* from the sea) and Tables S8 and S9 (*G. niger* from the Marchica lagoon). The obtained results revealed significant positive correlations ($p<0.05$) between all the parameters.

The first PCA performed on the morphometric and meristic data allowed the identification of a distinct stratification based on the origin of the individuals (see Supplementary material

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Figure S2).The first two axes are the most informative, representing respectively 78.11% and 9.47% of the total inertia, totalising 87.58% of the total variation. Overall, the first axis is correlated to morphometric characteristics, while the second axis is correlated to meristic characteristics.

The regression of PC1 scores for the different individuals against SL (Fig. 6) revealed a strong correlation between PC1 and SL. The PCA based on the residuals, revealed separation related to geographic origin of specimens (adjacent Moroccan Mediterranean and the Marchica lagoon) without clear distinction related to the colour (clear vs dark specimens) is visible.

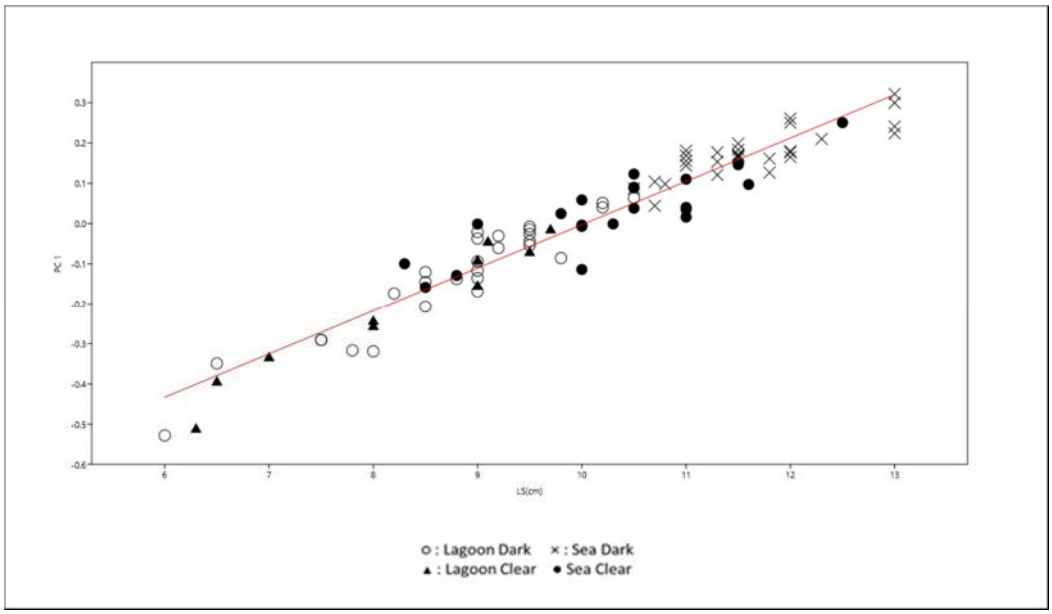

Fig. 6: Linear regression of the principal component score axis (PC1) from morphometric measurements on the log standard length of *Gobius niger* with projection of phenotypic groups.

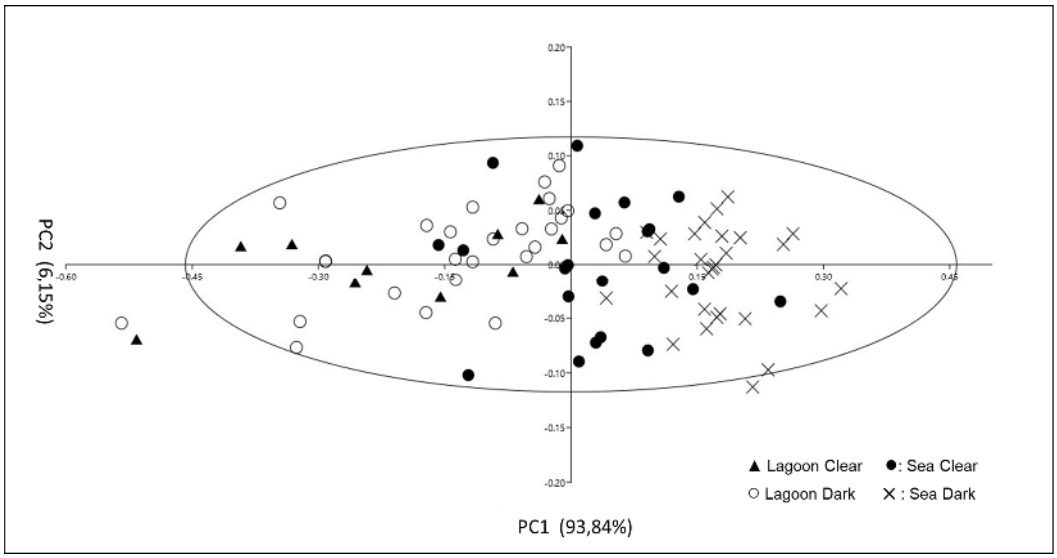

Fig. 7: Principal Component Analysis on the residuals from the regression of PC1 and SL of *Gobius niger* with projection of phenotypic groups.

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Genetic differentiation

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

Diversity measures, calculated for the four groups (Sea dark, Sea clear, Lagoon dark, Lagoon clear), are reported in Table 2. The marine population with a dark color shows the highest total number of haplotypes (15) while the population from the lagoon with a clear colour shows the lowest number of haplotypes (6). The highest number of segregating sites (16) is recorded for the population of the lagoon with a dark colour the while the population of the lagoon with clear color shows the lowest number of segregating sites (8). The nucleotide diversity (π) of the four groups in this study ranges from ± 0.00382 to ± 0.00502 while haplotype diversity (h) ranged from ± 0.81905 to ± 0.9365. Neutrality test values (Fu & Li's F and Tajima's D) are negative and significant.

Table 2. Results of the haplotype diversity analysis of the four groups of the species *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	Fu & Li's F	Tajima's D
Sea clear	21	14	11	0.81905	0.00467	-2.53758	-1.71526
Sea dark	29	15	15	0.87685	0.00382		
Lagoon clear	10	8	6	0.88889	0.00502		
Lagoon dark	28	16	12	0.93651	0.00432		

The maximum parsimony network shows four ancestral haplotypes (numbers 22, 29, 35, 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.

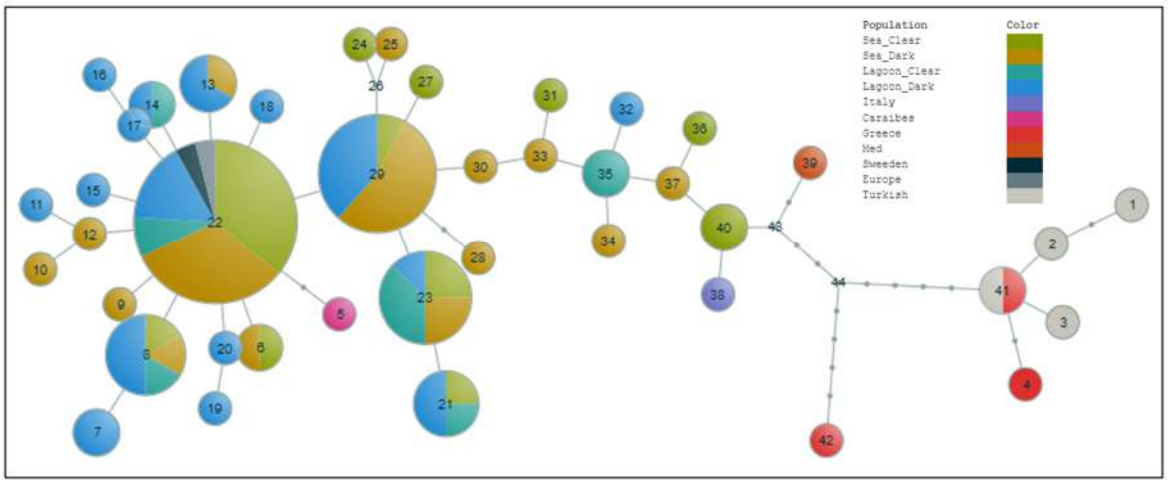

Fig. 8: Haplotype networks constructed from 16S mtDNA sequences of *Gobius niger*. The size of a particular circle reflects the haplotype frequency. The numbers indicate the nodes.

The pairwise F_{ST} comparisons show, in most cases, low values; F_{ST} p-values were all not significant (Table 3). This implies that there is no considerable degree of genetic differentiation between the different groups of *G. niger* inhabiting Marchica lagoon.

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Table 3. Results of the F_{ST} population structure analysis of *Gobius niger* from the Marchica lagoon and the adjacent Mediterranean coast of Morocco. p: p-value

Groups	Sea clear	Sea dark	Lagoon clear	Lagoon dark
Sea clear	*	F_{ST}:-0.02171 p: 0.89160 ± 0.0102	F_{ST}: -0.01659 P: 0.52148 ± 0.0173	F_{ST}: -0.00730 p: 0.53223 ± 0.0177
Sea dark	*	*	F_{ST}: 0.1161 p: 0.28125 ± 0.0136	F_{ST}: 0.00569 p: 0.27832 ± 0.0152
Lagoon clear	*	*	*	F_{ST}: 0.02781 p: 0.14258 ± 0.0104
Lagoon dark	*	*	*	*

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

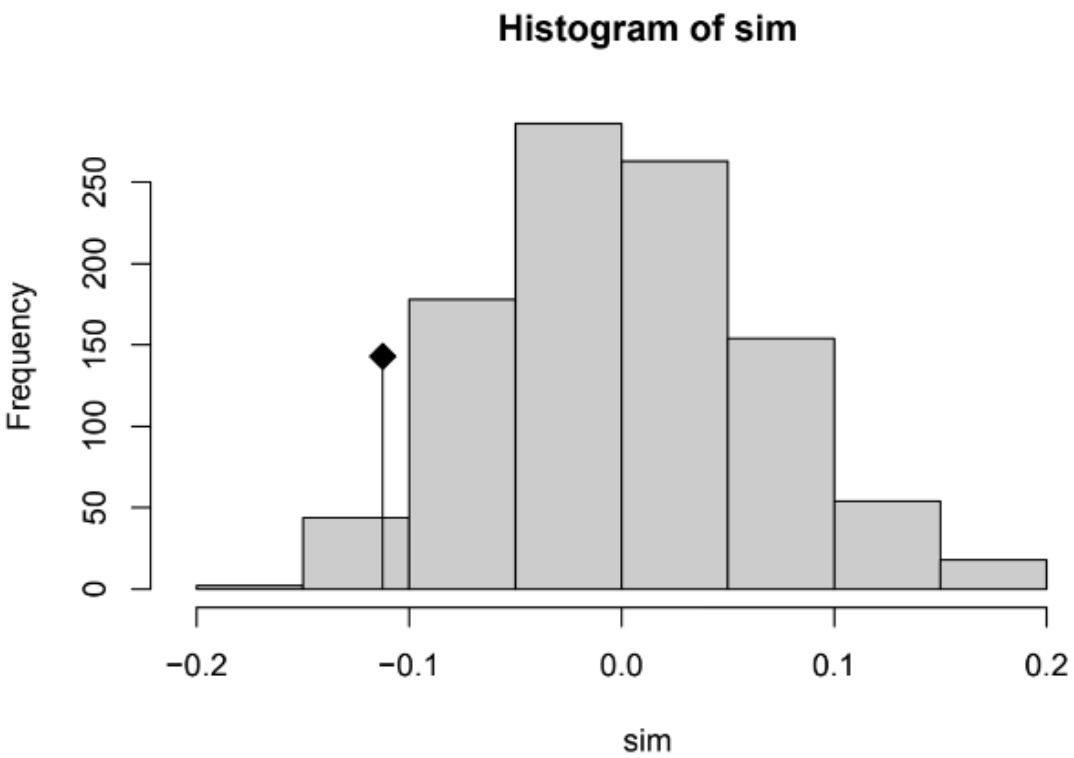

Fig. 9: Histogram of the Mantel test assessing the relationship between genetic and morphologic distance for *Gobius niger*. Sim: simulations; Frequency: frequency values of the correlation between the genetic and morphologic distances.

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.

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Discussion

Spatial and temporal distribution of Gobius niger *in the Marchica lagoon and environmental drivers*

The spatial and temporal distribution of *G. niger* evidenced that the species is long-lasting 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. This is consistent with the ecology of the species in other Mediterranean coastal lagoons where it prefers shallow habitats (<1.5m depth) on sandy and sandy-muddy vegetated beds (Kara & Quignard, 2019). Moreover, its permanent presence in the Marchica lagoon supports its supposed ecological status as sedentary fish (Franco *et al.*,2008a, 2008b, 2012, Kara & Quignard, 2019; Selfati *et al.*, 2019), being among the most frequent fish (62.8%) 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, 1979; 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 sedentary fish, *G. niger* is a suitable candidate to monitor 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 (Maradonna & Carnevali, 2007; Ramsak *et al.*, 2007; Barucca *et al.*, 2006; Migliarini *et al.*, 2005; Maradonna *et al.*, 2004; Fossi *et al.*, 1989; Katalay & Parkak, 2002; Barucca *et al.*, 2006).

Overall, according to our results, depth and temperature are important predictors explaining the distribution of *G. niger* in the lagoon, with depth being the most contributing variable. 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 lagoon inner margins (Selfati *et al.*, 2019). Moreover, depth is a key factor for understanding the structure of fish assemblages in the Marchica lagoon, but has to be seen as a variable that acts in concert with other factors such as vegetation cover, pH, temperature and dissolved oxygen for bentho-demersal fish, and salinity and suspended matter for pelagic fish (Selfati *et al*. 2019). The significant decrease of *G. niger* during winter could be related to low temperatures during this season leading to migration of the species to marine waters (Mozzo, 1968). Another explanation proposed by Arruda *et al.*, (1993) in the Ria de Aveiro Lagoon (Portugal) is the massive mortality following the spawning period. However, in-depth investigations are required to verify these hypotheses.

Comparison of goby populations

Morphometric patterns

In the Moroccan Mediterranean, the black goby has been previously reported both in the lagoon and in the open sea (Aloncle, 1951; Selfati *et al.*, 2019). The work of Böhlke & Robins (1968), Hoese (1971, 1983), Bath (1973), Akihito (1986), Gill *et al.*, (1992), and more recently by Bouchereau *et al.* (2000) and Kovačić & Golani (2007) has shown that the SL of the head and trunk is very useful in the classification of genera and species of Gobiidae. The difference

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in size between individuals from the lagoon and their marine counterparts is probably due to the differential depth of 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 by a larger body size (Harvey & Stewart, 1991).

It has been reported that there are light and dark specimens of *G. niger*. This coloration may be related to sex and/or age as the colour of *G. niger* is generally darker in males than females and becomes very dark with age (Bodilis & Le Bris, 2018; De Casabianca & Kiener, 1969).

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 dispersal of gametes, zygotes, larvae and adults (Giovannotti et al 2009).

Our study revealed the presence of common haplotypes shared by the four recognized groups based on the combination of colour pattern and habitat. According to Bortone *et al.* (2005), *G. niger* is considered as 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 lagoon and sea. F_{ST} values are used to conclude the level of gene flow (Shyama Sundari Devi Chanthran *et al.*, 2020). According to Wright (1965), populations with F_{ST} of 0–0.05 show a small differentiation, 0.05–0.15 is considered as moderate differentiation, 0.15–0.25 as important differentiation and values greater than 0.25 as very important differentiation. The occurrence of common haplotypes between the groups and small to moderate differentiation based on F_{ST} results suggests unrestricted gene flow with no relation to the colour form and habitat.

Parasites of G. niger

Monogenean parasites are one of the largest groups of Platyhelminthes characterised 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 and Volckaert (2005) *Gyrodactylus* species parasitize on 19 orders of fresh and marine bony fishes. To date, three *Gyrodactylus* spp. parasitizing *G. niger* have been recorded: *Gyrodactylus flesi* Malmberg, 1957; *G. proterorhini* Ergens, 1967 and *G. niger* sp. Huyse *et al.* 2003 from North Sea (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 could be explained by environmental conditions. Another explanation of this absence is the immune system of fishes. Indeed, Zander *et al.* (1999) and Zander (1993) 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.

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Conclusion

Gobius niger was revealed to be a year-long present fish in the Marchica lagoon with the higher abundances recorded in the shallow bottoms of the lagoon inner margins on a variety of mostly vegetated substrates and with general decrease in abundances during winter. Depth is a key factor for understanding the spatial patterns of *G. niger* in Marchica lagoon, but has to be seen as a variable that acts in concert with other factors such as vegetation cover, pH, and temperature among others. 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 counterparts from the adjacent Mediterranean coast of Morocco revealed that specimens caught at the sea are of a bigger size compared to the ones from the lagoon without any effect of the colour morphs observed (dark vs clear). Moreover, the absence of population structuring, low genetic diversity and common haplotypes between the two populations indicates no apparent restriction in the gene flow between the two populations. Moreover, the morphometric differences and colour morphs observed seems to be unrelated to genetic differences.

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Supplementary material

The following supplementary material is available for this article:

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

Table S2. Meristic characters of clear *Gobius niger* individuals from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S3. Meristic characters of dark *Gobius niger* individuals from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

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

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

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Table S6. Allometric relationships between various numerical characters measured in clear *Gobius niger* from the adjacent Moroccan Mediterranean Sea. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter, N: number; r: correlation coefficient.

Table S7. Allometric relationships between various numerical characters measured in dark *Gobius niger* from the adjacent Moroccan Mediterranean Sea. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter, N: number; r: correlation coefficient.

Table S8. Allometric relationships between various numerical characters measured in clear *Gobius niger* from Marchica lagoon. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter, N: number; r: correlation coefficient.

Table S9. Allometric relationships between various numerical characters measured in dark *Gobius niger* from Marchica lagoon. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter, N: number; r: correlation coefficient.

Figure S1. Phylogenetic tree including all the species of Gobius that are represented on GenBank by a targeted a fragment of the 16S rRNA gene.

Figure S2. Principal Component Analysis of morphological variables of the 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 and ventral fin, VF) with projection of phenotypic groups.

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Supplementary Materials

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

Species	Accession number	Locations
Gobius niger	KJ128783	Sweeden
	KF415385	Europe
	EF218645	Italy, Ancona
	AF067269	Mediterranean
	FJ460203	Turkish
	FJ460202	Turkish
	FJ460201	Turkish
	FJ460192	Turkish
	AF491118	Caraibes
	ON847339	Greece
	ON847340	Greece
	ON847341	Greece
	ON847342	Greece
Gobius paganellus	AF518216	Unpublished
	EF218651	Italy, Ancona
	FJ460204	Turkish
	AF067271	Mediterranean
Gobius bucchichi	EF218642	Italy, Napoli
	FJ460197	Turkish
	AF067268	Mediterranean
Gobius cobitus	EF218644	Italy, Ancona
	FJ460198	Turkish
Gobius cruentatus	EF218641	Italy, Napoli
Gobius auratus	AF067267	Mediterranean
Gobius xanthocephalus	AF491117	Spain, Gran Canaria, Puerta Ricco
Gobius vitatus	GQ485305	Turkish
Gobius Couchi	FJ460199	Turkish
Gobius geniporus	FJ460200	Turkish

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Table S2. Meristic characters of clear *Gobius niger* individuals from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean

Meristic characters	**N**	**Extremes**	**m**	**M**	**S**	**Sm**
Rays on the first dorsal fin	30	6	6	6	0	0
Rays on the 2nd dorsal fin	30	13-14	13.7	14	0.45	0.08
Rays at the anal fin	30	12-13	12.46	12	0.49	0.09
Rays on the pectoral fin	30	15-17	16.43	17	0.66	0.12
Rays at the ventral fin	30	9-11	9.53	9	0.61	0.11

Table S3. Meristic characters of dark *Gobius niger* individuals from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Meristic characters	**N**	**Extremes**	**m**	**M**	**S**	**Sm**
Rays on the first dorsal fin	30	6	6	6	0	0
Rays on the 2nd dorsal fin	30	13-14	13.83	14	0.37	0.06
Rays at the anal fin	30	11-13	12.43	12	0.55	0.10
Rays on the pectoral fin	30	16-18	16.93	17	0.62	0.11
Rays at the ventral fin	30	9-11	10.13	10	0.56	0.10

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

Meristic characters	**N**	**Extremes**	**m**	**M**	**S**	**Sm**
Rays on the first dorsal fin	30	6	6	6	0	0
Rays on the 2nd dorsal fin	30	13-14	13,48	13	0,49	0,10
Rays at the anal fin	30	12-13	12,4	12	0,48	0,10
Rays on the pectoral fin	30	16-18	16,32	16	0,54	0,11
Rays at the ventral fin	30	9-10	9,12	9	0,32	0,06

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

Meristic characters	**N**	**Extremes**	**m**	**M**	**S**	**Sm**
Rays on the first dorsal fin	30	6	6	6	0	0
Rays on the 2nd dorsal fin	30	13-14	13,73	14	0,44	0,08
Rays at the anal fin	30	12-13	12,66	13	0,47	0,08
Rays on the pectoral fin	30	15-17	16,16	16	0,46	0,08
Rays at the ventral fin	30	9-10	9,10	9	0,30	0,05

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Table S6. Allometric relationships between various numerical characters measured in clear *Gobius niger* from the adjacent Moroccan Mediterranean Sea. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter, N: number; r: correlation coefficient.

Allometry equations **Log y = a Log x + Log b**	**y (cm)**	**Extremes y (cm)**	**x (cm)**	**Extremes x (cm)**	**N**	**R**	**p-value**
Log TL = 1,2571 Log SL - 0,4726	12,27	9-15	10,13	8-12,5	30	0,97	<0,05
Log HL = 0,266 Log SL +0,1199	2,811	2,3-3,5	10,13	8-12,5	30	0,85	<0,05
Log BH = 0,1249 Log SL +1,134	2.40	1,5-3	10,13	8-12,5	30	0,41	<0,05
Log ED = 0,0895 Log HL +0,1312	0,38	0,3-0,5	2,81	2,5-3,5	30	0,50	<0,05
Log SnL = 0,3621 Log HL -0,0933	0,92	0,6-1,3	2,81	2,5-3,5	30	0,64	<0,05

Table S7. Allometric relationships between various numerical characters measured in dark *Gobius niger* from the adjacent Moroccan Mediterranean Sea. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter, N: number; r: correlation coefficient.

Allometry equations **Log y = a Log x + Log b**	**y (cm)**	**Extremes y (cm)**	**x (cm)**	**Extremes x (cm)**	**N**	**R**	**p-value**
Log TL = 1,0462 Log SL + 2,0031	14.24	12.5-16	11,7	10,5-13,5	30	0,89	<0,05
Log HL = 0,2838 Log SL +0,0364	3.35	2,8-4	11,7	10,5-13,5	30	0,71	<0,05
Log BH = 0,6438 Log SL +0,17	2,60	2,2-3,1	11,7	10,5-13,5	30	0,64	<0,05
Log ED = 0,0316 Log HL +0,3306	0,43	0,4-0,5	3,35	2,8-4	30	0,36	<0,05
Log SnL = 0,3223 Log HL +0,2585	1,19	1-1,5	3,35	2,8-4	30	0,59	<0,05

Table S8. Allometric relationships between various numerical characters measured in clear *Gobius niger* from Marchica lagoon. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter, N: number; r: correlation coefficient.

Allometry equations **Log y = a Log x + Log b**	**y (cm)**	**Extremes y (cm)**	**x (cm)**	**Extremes x (cm)**	**N**	**R**	**p-value**
Log TL = 1.2799 Log SL -0.5042	9.97	6.3-13	8.18	5.4-10.2	30	0.99	<0.05
Log HL = -0.1034 Log SL +0.2838	2.22	1.5-2.8	8.18	5.4-10.2	30	0.96	<0.05
Log BH = 0.2469 Log SL -0.3854	1.63	0.8-2.3	8.18	5.4-10.2	30	0.87	<0.05
Log ED = 0.0449 Log HL+0.2323	0.33	0.3-0.4	2.22	1.5-2.8	30	0.40	<0.05
Log SnL = 0.3407 Log HL+0.0396	0.79	0.5-1.2	2.22	1.5-2.8	30	0.97	<0.05

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Table S9. Allometric relationships between various numerical characters measured in dark *Gobius niger* from Marchica lagoon. SL: standard length; TL: total length; HL: head length; BH: body height; ED: eye diameter. N: number; r: correlation coefficient.

Allometry equations **Log y = a Log x + Log b**	**y (cm)**	**Extremes y (cm)**	**x (cm)**	**Extremes x (cm)**	**N**	**R**	**p-value**
Log TL = 1.2178 Log SL+ 0.1024	10.86	7.3-13	8.83	6-10.5	30	0.98	<0.05
Log HL = 0.3188 Log SL -0.4324	2.38	1.5-3	8.83	6-10.5	30	0.85	<0.05
Log BH = 0.2445 Log SL -0.3695	1.79	1.1-2.4	8.83	6-10.5	30	0.79	<0.05
Log ED = 0.0426 Log HL +0.2251	0.32	0.3-0.4	2.38	1.5-3	30	0.36	<0.05
Log SnL= 0.3034 Log HL +0.227	0.95	0.5-1.2	2.38	1.5-3	30	0.80	<0.05

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Figure S1. Phylogenetic tree including all the species of Gobius that are represented on GenBank by a targeted a fragment of the 16S rRNA gene.

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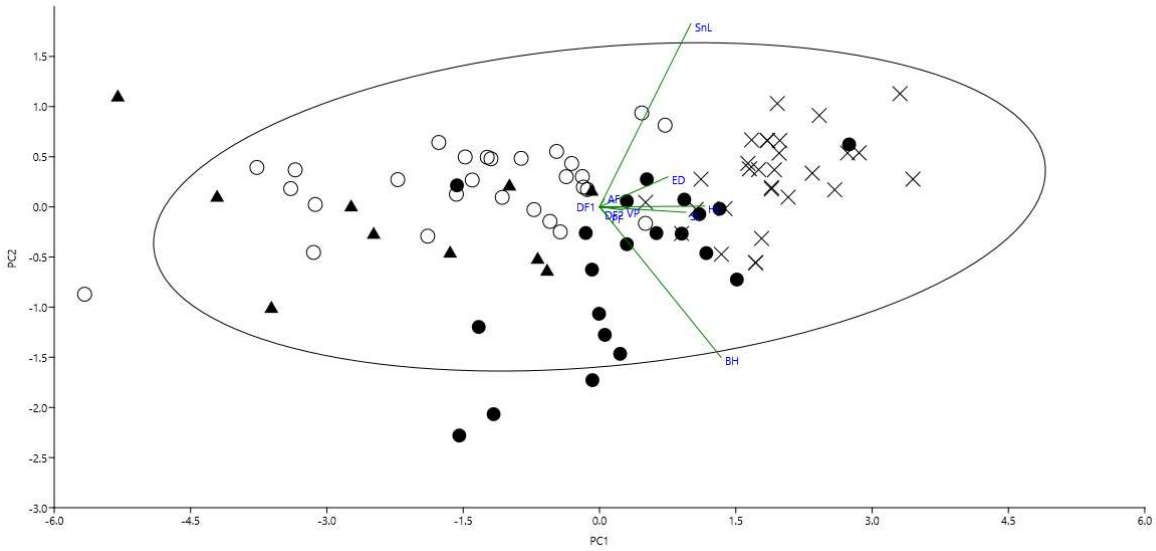

Figure S2. Principal Component Analysis of morphological variables of the Gobius niger (standard length, SL ; body height, BH; head length, HL; snout length, SnL; eye diameter, ED; first dorsal fin, DF1; second dorsal fin, DF2; anal fin, AF; pectoral fin, PF and ventral fin, VF) with projection of phenotypic groups.