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Very high genetic fragmentation in a large marine fish, the meagre *Argyrosomus regius* (Sciaenidae, Perciformes): impact of reproductive migration, oceanographic barriers and ecological factors

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Abstract – The meagre *Argyrosomus regius* is a large Sciaenid fish known to reproduce in the eastern Atlantic and Mediterranean Sea in just five distinct and restricted geographic areas: along the Mauritanian coast and at estuary openings (Gironde, Tagus, Guadalquivir and Nile). The biological traits of *A. regius* (high dispersal capabilities, high fecundity, long larval phase, overlapping generations, reproduction until 40 years of age) are, in principle, favourable to high gene flow, which should lead to genetic homogeneity over large geographic scales. Nevertheless, the high geographic distances between the few reproductive areas leads one ask whether there is genetic differentiation in this species. In the present study, the genetic differentiation of the wild *A. regius* was investigated across most of its natural range from the Atlantic Ocean (France, Portugal, Spain, Mauritania) to the Mediterranean Sea (Egypt, Turkey), using 11 microsatellite markers previously identified in another Sciaenid, the red drum *Sciaenops ocellatus*. At least two very distinct groups could be identified, separated by the Gibraltar Strait. Genetic divergences (F_{ST} values) were intermediate between the Atlantic samples (0.012–0.041), high between Egypt and the Atlantic (0.06–0.107) or Aegean Sea (0.081) and extremely high between the Aegean Sea and the Atlantic (0.098–0.168). *A. regius* exhibited a very high level of genetic differentiation rarely reported in marine fishes. These results also demonstrate the existence of a sixth independent spawning area in the Menderes delta (Turkey). Factors potentially involved in this very high genetic fragmentation are discussed, including physical barriers, glaciation pulses and biological traits.

Keywords: Genetic variation / Population differentiation / Linkage disequilibrium / Microsatellites / Sciaenids / Drum / *Umbrina* / *Pseudotolithus* / *Argyrosomus*

1 Introduction

Marine fishes generally exhibit limited genetic differentiation across large geographic distances (>1000 km). This pattern has been attributed to their high rates of dispersal and movement during both nektonic and planktonic phases (Gyllensten 1985; Ward et al. 1994). The level of within-species differentiation also depends on physical or biological barriers such as hydrology, oceanic fronts, geomorphology, historical sea-level variation and animal behaviour, which interact with complex species-specific life history traits. Therefore, within-species differentiation is difficult to predict even if such pattern is known for other closely related conspecific or confamilial species (Naciri et al. 1999; Patarnello et al. 2007).

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Table 1. Description of the twelve *Argyrosomus regius* collection sites by geographic origin, locality, coordinates, abbreviation, size, date of collection, mean standard body length (cm ± SD), known or estimated (in bracket) age according to Hermas (1995), and origin (Exp: experimental fishery; Fishery: at fisheries landings; Aquac: from aquaculture of wild fish).

Geographic origin	Locality	Coordinates	Abbreviation	*N*	Date of collection	Standard body length (cm)	Age (years)	Origin
Atlantic Ocean	Gironde estuary, Mortagne, France	45° 27′ N, 52° 34′ W	France-1 (F1)	35	25 June 2008	19.8 ± 2.2	2	Exp
	Gironde estuary, St Seurin sur Dizet, France	45° 29′ N, 55° 23′ W	France-2 (F2)	37	26 June 2008	35.6 ± 1.2	3	
	Tagus estuary and Peniche, Portugal	39° 21′ N, 9° 22′ W	Portugal-1 (P1)	37	Sept. 2005 to May 2007	83.3 ± 30.9	2–12	Fishery
	South coast (Algarve), Portugal	37° 11'N, 7° 24′ W	Portugal-2 (P2)	30	Aug. 2005 to Jan. 2007	84.9 ± 40.5	2–23	
	Guadalquivir estuary, Spain	36° 57′ N, 6° 14′ W	Guadalquivir (S1)	30	9 Febr. 2009	Data not available	4	Aquac
	Nouakchott, Mauritania	18° 06′ N, 16° 01′ W	Mauritania-1 (M1)	12	19 May 2008	142 ± 8	(>15)	Fishery
	Nouadibou, Mauritania	20° 54′ N, 17° 02′ W	Mauritania-2 (M2)	9	21 May 2008	139 ± 13	(>14)	
	Nouamghar, Mauritania	19° 21′ N, 16° 30′ W	Mauritania-3 (M3)	12	22 May 2008	145 ± 13	(>14)	
	Arkais, Mauritania	20° 07′ N, 16° 15′ W	Mauritania-4 (M4)	29	23 May 2008	83 ± 15	(>6)	
Eastern Mediterranean Sea	Port Said (Farm 1), Egypt	31.21′ N, 32° 02′ E	Egypt-1 (E1)	30	12 Oct. 2009	28.5 ± 2.9	0	Aquac
	Port Said (Farm 2), Egypt	31.21′ N, 32° 02′ E	Egypt-2 (E2)	30	12 Oct. 2009	33.7 ± 2.8	0	
	Menderes Delta, Turkey	37.32′ N, 27° 10′ E	Turkey (T1)	30	7 March 2009	115 ± 10	10	

The meagre *Argyrosomus regius* (Asso 1801) is one of the world's largest Sciaenids, attaining over 180 cm total length and 50 kg body weight (after FishBase.org). This coastal semi-pelagic species is distributed in the eastern Atlantic Ocean, from the Bay of Biscay to the coast of Senegal, and across the Mediterranean Sea, Black Sea and Gulf of Suez. Planktonic eggs (990 μm diameter) are spawned in open water and hatched within 48 h. Mouth opening is observed 2–3 days post hatch and yolk sac absorption within 7 days post hatch (Tixerant 1974). Planktonic larvae develop in shallow lagoons and over mudflats when the temperature exceeds 20 °C (Quéro and Wayne 1987). Juveniles migrate and spread toward deeper waters in their second year (60–200 m, 12 °C). The reproductive biology of *A. regius* combines several specific reproductive traits (Tixerant 1974; Quéro and Vayne 1987; Hermas 1995; Prista et al. 2009). Adults migrate to coastal reproductive areas (10–15 m deep with high water flow associated with estuaries and/or tides) to spawn when temperatures reach 13 to 23 °C. The species exhibits also very high fecundity, late first reproduction (7 years old), long generation interval (> 40 years), overlapping generations, aggregation and schooling migration in nearshore waters. More over, only five restricted coastal spawning areas have been documented to date in the Lévrier Bay and the Banc d'Arguin (Mauritania) and at the opening of the Gironde (France), the Tagus (Portugal), the Guadalquivir (Spain) and the Nile (Egypt) estuaries (Tixerant 1974; El-Hehyawi 1974; Costa 1986; Quéro and Vayne 1987; Quéro 1989a, 1989b; González-Quirós et al. 2011).

Most of the above-mentioned reproductive traits favour low or absent genetic differentiation, while the high geographic distances between the only five reproduction areas would act in the opposite direction. However, no genetic information has been reported to date for *A. regius*.

The objective of this study was thus to characterise the genetic variability of *A. regius* across its native range, using microsatellite nuclear markers previously isolated from another Sciaenid, the red drum *Sciaenops ocellatus* (Linnaeus 1766) by Renshaw et al. (2006). Two further Sciaenid species that live sympatrically with *A. regius* were also genotyped with the same markers to assess potential misidentification: the shi drum *Umbrina cirrosa* (Linnaeus 1758) sympatric in the Mediterranean Sea and the law croaker *Pseudotolithus senegallus* (Cuvier 1830) sympatric along the west coast of Africa. The results are expected to provide useful information for the preservation of wild stocks, improvements in fishery management and initiation of breeding programs for *A. regius* aquaculture.

2 Materials and methods

2.1 Sample collection

Fin clips from 378 wild *A. regius* were collected from twelve different locations in the Atlantic Ocean and eastern Mediterranean Sea (Table 1, Fig. 1) by experimental fishery (in F1 and F2) or obtained from catches landed by local fishermen (in P1, P2 and M1 to M4) or from fish farms rearing wild fish (S1, E1, E2, T1). Further information is given per site of collection:

1. Sites F1 and F2 (France): fish from two age groups (2 years of age for F1 and 3 years of age for F2) were captured in 2008 in the Gironde estuary at Mortagne (F1) and at St Seurin d'Uzet (F2) using the IRSTEA catamaran research vessel, *L'Esturial* equipped with a bottom trawl (mesh size: 40 mm).
2. Site S1 (Spain): wild fish from 4 years of age were collected in 2008 in the FMD hatchery (Oléron Island, France). These fish were captured at 5–10 g in the Guadalquivir estuary in the summer 2005 by PIMSA fish farm (Seville, Spain).
3. P1 and P2 (Portugal): Fish came from two commercial landing areas located along the western (Tagus estuary and Peniche) and southern coasts (Quarteira to Vila Real de Santo António) of Portugal. Samples from both sites included juveniles and adults collected from 2005 to 2007.

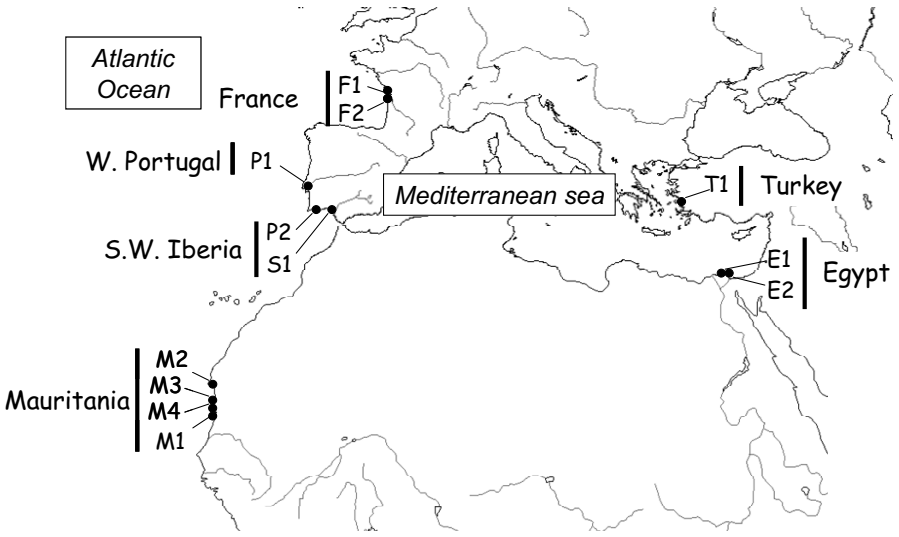

Fig. 1. Geographic locations of the twelve collection sites (abbreviations: F1, F2, etc.) and populations (full name: France, etc.) of meagre *Argyrosomus regius* samples. Abbreviations, localities and coordinates are given in Table 1.

4. Sites M1 to M4 (Mauritania): fins were collected in 2008 at commercial landing sites located at Nouakchott and Nouadhibou fishing harbours and in Nouamghar and Arkais Imragen villages (*Parc National du Banc d'Arguin*). The samples consisted of adult fish except in M4.
5. Sites E1 and E2 (Egypt): the samples were collected in 2009 from two fish farms located along the Manzalla Lake, close to Port Said. The fish consisted of wild juveniles captured as described by Sadek et al. (2009) between February and March 2008 along the Mediterranean coast between Alexandria and Port Said.
6. Site T1 (Turkey): fins were collected in 2009 from wild fish reared in captivity in the Egemar Su Ürünleri A.Þ. fish farm (Akbük-Didim, Aydın, Turkey). Fish consisted of mature individuals grown in the fish farm since their capture at 5–10 g in the lagoons of the Menderes Delta, south of Izmir during July and August 2000.

The shi drum (*U. cirrosa*) samples were collected in Turkey (n = 31) in the same geographical area as the *A. regius* samples. The law croaker (*P. senegallus*) samples were collected in Mauritania in Nouadhibou (n = 14) and in Arkais (n = 3), on the same days as the *A. regius* samples.

2.2 PCR amplification and microsatellite typing

All samples were stored in 95% alcohol and genotyped using 14 red drum microsatellites (Renshaw et al. 2006) combined to make two new panels: Soc11, Soc140, Soc400, Soc416, Soc423, Soc428, Soc592, Soc593 in panel 1 and Soc35, Soc44, Soc156, Soc410, Soc412, Soc432 in panel 2. Polymerase chain reaction (PCR) amplifications were performed in a final volume of 10 μl using a Qiagen® Multiplex PCR Kit and 50–100 ng of template DNA. Reactions were run for 30 cycles in an MJ thermal cycler (Model PTC-200). The PCR amplifications included an initial activation step at 95 °C for 15 min, denaturation at 94 °C for 30 s, primer annealing at 60 °C for 90 s, extension at 72 °C for 1 min and final extension at 60 °C for 30 min. After PCR amplification, an applied biosystems 3 730 xl DNA analyser with GeneMapper Analysis software (Applied Biosystems) was used to analyse the fluorescently tagged fragments for length polymorphisms.

2.3 Data analysis

2.3.1 Genetic variability and departure from Hardy-Weinberg equilibrium

The mean number of alleles per locus (*NA*) and the observed (H_{obs}) and unbiased expected (H_{exp}) heterozygosity (Nei 1978) were computed for each collection site and locus using Genetix 4.05.2 (Belkhir et al. 2004). The departure of genotypic frequencies from the expectations of Hardy-Weinberg equilibrium (HWE) was estimated within each site by the inbreeding coefficient or Wright's fixation index (F_{IS}) using Weir and Cockerham's (1984) f-estimator. The significance of the F_{IS} greater than zero (i.e. consistency with the null hypothesis on HWE) was estimated after 10 000 random allelic permutations and using simple Bonferroni procedure (Rice 1989) to correct for multiple testing and avoid type-1 errors (Rice 1989). In *A. regius*, the presence of null alleles or other scoring errors were estimated for all loci and collection sites using the MICRO-CHECKER program version 2.2.3 (van Oosterhout et al. 2004). The program uses the Monte Carlo simulation method to generate expected allele size difference frequencies and to compare the estimated null allele frequency using four different methods.

2.3.2 Genetic differentiation between populations and phylogenetic relationships

The differentiation between collection sites was estimated using Weir and Cockerham's (1984) global fixation index

(F_{ST}) estimator. F_{ST} were computed between sites of collection using Genetix 4.05.2 (Belkhir et al. 2004). Study-wide significance levels across collection sites were adjusted using 10 000 permutations on individual genotypes for the simple Bonferroni procedure.

The twelve collection sites were grouped into six new collection sites on the basis of the lack of significant pair-wise F_{ST} values between some of them and to increase the number of fish per site to 50 as recommended by Ruzzante (1998). The Mauritanian sites were pooled and treated as one population because of the limited number of samples per collection site, their close geographic proximity and their lack of genetic differentiation (though M4 was significantly different from M3 it had no transitivity with M2 and M1). The six new collection sites were identified as: "Egypt" (E1 + E2), "France" (F1 + F2), "Mauritania" (M1 + M2 + M3 + M4), "Southwest Iberia" (S1 + P2), "West Portugal" (P1) and "Turkey" (T1). These groupings were used in all further analyses and comparisons.

The same genetic estimators (H_e, H_{obs}, NA, number of alleles, number of fish genotyped, F_{IS}, F_{ST}) and statistical tests were then computed. Allelic richness (AR), representing a measure of the number of alleles independent of the sample size, was estimated using Fstat 2.9.3.2. (Goudet 1995). Difference in mean allelic richness and heterozygosity among the 6 collection sites were estimated using Friedman non-parametric test. The phylogenetic tree was drawn in MEGA 4.0 (Tamura et al. 2007) based on the $D_{Reynolds}$ genetic distances and using the neighbour-joining algorithm (Saitou and Nei 1987).

Different methods exist to estimate effective population size (Ne) based on heterozygote excess, temporal variation, linkage disequilibrium or the Bayesian method and can produce different results. As the estimation of Ne was not the main objective of the present work, estimation was only made based on non-random gametic linkage disequilibrium (LD) using *LDNe* software (Waples and Do 2008). Minimum allelic frequency was fixed at 0.05, as the less biased frequency reported and putative 95% confidence intervals calculated with parametric or jackknife methods.

3 Results

3.1 Genotyping of markers in each species

- *Pseudotolithus senegallus*: three of the 14 genetic markers did not amplify (Soc35, Soc416, Soc428) and 4 markers were monomorphic (Soc140, Soc156, Soc400, Soc592). The last 7 markers could be amplified and had a maximum of 4 alleles per marker (Soc11, Soc44, Soc410, Soc412, Soc423, Soc432, Soc593). Soc410 exhibited 4 alleles not observed in *A. regius*. Four of the seventeen fish sampled did not amplify at any locus. The F_{IS} was not estimated for this species due to the low number of alleles observed per fish.
- *Umbrina cirrosa*: three markers did not amplify (Soc423, Soc428 and Soc593) and 3 were monomorphic (Soc 44, Soc140, Soc400). Four markers exhibited more than 3 alleles (Soc416, Soc35, Soc156 and Soc432). A new allele not reported in *A. regius* was observed for each marker. The four last markers showed a good capacity for amplification and also 1 to 3 alleles not observed in *A. regius*: Soc11 (3 new alleles), Soc410 (2 new alleles), Soc412 (1 new allele) and Soc592 (3 new alleles). The F_{IS} values calculated for each marker indicated an excess of homozygotes for most of the markers (data not shown).
- *Argyrosomus regius*: the marker Soc416 amplified badly and the markers Soc 400 and Soc410 were monomorphic. These markers were not used in the genetic analysis of *A. regius*. One specimen collected in Mauritania identified as *Pseudotolithus senegallus* based on its genotype characteristics was discarded from the data analyses.

Overall, eleven of the fourteen red drum microsatellites used in this study proved useful for further genetic investigations in *A. regius*: Soc11, Soc140, Soc423, Soc428, Soc592, and Soc593 from panel 1; and Soc35, Soc44, Soc156, Soc412 and Soc432 from panel 2.

3.2 Genetic variability of *A. regius*

The number of alleles per locus varied from 3 to 31 (Table 2). Soc593 and Soc156 exhibited the lowest number of alleles (3 and 4 respectively), and Soc11, Soc35, Soc44, Soc412 and Soc428 the highest (14, 31, 17, 24, and 25, respectively).

The genetic characteristics (H_e, H_{obs}, NA, F_{IS}) and the number of fish genotyped in the twelve collection sites are presented in Table 3 (upper section). The mean number of alleles per site varied from 4.09 in T1 to 8.63 in M4, with a mean for all sites of 6.28 ± 1.15 (mean ± SD). H_{obs} within site for all markers varied from 0.47 ± 0.28 in T1 to 0.67 ± 0.19 in M3 and H_{exp} varied from 0.46 ± 0.24 in T1 to 0.65 ± 0.20 in M1.

Null alleles were only detected for the loci Soc44 in F1 and E1, Soc423 in T1 and E2 and Soc 412 in E1. Only the E1 ($p < 0.001$) and M4 ($p < 0.05$) collection sites showed departure of genotypic frequencies from the expectations of HWE equilibrium.

F_{ST} pair-wise comparisons revealed no significant differences between: F1 and F2, Mauritanian sites (except between M3 and M4), S1 and P2 or between E1 and E2 (Table 4).

The genetic characteristics (H_e, H_{obs}, NA, AR and F_{IS}) and the number of fish genotyped in the six collection sites are presented in Table 3 (lower section). The mean number of alleles per site varied from 4.09 in "Turkey" to 11.00 in "Mauritania", with a mean for all sites of 6.98±2.37 (mean ± SD). The allelic richness differed significantly between sites ($p < 0.001$) and varied from 4.09 to 8.72. It was highest for the sites in "Mauritania" and "SW Iberia", intermediate in "France" and "W Portugal" and the lowest in "Egypt" and "Turkey". H_e differed significantly between sites ($p > 0.002$), and within-site H_{obs} for all markers varied from 0.47 ± 0.28 in T1 to 0.64 ± 0.20 in F, and H_{exp} varied from 0.46 ± 0.24 in T1 to 0.65 ± 0.20 in M.

Heterozygote or homozygote excesses are given in Table 3 (lower section). When all markers were considered, only the Egyptian site showed a significant heterozygote deficiency. Null alleles were detected for Soc423 in Egypt and Turkey, for Soc11 in SW Iberia and for Soc44 in Egypt.

Highly significant F_{ST} differentiations ($p < 0.002$) were observed between all 6 collection sites (Table 5, above the

Table 2. Mean number of alleles per microsatellite marker (±SD) scored in meagre *Argyrosomus regius*, law croaker *Pseudotolithus senegallus* and shi drum *Umbrina cirrosa* genotyped in this study, taking the red drum *Sciaenops ocellatus* genotyped by Saillant et al. (2009) as a reference. * marker not used for genotyping by Saillant et al. (2009).

Microsatellites	*Argyrosomus regius*	*Pseudotolithus senegallus*	*Umbrina cirrosa*	*Sciaenops ocellatus*
Soc11	14	3	7	10
Soc35	31	–	5	*
Soc44	17	2	1	24
Soc140	6	1	1	4
Soc156	4	1	4	5
Soc412	24	3	5	25
Soc423	6	3	–	18
Soc428	25	–	–	28
Soc432	8	4	3	9
Soc592	7	1	7	*
Soc593	3	2	–	*
Number of fish sampled	361	17	31	45
Mean number of alleles per locus for all markers	13.2 ± 9.7	2.2 ± 1.1	4.1 ± 2.4	15.4 ± 9.6
Mean number of alleles per locus with only the Saillant et al. (2009) common markers	13.2 ± 9.7	2.4 ± 1.1	3.5 ± 2.3	15.4 ± 9.6

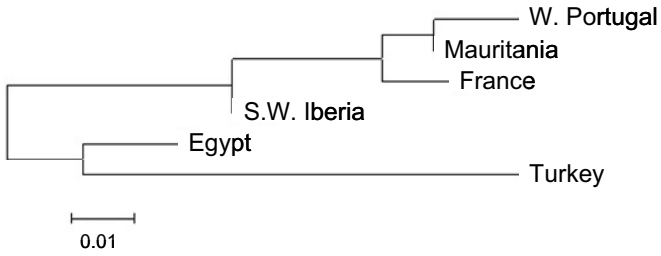

Fig. 2. Evolutionary relationships of the six *Argyrosomus regius* populations. The optimal tree is inferred by Neighbour-Joining with a sum of branch length. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA 4.

diagonal). The lowest F_{ST} values were found between the Atlantic samples (values from $0.012 < F_{ST} < 0.041$). Differentiation was moderate between the "Egypt" sample and all other populations ($0.061 < F_{ST} < 0.107$). The highest F_{ST} values were found between "Turkey" and all other sites ($0.081 < F_{ST} < 0.168$), the highest of these being observed between "Turkey" and "W Portugal". Within the Mediterranean, despite a relative geographical proximity, high genetic differentiation was also observed between the two samples from "Egypt" and "Turkey" (0.081). The phylogenetic tree (Fig. 2) illustrates the subdivision of *A. regius* populations into two distinct groups between Atlantic and Mediterranean.

Estimates of effective population size (*Ne*) are given in Table 6. Upper *Ne* values could not be differentiated from infinity (under the 95% confidence interval) for the populations from France, W Portugal and SW Iberia. The population from Mauritania showed a more limited *Ne* value of 111.0 ($61.6 < Ne < 330.3$). Only the population from Turkey presented a very low *Ne* of 17.4 ($10.3 < Ne <$ and 33.6).

4 Discussion

4.1 Genetic structuring

Genetic characterisation was made with microsatellite markers isolated from another species. The very limited number of null alleles in *A. regius* confirms the potential for cross-amplification between Sciaenids (Turner et al. 1998) and ability to identify *U. cirrosa* and *P. senegallus* species. Incidentally, *A. regius* presents a lower mean number of alleles per locus per population than the red drum (Saillant et al. 2009).

The non significant F_{ST} values between different collection sites justified their pooling into six distinct new sites. Among the Mauritanian sites, a sampling artefact can be suspected for sample M4, as it is differentiated from one Mauritanian sample but not the other two. A hierarchical analysis of molecular variance could have been performed to account for regions and collection sites within regions, but we estimated that genetic differentiation was so high that any type of statistical method would give the same kind of results. Similarly, we did not estimate the effect of geographic distance on genetic differentiation since the geographic distances between sites are so great and different that this effect is obvious.

A. regius can be divided into at least two very distant genetic groups: Atlantic and Mediterranean. Our results reveal the existence of a previously unknown sixth distinct reproductive area for the species in the Aegean Sea, at the mouth of the Menderes river delta in Turkey. Interestingly, the SW Iberia population is somewhat intermediate between the Atlantic and Mediterranean, which is in accord with its geographical location. Reproduction has been also suggested in Morocco

Table 3. Genetic variability and F_{IS} estimates at 11 microsatellite loci in twelve *Argyrosomus regius* collection sites (upper panel) or the same samples pooled into six populations (lower panel). *N*: number of individuals; *NA*: mean number of alleles per locus; *AR*: Allelic richness estimated for $n = 27$; H_{exp} = Nei's (1987) gene diversity (across-locus standard deviation in brackets); H_{obs}: observed heterozygosity; F_{IS} : heterozygote deficiency within collection site (upper part of the Table) or within population (lower part of the Table) for all loci or per locus. Significant levels after Bonferroni correction (Rice 1989) are shown in bold: * $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$. F_{IS} values with null allele are underlined.

	N	*NA*	ArR	*He*	*Ho*	Multilocus F_{IS}	*p*-value	Soc11	Soc35	Soc44	Soc140	Soc156	Soc412	Soc423	Soc428	Soc432	Soc592	Soc593
All sites																		
Egypt-1 (E1)	30	5.81		0.54 (0.23)	0.49 (0.22)	0.114	**<0.001**	–0.035	0.096	**0.444*****	**0.275***	–0.074	**0.191***	0.069	–0.050	0.117	0.116	–0.094
Egypt-2 (E2)	30	5.72		0.54 (0.25)	0.53 (0.27)	0.032	0.147	0.104	–0.078	–0.081	0.073	0.151	0.084	**0.335****	**0.106***	–0.167	–0.031	**–0.035***
Spain (S1)	28	7.72		0.61 (0.20)	0.61 (0.23)	0.005	0.448	0.118	–0.087	–0.026	0.112	–0.058	–0.024	–0.059	–0.119	–0.070	**0.176***	0.142
France-1 (F1)	35	6.27		0.64 (0.16)	0.61 (0.22)	0.041	0.095	–0.026	–0.111	**0.299****	0.004	**0.413***	–0.031	–0.185	–0.011	0.072	**0.283****	–0.084
France-2 (F2)	37	6.45		0.63 (0.18)	0.66 (0.21)	–0.031	0.863	–0.072	**0.114***	–0.172	–0.083	–0.102	–0.075	0.004	–0.100	0.040	–0.040	**0.254***
Mauritania-1 (M1)	12	6.72		0.65 (0.20)	0.66 (0.21)	–0.052	0.865	–0.038	–0.038	–0.052	**0.262***	–0.100*	–0.094	–0.308	–0.051	0.009	–0.213	0.009
Mauritania-2 (M2)	9	6.36		0.63 (0.19)	0.61 (0.22)	0.058	0.141	0.138	0.096	0.130	0.158	–0.067	–0.142	0.094	**0.085***	–0.191	–0.032	**0.400***
Mauritania-3 (M3)	12	5.63		0.62 (0.19)	0.67 (0.19)	–0.041	0.761	–0.151	0.034	0.2090	–0.054	–0.158	–0.234	0.172	–0.073	0.074	–0.222	–0.200
Mauritania-4 (M4)	29	8.63		0.63 (0.21)	0.61 (0.20)	0.051	**0.037**	0.094	0.067	0.000	0.029	–0.120	**0.119***	–0.206	**0.118***	0.075	–0.006	**0.263***
Portugal-1 (P1)	28	5.36		0.60 (0.19)	0.59 (0.21)	0.031	0.189	**0.197****	0.018	0.087	–0.103	**0.286***	**0.160***	–0.175	–0.085	0.033	–0.046	0.044
Portugal-2 (P2)	25	6.63		0.59 (0.21)	0.61 (0.21)	–0.008	0.571	**0.226****	0.033	0.058	–0.163	–0.095	–0.065	–0.155	0.029	–0.061	0.010	–0.116
Turkey (T1)	27	4.09		0.46 (0.24)	0.47 (0.28)	0.003	0.464	**0.198***	0.070	0.118	–0.040	–0.106	–0.027	**0.546*****	–0.206	–0.097	–0.138	–0.083
Pooled sites																		
Egypt (E1 + E2)	60	6.45	5.69	0.55 (0.24)	0.51 (0.24)	0.073	**0.002****	0.033	0.021	**0.185****	**0.174***	0.059	**0.135***	**0.195****	0.033	–0.027	0.039	–0.067
SW Iberia (P2 + S1)	53	7.72	7.17	0.61 (0.20)	0.61 (0.23)	0.003	0.455	**0.177***	–0.033	0.020	–0.021	–0.084	–0.041	–0.102	–0.052	–0.055	0.102	0.091
France (F1 + F2)	72	7.27	6.13	0.64 (0.17)	0.64 (0.20)	0.006	0.399	–0.047	0.003	0.045	–0.036	**0.159***	–0.043	–0.087	–0.056	0.051	**0.141***	0.082
Mauritania (M1 to M4)	62	11.00	8.72	0.65 (0.20)	0.64 (0.19)	0.022	0.131	0.068	0.064	0.058	0.071	–0.140	–0.038	–0.099	0.038	0.016	–0.028	0.140
W Portugal (P1)	28	5.36	5.33	0.60 (0.19)	0.59 (0.21)	0.032	0.196	**0.197****	0.018	0.087	–0.103	**0.286***	**0.160***	–0.175	–0.085	0.033	–0.046	0.044
Turkey (T1)	27	4.09	4.09	0.46 (0.24)	0.47 (0.28)	0.003	0.464	**0.198***	0.070	0.118	–0.040	–0.106	–0.027	**0.546*****	–0.206	–0.097	–0.138	–0.083

Table 4. Pair-wise estimates of F_{ST} values among the twelve *Argyrosomus regius* collection sites: Weir and Cockerham (1984) θ-statistics above the diagonal and significance below the diagonal after simple Bonferroni correction (Rice 1989).

	France-1 (F1)	France-2 (F2)	Portugal-1 (P1)	Portugal-2 (P2)	Spain (S1)	Mauritania-1 (M1)	Mauritania-2 (M2)	Mauritania-3 (M3)	Mauritania-4 (M4)	Egypt-1 (E1)	Egypt-2 (E2)	Turkey (T1)
France-1 (F1)		0.003	0.021	0.023	0.035	0.021	0.026	0.033	0.038	0.112	0.108	0.159
France-2 (F2)	0.186		0.033	0.020	0.033	0.019	0.024	0.029	0.038	0.090	0.089	0.137
Portugal -1 (P1)	0.004	< 0.001		0.015*	0.014	0.044	0.019	0.044	0.034	0.109	0.011	0.168
Portugal.-2 (P2)	0.002	< 0.001	< 0.011		0.008	0.019	0.019	0.038	0.025	0.062	0.055	0.119
Spain (S1)	< 0.001	< 0.001	< 0.011	0.076		0.034	0.030	0.039	0.039	0.096	0.081	0.146
Mauritania-1 (M1)	0.019	0.009	< 0.001	0.017	0.002		0.007	0.001	0.006	0.068	0.065	0.113
Mauritania-2 (M2)	0.016.	0.009	0.005	0.039	0.006	0.693		0.023	0.009	0.057	0.061	0.118
Mauritania-3 (M3)	0.008	< 0.001	< 0.001	< 0.001	< 0.001	0.449	0.053		0.042	0.093	0.079	0.126
Mauritania-4 (M4)	< 0.001	< 0.001	< 0.001	< 0.001	<0.001	0.169	0.874	0.002		0.063	0.065	0.115
Egypt-1 (E1)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		0.001	0.093
Egypt-2 (E2)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.506		0.075
Turkey (T1)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Table 5. Pair-wise estimates of F_{ST} values among the six *Argyrosomus regius* populations: Weir and Cockerham (1984) θ-statistics above the diagonal and significance below the diagonal after simple Bonferroni correction (Rice 1989).

Samples	France	W Portugal	Mauritania	SW Iberia	Egypt	Turkey
France		0.026	0.026	0.025	0.099	0.140
W Portugal	<0.001		0.041	0.012	0.107	0.168
Mauritania	< 0.001	< 0.001		0.024	0.061	0.098
SW Iberia	0.003	< 0.001	< 0.001		0.073	0.126
Egypt	< 0.001	< 0.001	< 0.001	< 0.001		0.081
Turkey	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Table 6. Effective population size (*Ne*) estimates for the six *Argyrosomus regius* populations based on linkage disequilibrium estimated with parametric and jackknife methods (Waples and Do 2008). *Ne*: mean effective population size, upper and lower 95% confidence interval are shown. * Negative value, estimated by the *LDNe* software, suggests *Ne* is not different from infinity.

Samples	*Ne*	Parametric method		Jackknife method	
		Lower	Upper	Lower	Upper
France	−634.1*	394.1	Large	281.3	Large
W Portugal	−117.5*	250.2	Large	136.0	Large
SW Iberia	−722.1*	182.5	Large	99.3	Large
Mauritania	111.0	68.7	237.2	61.6	330.3
Egypt	531.5	292.3	Large	151.6	Large
Turkey	17.4	10.7	31.8	10.3	33.6

(Hermas 1995) and in several other cryptic areas (Costa pers. comm.; Champagnat and Domain 1978; Dieuzeide 1929; Chakroum et al. 1983; Quéro 1989b).

4.2 Why such high genetic differentiation?

A. regius F_{ST} values are very high and in the highest ever reported in marine fish from this the same geographic area (Bonhomme et al. 2002; Nielsen et al. 2003, 2004; Alarcón et al. 2004; Kotoulas et al. 2006; Maggio et al. 2009; Gallarza et al. 2009; González-Wangüemert et al. 2010) or in smaller Sciaenids (Lankford et al. 1999; Gold et al. 2001; Ward et al. 2007; Han et al. 2008; Xiao et al. 2009). Such F_{ST} values have only been previously reported for coastal marine species strongly affected by post-glacial recolonization (Wilson and Veraguth 2010) or for populations separated by the Atlantic ocean (Ball et al. 2007) or by the Indian Ocean for another *Argyrosomus* species, the mulloway *A. japonicus* (Archangi 2008).

Geographic and hydrological barriers were advocated to explain genetic differentiation in marine fish. The Cape Sagres separates the two Portuguese populations, which are less than 200 km apart. The Gibraltar Strait-Alboran Sea zone and the Siculo-Tunisian Strait limit genetic exchanges between the Atlantic Ocean and the southeast Mediterranean Sea (see Naciri et al. 1999; Barhi-Sfar et al. 2000; Patarnello et al. 2007). In the northern waters of the Aegean Sea, the lowest salinity and the coolest temperature induce a cyclonic circulation, causing its isolation from Egypt (Barhi-Sfar et al. 2000). These barriers may have played an important role in the recent past of the species.

The subdivision into the two Atlantic and Mediterranean groups and the lower allelic richness and effective sizes of the Mediterranean populations could be due to the effects of vicariance, limited introgression after secondary contact, and/or population expansion following the successive coolings and warmings of the Mediterranean Sea during previous interglacial phases of the Quaternary (Borsa et al. 1997; Bianchi and Morri 2000; Patarnello et al. 2007). Even though the present results do not demonstrate the impact of Pleistocene glaciation per se, these glaciations could have also restricted *A. regius* area in the Atlantic to a more southern part than its

present distribution. Crowley (1981) estimates very low summer temperature (9.2 °C) in northern Portuguese waters at the time of the 18 000-year B.P. during the last glacial maximum. Such a low temperature makes the reproduction of *A. regius* in the Tagus and the Gironde estuaries theoretically impossible at this time. These northern populations could, therefore, result from a recent expansion in the North Atlantic Ocean, which is in agreement with their lower allelic richness. In the Atlantic, this result confirms the early Tixerant (1974) hypothesis of differentiation between France and Mauritania based on differential otolith growth. In the Mediterranean Sea, the genetic differentiation between the two populations is in agreement with the two biogeographic areas defined by Bianchi and Morri (2000) as "North Aegean" and "Gulf of Gabes to Levant Sea".

Biological factors have also been put forward to explain the genetic differentiation of marine organisms. However, early life-history traits (egg type, pelagic larval duration, and inshore-offshore spawning) have a limited involvement in the genetic differentiation of marine fishes (Gallarza et al. 2009). For *A. regius*, Tixerant (1974) and Quéro (1989a) pointed out factors that varied between Mauritania, Egypt and France, such as the difference in water salinity. They also identified differences in the duration and the time of reproduction (3 weeks in early June in the Gironde, the 2 months of February and March in Egypt, and 9 months from October to June in Mauritania). But among all the biological factors, reproductive migration is probably the most important factor limiting adult movement and, therefore, limiting gene flow between reproductive areas. This factor is reinforced by two others. The first is the limited number of potentially favourable sites for *A. regius* reproduction and for long term settlement. The need for an actual estuary was questioned by Tixerant (1974), as there is no estuary in Mauritania. A site favourable for reproduction would require intermediate temperature (14 °C to 23 °C), with an optimal window (19 °C to 21 °C) for reproduction and successful larval recruitment (Quéro and Vayne 1987; Quéro 1989a), high water flow and minimal water depth for spawning (>10−15 m). The second requirement is the need for extensive mudflats (in the Atlantic) or lagoons (in the Mediterranean) habitats close to the reproduction sites to provide a suitable environment for larval recruitment and juvenile growth (Quéro and Wayne 1987). The Gironde, Tagus, Guadalquivir and Menderes estuaries, the sea areas at the opening of the Nile river delta and the large mudflats in the Banc d'Arguin in Mauritania fulfil these basic requirements.

4.3 Potential biases

In this study, temporal variation in allele frequencies may be confounded with spatial variation because samples were composed of very different age groups among and within sites. However, the magnitude of the divergence between regions is so large that some noise due to temporal genetic variation is unlikely to have a great impact on the observed patterns of population structure reported here.

Bias could potentially arise from rearing wild fish in captivity by artificial selection for survival even if we estimate genetic variability of neutral markers. Only the population from Egypt had a significant heterozygote deficiency when all loci were considered. Inbreeding can be excluded, as heterozygote deficiency was not the case at all loci. As heterozygote deficiency was also estimated before the pooling of the two collection sites, Wahlund effect (presence of different genetic stocks in a single sample) seems improbable as such a hypothesis would imply that each of the two Egyptian stocks would have been composed of different origins in the same ratio. Assortative mating can also be excluded as *A. regius* is a mass spawner (Tixerant 1974). An effect of artificial selection during captivity would require a strong association between the potentially neutral loci and survival, which is unrealistic with the limited number of markers used. Only a more detailed investigation on wild captured cohorts among different Egyptians coastal lakes could allow conclusions about these factors. In any case, the heterozygote deficiency observed in the Egyptian samples does not interfere with the general conclusion that there is very high geographic differentiation.

4.4 Effective population sizes

The estimations of population size differ greatly: from infinity (France, W Portugal, SW Iberia) to very limited (Turkey). Several factors need to be considered for the interpretation of these results.

Ne estimates were obtained from populations composed of different age groups (W Portugal, SW Iberia and Mauritania), a single age group (Egypt, Turkey) or two age groups (France). If estimates in the former three populations represent approximations of *Ne* (effective size for the generation), the *Ne* estimates from the latter three populations most probably reflect N_b (effective number of breeders having produced the sample) than N_e (Waples 2005). It can then be argued that the assumption of the statistical models based on discrete generations is severely violated as *A. regius* reproduces with overlapping generations until fourty years of age.

The long generation interval and overlapping generations are also factors that could cause genetic disequilibrium between age groups and underestimation of the true effective number of parents ("sweepstake recruitment hypothesis", Hedgecock 1994). Preliminary results from F1 and F2 yearly cohorts do not provide much if any support for this hypothesis as the two cohorts collected from the same population exhibited neither genetic differentiation nor heterozygote deficiency (caused in this case by temporal Wahlund effect).

High temporal heterogeneity in effective population size was also associated with a very low ratio of individuals producing new young fish to adult census population size in another large Sciaenid, the red drum *Scianops ocellatus* (Turner et al. 2002). The high variation in *A. regius* captures (i.e. from 350 t in 1992 to 12 200 t in 2001 in Mauritania or from 35 t in 1985 to 1356 t in 2006 in France, FAO data) could also result and/or create unequal reproductive success and fluctuation in population size between cohorts (Hedrick 2005).

Finally, all populations show very limited lower bounds of effective size, inferior to 500 (Table 6), which is very low for a marine fish and may indicate a population risk for long term viability (Franklin and Frankham 1998). Among the six populations identified, Turkey and Mauritania had a finite upper

estimate of *Ne* effective (33.6 or 330.3). For the population from Turkey, that is also the less variable and the more genetically differentiated, the actual small surface of the Menderes delta, and its shallow lagoons for the juvenile development, could explain the limited annual captures (< 50 t, Gamsiz K. pers. comm.). The Holocene sea-level variations (Ergin et al. 2007; Kazanci et al. 2009) are also factors that have greatly restricted the Menderes delta surface and potentially limited reproductive capacities and therefore *Ne*. The high genetic variation of the population from Mauritania is opposite to its limited *Ne* estimate. Since the Mauritanian "sample" combined small numbers of fish collected at four locations, the *Ne* estimate could be biased downward by mixture disequilibria from a two-locus Wahlund effect (see discussion in 4.3. about F_{ST} values and genetic differentiation between Mauritanian sites) and underestimate the real *Ne*, which should be considered higher.

More generally, the meagre shared several biological factors with the ten other large Sciaenids already threatened and for which the protection of seasonal aggregation areas and nursery grounds was advocated (Cisneros-Mata et al. 1995; Sadovy and Cheung 2003; Liu and Sadovy de Mitcheson 2008). The limited values of *Ne* and the very long distances known between reproduction areas should be considered in the meagre management, as recent river water pollution, modification of water flow and overfishing at 1^+ year of age were reported as factors associated with decreases in meagre abundance (Bebars et al. 1996; Oczkowski and Nixon 2008; Kazanci et al. 2009; Sourget and Biais 2009; Morales-Nin et al. 2012).

5 Conclusion

This study provides the first genetic characterisation of *Argyronomus regius* across most of its natural range. The species is genetically highly structured, with a degree of differentiation rarely reported in a marine fish. The high genetic fragmentation highlights the genetic originality of each population and the need to consider their management regionally. This work also demonstrates the existence of a previously unknown reproductive area in the Aegean Sea. Future studies, including the genetic characterisation of fish from other areas (Morocco, Balearic Islands, etc.) or other cryptic populations using microsatellite markers or mtDNA could offer further insight about *A. regius* ecology, biodiversity and recent evolution. These data will be useful for its preservation and for its exploitation by fisheries and aquaculture.

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