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Phylogeographic structure and demographic patterns of brown trout in North-West Africa

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ABSTRACT

The objectives of the study were to determine the phylogeographic structure of brown trout (*Salmo trutta*) in Morocco, elucidate their colonization patterns in North-West Africa and identify the mtDNA lineages involved in this process. We also aimed to resolve whether certain brown trout entities are also genetically distinct. Sixty-two brown trout from eleven locations across the Mediterranean and the Atlantic drainages in Morocco were surveyed using sequence analysis of the mtDNA control region and nuclear gene LDH, and by genotyping twelve microsatellite loci. Our study confirms that in Morocco both the Atlantic and Mediterranean basins are populated by Atlantic mtDNA lineage brown trout only, demonstrating that the Atlantic lineage (especially its southern clade) invaded initially not only the western part of the Mediterranean basin in Morocco but also expanded deep into the central area. Atlantic haplotypes identified here sort into three distinct groups suggesting Morocco was colonized in at least three successive waves (1.2, 0.4 and 0.2–0.1 MY ago). This notion becomes more pronounced with the finding of a distinct haplotype in the Dades river system, whose origin appears to coalesce with the nascent stage of the basal mtDNA evolutionary lineages of brown trout. According to our results, *Salmo akairos*, *Salmo pellegrini* and "green trout" from Lake Isli do not exhibited any character states that distinctively separate them from the other brown trout populations studied. Therefore, their status as distinct species was not confirmed.

1. Introduction

The brown trout (*Salmo trutta*, in this study considered as a single species except for *Salmo salar*, *Salmo obtusirostris* and *Salmo ohridanus*) is the most widely distributed Palaearctic freshwater fish. It is native across Europe, including Iceland, and parts of western and central Asia, with a natural range that extends southwards even to the Atlas Mountains of North-West Africa (Pellegrin, 1921, 1924a; Azeroual, 2003). Up until the Miocene, the inland water ichthyofauna of North-West Africa had a tropical character, after which it became Palaearctic in composition (Greenwood, 1974). Due to unsuitable tropical warm conditions in seas surrounding the Iberian Peninsula and northern Africa (Pérès, 1985), it is believed that brown trout did not arrive in this region before the onset of the Pleistocene cooling. During the warm interglacial periods, brown trout in North-West Africa largely became extinct with some populations retreating to higher altitudes, while during cold periods, the distribution range advanced southwards. Thus brown trout from more northerly locations expanded their range along the Atlantic coast as far south as the Draa river basin at a latitude of about 28°N, and along the Mediterranean zone eastwards to Algeria and even Sicily (Schöffmann et al., 2007; Cortey et al., 2009). Due to cycling of glacial periods, the southward expansion of brown trout into the coastal rivers of the Iberian Peninsula and North-West Africa appears to have repeated several times (Cortey et al., 2009).

Brown trout in Morroco of both the Atlantic and the Mediterranean basins have been assigned, on the basis of a limited sample-set (Bernatchez, 2001; Suarez et al., 2001), to the southern clade of the Atlantic mtDNA lineage, largely present in the Iberian peninsula (Cortey et al., 2009). This assignment is centered on the concept of major evolutionary lineages based upon mtDNA analysis, e.g., Atlantic (AT), Danube (DA), Mediterranean (ME), Adriatic (AD), *marmoratus* (MA) (Bernatchez et al., 1992) and Duero (DU) (Suarez et al., 2001).

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In Morocco, autochthonous brown trout presently occur in the High Atlas above an altitude of 1500 m, in the Middle Atlas above 1200 m, and in the Mediterranean tributaries of the Rif Mountains, where they reside at an altitude of about 400 m (Azeroual, 2003; unpublished data). Yet in the middle years of the last century, the lower altitude limit of brown trout residence in the Middle Atlas was found to be about 900 m (Kerans, 1962). In the western High Atlas Mountains, the southernmost natural distribution of brown trout is nowadays confined to a latitude of 30° 50′N (River Nfiss of the Tensift basin), and 31° 02′ (Lake Ifni of the Sous basin). Curiously, one brown trout population inhabits the headwaters of a tributary of the River Ziz, which rises on the south slope of the High Atlas and drains into the Sahara Desert (Ilahiane, 1996; Hammada, 2007). Isolated lake-dwelling brown trout still exist in two lakes of the High Atlas: the recently described "dwarf trout", *Salmo akairos* of Lake Ifni (Delling and Doadrio, 2005) and the "green trout" of Lake Isli (Vivier, 1948; Mouslih, 1987). Another lake-dwelling trout, also described as a distinct species *Salmo pallaryi*, was reported from the high mountain Lake Aguelmame Sidi Ali in the Middle Atlas (Pellegrin, 1924a,b) and from the nearby lake Tiguelmamine N'Ait Mahi (Vivier, 1948). However, this trout disappeared soon after the introduction of carp (*Cyprinus carpio*) in 1934 (Mouslih, 1987; Schöffmann, 1993). *Salmo macrostigma* (Duméril, 1858), first described from Oued el Abaïch in north-eastern Algeria, was described also in Morocco from the River Tigrigra of the Sebou basin, the upper River Moulouya with its tributaries Ansegmir and Berrem, and the River Melloulou, a tributary to the lower Moulouya river system (Pellegrin, 1924a; Joleaud, 1938). The name *S. macrostigma* has been applied also to several peri-Mediterranean trout populations (Bianco, 1994; Kottelat, 1997) though without any demonstrative analysis. Another morphologically distinct species, *Salmo pellegrini*, was described from the stream Ourika, south of the city of Marrakech (Werner, 1931) and characterized by an appearance intermediate between *S. pallaryi* and *S. macrostigma*.

In the course of the twentieth century, many native brown trout populations in Morocco became drastically reduced or extirpated due to human activities. The predominant threats to the remaining populations are increased water use, environmental degradation due to deforestation and overgrazing, road and dam constructions, water pollution and global warming. Some locations have been stocked with a domestic brown trout strain originating from River Oum Er Rbia upstream wild brown trout at the state hatchery of Azrou (Centre National d'Hydrobiologie et de Pisciculture, CNHP, unpublished report).

The primary objectives of this study are to determine phylogeography of brown trout in Morocco, elucidate their colonization patterns and identify the lineages involved. In this context we examine the proposed hypothesis of colonization of Sicily with brown trout via North-West Africa.

A further aim is to genetically profile *S. pellegrini* (River Ourika), *S. akairos* (Lake Ifni) and "green trout" (Lake Isli) and determine if they represent genetically distinct entities.

2. Material and methods

2.1. Material

Between 1998 and 2010, 62 brown trout from eleven locations across the Mediterranean and the Atlantic river basins in Morocco were fin-clipped (Table 1, Fig. 1). Allegedly unstocked locations were selected based upon information published in the literature (Schöffmann, 1993; Kottelat, 1997; Bernatchez, 2001; Delling and Doadrio, 2005) and the authors' personal observations. The highly limited range of brown trout in Morocco—it is confined mainly to remote and inaccessible small streams in the Atlas and Riff Mountains—made sampling extremely difficult and, crucially, contributed to a relatively small number of sampling sites.

Total DNA was isolated from fin tissue preserved in 96% ethanol following the protocol of Medrano et al. (1990).

Brown trout samples from Sicily (see Schöffmann et al., 2007) were reanalyzed in order to obtain comparable sequences of mtDNA CR.

2.2. Mitochondrial DNA amplification, sequencing and data analysis

Mitochondrial DNA CR was amplified by polymerase chain reaction (PCR) using primers 28RIBa (Sušnik et al., 2001) and HN20 (Bernatchez and Danzmann, 1993). Each 30 μl reaction included 1 μM of each primer, 0.2 μM dNTP, 1.5 μM $MgCl_2$, 1× PCR buffer, 1 U *Taq* polymerase (Applied Biosystems, Foster City, CA, USA) and 50 ng of genomic DNA. Amplified DNA fragments were run on a 1.5% gel and subsequently dissected and isolated from the gel using the QIAEX II gel Extraction Kit (QIAGEN, Hilden, Germany). The conditions for PCR were initial denaturation (95 °C, 3 min) followed by 30 cycles of strand denaturation (94 °C, 45 s), primer annealing (52 °C, 45 s) and DNA extension (72 °C, 2 min). All PCR amplifications were performed in a programmable thermocycler GeneAmp® PCR System 9700 (Applied Biosystems).

All sequencing reactions were prepared using a BigDye Terminator Ready Reaction Mix (Applied Biosystems) according to the manufacturer's recommendations. The control region fragment was sequenced from both directions using PCR primers. Termination PCRs were performed in a programmable thermocycler under the following conditions: 10 s denaturation at 96 °C, 5 s annealing at 50 °C and 4 min extension at 60 °C, repeated for 30 cycles. The amplified, fluorescently labeled and terminated DNA was salt-precipitated and analysed with an ABI Prism 3130 xl automated sequencer.

Table 1
Sample sites and sizes (*N*), mtDNA haplotypes, and corresponding river system and drainages (AT, Atlantic; ME, Mediterranean). Sample numbers correspond to those in Fig. 1. For details about the samples from Sicily, see Schöffmann et al. (2007).

Sample No.	Location	*N*	mtDNA haplotype	River system/basin	Drainage
1	Lake Isli	4	ATM2	Oued Melloul → Oum Er Rbia	AT
2	Melloul	4	ATM6	Oum Er Rbia	AT
3	Ourika	5	ATM4	Tensift	AT
4	Ait Mizane	12	ATM1, ATcs25	Reghaya → Tensift	AT
5	Lake Ifni	7	ATM3	Tifnout → l'oued Souss	AT
6	M-Goun	2	Dades	Draa	AT
7	Dades	12	Dades	Draa	AT
8	Zaouia-Sidi-Hamza	5	ATM7	Ziz	AT
9	Berrem	4	ATM4	Moulouya	ME
10	Kanar	3	ATcs33	Medit. Sea basin	ME
11	Adelma	4	ATM5	Medit. Sea basin	ME