

## Genetic verification of native brown trout from the Persian Gulf (Catak Cay River, Tigris basin)

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Analysis of the mtDNA control region in six individual brown trout *Salmo trutta* from the Catak Cay River (Tigris basin) revealed a single, new, haplotype, 1.0 to 1.5% divergent from five other Da lineage haplotypes analysed, that groups with the Danubian clade with low bootstrap support. This highly divergent haplotype combined with unique phenotypic characteristics underscores the novelty and native status of this population, which has probably been isolated from other brown trout lineages for at least several hundred thousand years.

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The evolutionary history of brown trout *Salmo trutta* L. has been extensively studied with molecular genetic approaches throughout its natural range of distribution (Bernatchez *et al.*, 1992; Apostolidis *et al.*, 1997; Weiss *et al.*, 2000; Bernatchez, 2001). All such studies have demonstrated five distinct phylogenetic lineages (defined by mtDNA sequences), four of which correspond to major basins [Atlantic, Mediterranean, Adriatic and Danube (Black/Caspian/Aral Seas)] and one to the distinct phenotype commonly known as marble trout *Salmo trutta marmoratus* Cuvier, limited to the Adriatic basin. These lineages presumably evolved in geographic isolation during the Pleistocene (Bernatchez, 2001). Despite the extensive literature no genetic based studies note that the brown trout may indeed be native to the headwaters of the Tigris and Euphrates Rivers of the Persian Gulf basin. Seven individual samples from three localities in the upper Euphrates River system (Tohma, Fyrat and Balikli) were typed for mtDNA control region (CR) variation in Bernatchez (2001) and haplotypes were phylogenetically assigned to the so-called Adriatic and Danubian lineages. Adriatic haplotype Ad6 found in the Tohma River was confined to this population, whereas the Da haplotypes from Fyrat and Balikli were previously found in diverse locations in the Black, Caspian and Aegean Sea basins. These results, however, are not discussed in Bernatchez (2001). The phylogeographic

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significance of diverse mtDNA lineages in the Tigris-Euphrates basin has never been addressed, nor the question of whether or not these haplotypes represent native material from the region.

The Euphrates and Tigris Rivers are the two major rivers flowing through Mesopotamia to the Persian Gulf. Over 1900 km long, the Tigris River rises from two sources near Elazig in Turkey, and joins the Euphrates River to form the Shatt Al-arab. Several reports note that brown trout were introduced into the Tigris-Euphrates basin (Coad, 1996a; Fishbase, 2003), but Heckel (1846–1849) reported that native brown trout may exist in the upper Tigris drainage. Later, Tortonese (1954) described a brown trout with a large red-spotted adipose fin from Catak Cay River, a small tributary of the Tigris River. In reports on the world distribution of the species (MacCrimmon & Marshall, 1968; MacCrimmon *et al.*, 1970), the possible native distribution into the Tigris-Euphrates system is mentioned only with a 'pers. comm' citation from R. Behnke, but no material from this basin is examined in Behnke (1965) nor is the basin specifically addressed in Behnke (1986).

In the present study the mtDNA control region variation was analysed from individual samples originating from the Catak Cay River, in order to assess their phylogenetic position within the species, and attempt to resolve the question of whether or not such populations are native to the Persian-Gulf basin (Tigris River).

Six samples of brown trout were collected from Catak Cay River (upper Tigris basin, eastern Turkey; 38°07' N; 42°48' E). Total DNA was isolated from fin clips, preserved in 95% ethanol, using the Wizard Genomic DNA Purification Kit (Promega). Approximately 1300 bp of the mtDNA CR were amplified using primers 28RIBa (Snoj *et al.*, 2000) and HN20 (Bernatchez & Danzmann, 1993). The PCR conditions were as follows: initial DNA denaturation (95°C, 3 min) and 30 successive cycles of strand denaturation (94°C, 45 s), primer annealing (52°C, 45 s) and DNA extension (72°C, 2 min). All DNA-amplifications were performed in a programmable thermocycler GeneAmp<sup>®</sup> PCR System 9700 (AB Applied Biosystems). A total volume of 30 µl contained 1 µM of each primer, 0.2 mM dNTP, 1.5 mM MgCl<sub>2</sub>, 1X PCR buffer, 1 U of *Taq* polymerase (PE Applied Biosystems) and 100 ng of genomic DNA. Amplified DNA fragments were run on a 1.5% agarose gel and were isolated from the gel using QIAEX II Gel Extraction Kit (QIAGEN). Sequencing reactions were prepared using BigDye Terminator Ready Reaction Mix (PE Applied Biosystems) according to the manufacturer's recommendations. The amplified, fluorescently labelled and terminated DNA was salt-precipitated and analysed on the ABI PRISM 310 automated sequencer.

Sequences of 5'- and 3'-end of the control region (a total of 737 bp) were aligned using the computer programme ClustalX (Thompson *et al.*, 1994). In addition to the new sequences, 15 haplotypes representing all five mtDNA lineages (Bernatchez *et al.*, 1992) were analysed (see Table I for accession numbers). Maximum parsimony (MP), neighbour-joining (NJ) and maximum likelihood (ML) analyses were performed using the computer programme PAUP (version 4.0b3 for Macintosh; Swofford, 2000) with *Salmo salar* L and *Salmo ohridanus* Steindachner as the outgroup. For MP, insertions or deletions (indels) were coded as a fifth character. A heuristic search (10 replicates) with TBR branch swapping was employed to find the shortest tree. For ML, a sequence

TABLE I. Accession numbers of 5'- and 3'- end of mtDNA CR haplotypes

Species and haplotype	Accession number	
	5'-end	3'-end
<i>Salmo trutta</i>		
Me1	M97983	M97982
Ma1	AY260522	AY260523
Ad1	AH012977	AY260516
Ad2	M97965	M97946
Ad3	AY260518	AY260519
Ad4	AY260520	AY260521
Da3	AY185571	AY185571
Da2	AY185570	AY185570
Da23a	AY185574	AY185574
Da9	AY185572	AY185572
Da1	AY185568	AY185568
At1	AF321990	M97970
At10	AY185577	AY185577
At2	M97971	M87970
At11a	AY185578	AY185578
<i>Salmo platycephalus</i>	AY260514	AY260515
<i>Salmo ohridanus</i>	AY260512	AY260513

evolution model was first chosen using the programme ModelTest Version 3.06 (Posada & Crandall, 1998) incorporated into PAUP, and then a heuristic search was used to find the shortest tree. For NJ, a Kimura two-parameter model was chosen. Supports for the nodes were obtained with 1000 bootstrap replicates for MP, NJ or ML analysis, whereby the fast stepwise addition method was used for ML.

All six individuals displayed a single haplotype, not yet described in the literature, designated 'Da26' (GeneBank Acc. No. AY736130 for 5'-end and AY736131 for 3'-end; Fig. 1). Based on the 737 bp of the CR used in this analysis, Da26 is 1.0 to 1.5% divergent (Kimura two parameter) from the five other Da lineage haplotypes analyzed, yet groups with the clade with low bootstrap support (Fig. 1). The haplotype tree, rooted with both *S. salar* and *S. ohridanus* reveals surprisingly low bootstrap support for the species *S. trutta* (Fig. 1). Moreover, the inclusion of the Da26 haplotype into the Danubian clade is not at all supported with the ML-based topology (data not shown).

To further assess the distinctiveness of the Da26 haplotype it was compared to the 22 published Da haplotypes in Bernatchez (2001) and Weiss *et al.* (2001), whereby only 310 bp of the 5' end of the CR could be used. With this data set, the Tigris River haplotype still revealed itself as highly distinct, being 1.3 to 2.3% from all others (data not shown). This 'within' lineage divergence can be put into perspective considering that the maximum pair-wise distance among individual haplotypes from all five mtDNA lineages (based on a Kimura two parameter model of 640 bp of CR) first reported in Bernatchez *et al.* (1992), was 1.92%. Thus, the isolation event that has led to the existence of the Da26

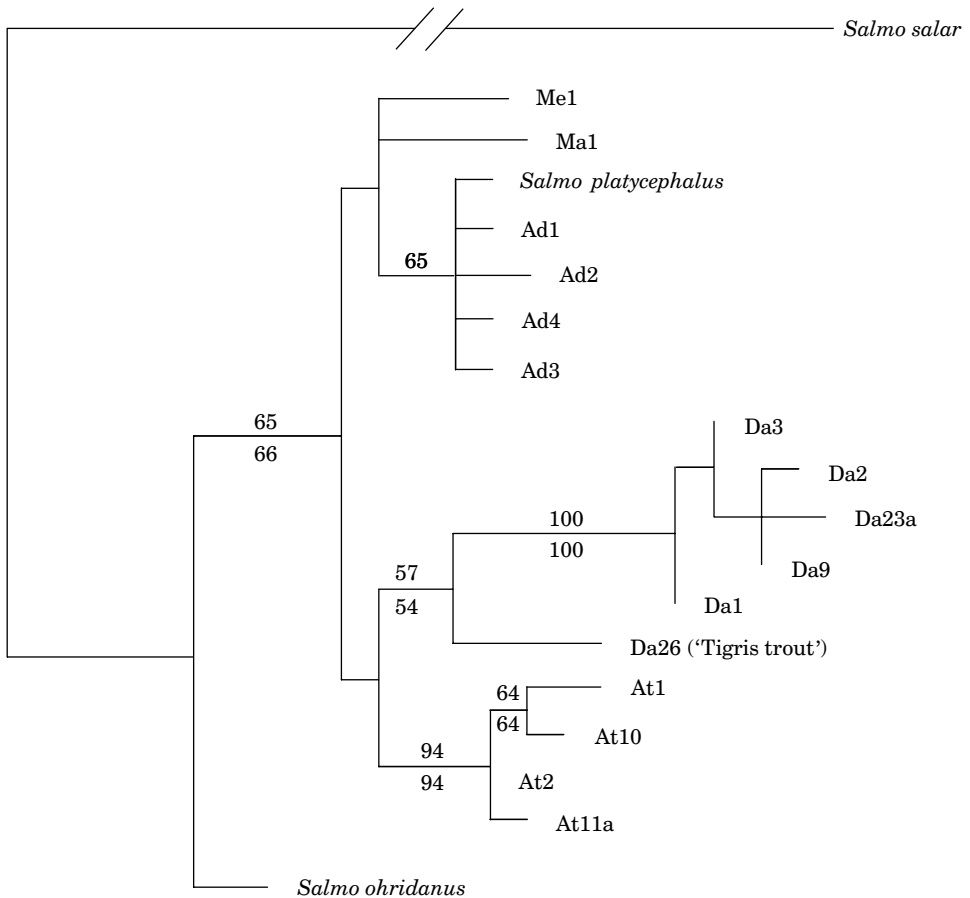


FIG. 1. Maximum parsimony-phylogram of mtDNA control region haplotypes. Node support is shown by per cent bootstraps for maximum parsimony consensus (1000 replicates) above and neighbour-joining (1000) below. The maximum-likelihood -based topology (not shown) was in general concordance, but lacked bootstrap support for the inclusion of Da26 in the Danubian clade (Model test: 'HKY + G' model of sequence evolution). Me, Mediterranean; Ad, Adriatic; Da, Danubian; Ma, *marmoratus*; At, Atlantic lineage within *Salmo trutta* species complex, as first presented in Bernatchez *et al.* (1992).

haplotype appears to be the oldest in the clade, perhaps predating the estimated age of expansion for the lineage (154 500–309 000 years ago) (Bernatchez, 2001) and approaching the age of divergence (1–2 million years ago) among the five major mtDNA lineages.

The presence of such a highly divergent haplotype in the upper Tigris River strongly supports the native status of the species in the Persian Gulf basin. Considering broader scale zoogeographical patterns, brown trout most probably reached the Persian Gulf basin through headwater capture events between the Black Sea and Tigris-Euphrates basins as hypothesized for primarily northern cyprinid genera (*Alburnoides*, *Alburnus*, *Aspius*, *Chalcalburnus*, *Chondrostoma* and *Leusicus*) (Coad, 1996b). But these genus-level similarities primarily relate to more ancient connections, that are believed to have occurred as early as the

Oligocene and continuously since the upper Miocene (Almaca, 1990). Events more likely related to the spread of brown trout in the region are clearly documented in the late Pliocene when the entire Black, Caspian and Aral Sea regions were interconnected by a series of lakes, which reached west across the Hungarian plains (Fink, 1966). The most recent large-scale connections can be further inferred by the presence of widespread haplotypes, such as Da3 and Da7, the former found in crystalline drainages of the Bohemian massif in north-eastern Austria, and as far east as the Aral Sea (Weiss *et al.*, 2000), and the latter found in the upper Euphrates basin as well as in numerous locations in the Black and Caspian Sea basins (Bernatchez, 2001). Similarly, the presence of an Adriatic basin haplotype in the Persian Gulf (one bp divergent from the common Ad1) must relate to relatively recent (*i.e.* late Pleistocene) hydrological events. While large scale extinctions associated with episodes of Quaternary desiccations are known in this region, and have probably contributed to the current isolation of many brown trout populations, Bânârescu (1991) notes that desiccation was less dramatic in the mountain tributaries of the Tigris-Euphrates. If all or most of the reported haplotypes in the Tigris-Euphrates basin are native, then clearly the region has undergone episodes of colonization over diverse historical time periods.

While these observations clearly support the plausibility of natural colonization corridors between the Ponto-Caspian and Persian-Gulf basins, it must be re-emphasized that the comparatively unique and old event relates to the presence of haplotype Da26 in the Catak Cay River of the upper Tigris. This population is also highly distinct phenotypically. The most striking features are an unusually large adipose fin and well-rounded margins of all other fins, particularly the anal fin (unpubl. obs.). These fin characteristics may very well be an adaptation to the very strong river currents, and virtual absence of pools or other substantial water velocity refuges. Body colouration is also highly distinct, being dominated by fine spots (black and red, or only red), strongly suggestive of brown trout from Liqvan Chay, Iran. Parr marks (nine to 11) are retained in adult (sexually mature) fish. Such a small, isolated, highly distinct and divergent population of brown trout represents a worthy unit of conservation within the evolutionary legacy of this highly polymorphic species.

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