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A very compact mt-DNA control region in the widely distributed goby *Pomatoschistus minutus* (Teleostei: Gobiidae)

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The control region of the mitochondrial genome was amplified and sequenced for six individuals of the gobioid fish *Pomatoschistus minutus*, from several European localities, and one specimen of the related *Deltentosteus quadrimaculatus*. The length of this region for the former species was found to be 773 bp, 7.1% shorter than that previously described as the most compact D-loop known among teleosts. Sequences from other fish have been compared and the largest gap falls in the section between the conserved sequence block and the pyrimidine tract. Alignment of *P. minutus* sequences was done with *D. quadrimaculatus*, whose control region length was 853 bp, and this gap was found to be of 61 bp. For the *P. minutus* sample, the intraspecific sequence divergence is 0.07%.

INTRODUCTION

The mtDNA control region is involved in the control of mtDNA replication and RNA transcription. It is also known as the displacement loop, or more simply the D-loop, because one of the two strands of the helix is displaced by the synthesis of a new strand during replication. Among teleosts, the length of this region is variable, ranging from 841 bp in *Rhinogobius maculofasciatus* (Gobiidae) (Chen et al., 1998) to about 2499 bp in *Dicentrarchus labrax* (Moronidae) (Cesaroni et al., 1997) and it often contains tandemly repeated segments (Hoelzel et al., 1994).

The sand goby, *Pomatoschistus minutus* (Pallas, 1770), is one of the most widely distributed gobiid fish of the temperate eastern Atlantic–Mediterranean zoogeographic region. It ranges from northern Norway to Morocco and throughout the Mediterranean, including the ancillary Baltic and Black Seas (Miller, 1986).

The four-spotted goby, *Deltentosteus quadrimaculatus* (Valenciennes, 1837), has a less extensive distribution in the Mediterranean and eastern Atlantic (southern Bay of Biscay to Mauritania) (Miller, 1986) and nothing is known at molecular level about this species.

Both species, *P. minutus* and *D. quadrimaculatus*, belong to the European sand goby clade, being synapomorphic in the absence of postmaxillary process on premaxilla, higher number vertebrae in both abdominal, caudal regions and dorsal pterygiophores (DP) spacing (McKay & Miller, 1997).

In this paper, the complete sequences of mtDNA control region for *P. minutus* from several European localities are discussed after comparison with others from different species of teleosts.

MATERIALS AND METHODS

The six specimens of *Pomatoschistus minutus* are from Amerlander (North Sea), Bergen (Atlantic), Oban (Atlantic), Plymouth (Atlantic), Mauguio (Mediterranean) and Venice (Adriatic) (Figure 1). The *Deltentosteus quadrimaculatus* was chosen as outgroup and was also collected in the northern Adriatic. All material was collected, donated, or bought in a fish market, over the period 1997–1998. Specimens were kept frozen at −50°C until extraction of DNA following the phenol-chloroform-isoamyl alcohol method as described in Stefanni (2000).

The fragment of mtDNA that includes the control region was amplified using a pair of primers, UG-PROA (5′–AACTCCC(G/A)CCCCTGG(C/T)(C/T)CCCAAA GCCAGCATTC–3′), MIN-FB (5′–TCAAAATTGATGG TAAAGTCAGGAC–3′) for *P. minutus*; UG-PROA and PMC-FB (5′–TTTCTAGG(C/G)CCCATGTTAACGTC TTCAG–3′) for *D. quadrimaculatus*. Another set of primers PMI-1A (5′–TACTGCTCACAGTGAATTATT CCTG–3′) and PMI-2B (5′–ACAATCTGCACCGCG GAGCAAGGAG–3′) was used for DNA sequencing. Most of the primers were designed by one of us (ISC), after comparison with sequences for other perciforms downloaded from GenBank. Primers PMI-1A and PMI-2B were designed from conserved sequences of *P. minutus* obtained from the DNA sequencing with primers UG-PROA and MIN-FB or PMC-FB.

The polymerase chain reaction (PCR) was performed in 100 μl reaction volume, following the proportions of reagents as described in Stefanni (2000). The PCR amplifications were carried out in a Perkin Elmer-Cetus thermal cycler using the following parameters: one cycle

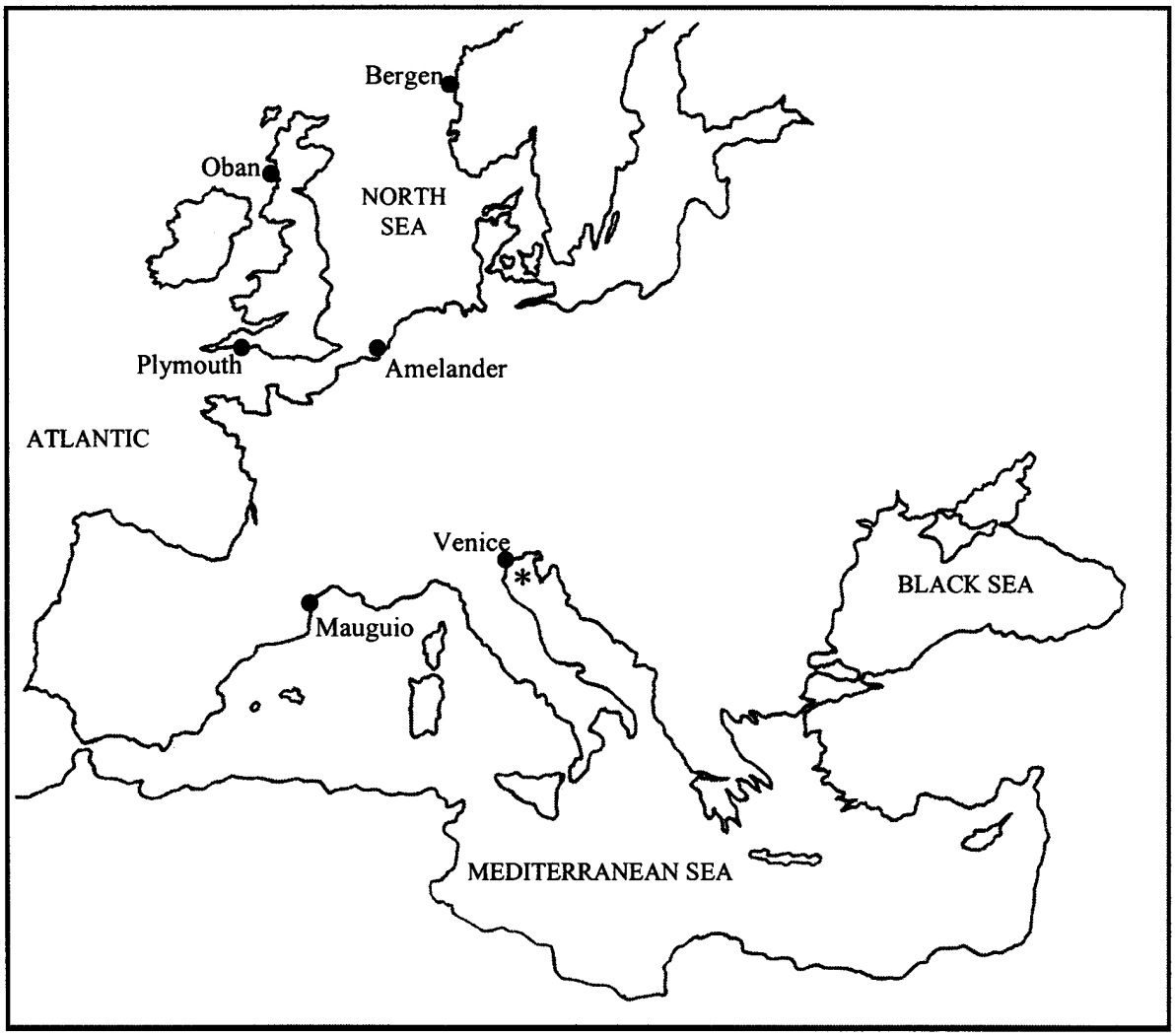

Figure 1. Map showing the localities where samples of *Pomatoschistus minutus* (●) and *Deltentosteus quadrimaculatus* (∗) were collected.

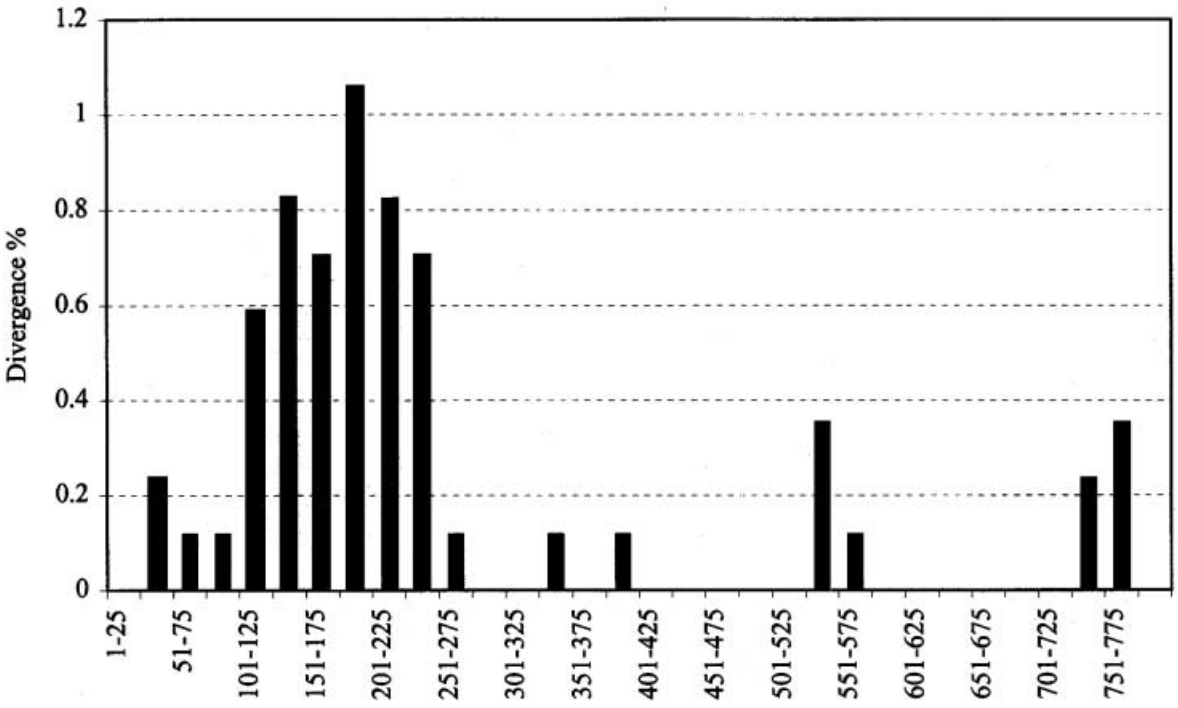

Figure 2. Histogram representing the percentage of divergence grouped for each 25 bp of mtDNA control region between *Pomatoschistus minutus* from the different sampling localities.

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of pre-denaturation at 94°C for 1 min, 30–40 cycles of denaturation at 94°C for 1 min, primer annealing at 46–50°C for 1 min, and extension at 72°C for 2–5 min.

The double-stranded DNA product was purified using the Genclean II Kit (Bio 101), and vacuum-dried DNA was resuspended in 20 μl of distilled water.

Automated sequencing was performed as recommended with a PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit for an ABI Model 373 DNA sequencer.

MtDNA sequences were aligned by using CLUSTAL X software package (Thompson et al., 1997). After minor manual adjustments, they were compared with published sequences of other teleosts to verify the boundaries and alignment of the control region.

RESULTS

Length of the control region is 773 bp for *Pomatoschistus minutus* (six individuals) and 853 bp for *Deltentosteus quadrimaculatus* (single specimen) and sequences are deposited in GenBank (accession numbers AY033004/15/26/31/37/43 and AY033003, respectively). In the case of *P. minutus*, comparison with other teleost sequences of the same mtDNA region, describe its D-loop as the most compact known so far, followed by *Rhinogobius* sp. (841 bp) (Chen et al., 1998), *D. quadrimaculatus* (853 bp) (present paper), *Microgadus tomcod* (853 bp), *Melanogrammus aeglefinus* (856 bp) and *Pollachius virens* (868 bp) (Lee et al., 1995).

All the sand gobies from the six different localities had identical length for the control region, and from a comparison, the overall intraspecific divergence is 0.07% (23.2% including *D. quadrimaculatus*). The percentage of divergence grouped for 25 bp of aligned mtDNA control region compared for the *Pomatoschistus minutus* only is presented in Figure 2. The first third of the sequence (1–275 bp), left domain, is the one showing the highest divergent rate (15.7% for *P. minutus* alone and 36.3% including *D. quadrimaculatus*). The central part of the sequence (276–525 bp) is the most conserved (near to 0% for the *P. minutus* and 14.7% for the complete data set). The value for the complete data set is affected by the large gap between the conserved sequence block (CSB-D) and the pyrimidine tract (PY) generated by the missing 61 bp in *P. minutus* in position 368 from the origin of the D-loop. In the central domain, excluding *D. quadrimaculatus* from the comparison, there are only two transitions, both affecting the Adriatic *P. minutus*. This tendency has been observed in several teleosts (Lee et al., 1995).

DISCUSSION

A length of 773 bp for the complete D-loop in *Pomatoschistus minutus* is now the shortest reported among teleosts, followed by the 841 bp described for two Asian gobioid species *Rhinogobius maculafasciatus* and *R. giurinus* (Chen et al., 1998).

This region is shorter in comparison to that of *Deltentosteus quadrimaculatus*, and *Rhinogobius* species because of a series of small deletions plus a larger one involving ~60 bp located in a non-structural section between the CSB-D and the PY. This gap has been detected from comparison of this mtDNA region with other teleost sequences obtained from the GenBank library. Apart from the conspicuous difference in length, the CSBs in *P. minutus* are still the same as in other perciforms, and the termination-associated sequences and PY, commonly observed in other vertebrates and fish, are also present.

The CSB-D block, contained into the central section of the D-loop, is involved in heavy strand replication, including initiation by a three-stranded displacement loop (Clayton, 1982), and it is described as the most universally conserved segment among fish families (Lee et al., 1995). However, the shortening of the conserved section that follows the CSB-D does not seem to interfere with the function of this region.

The length of the control region in teleosts varies substantially among species, chiefly because of the variable copy numbers of tandemly repeated sequences (Arnason & Rand, 1992; Cesaroni et al., 1997). These tandemly repeated sequences are found in the control region of many vertebrates, although their phylogenetic utility is not resolved yet (Hoelzel et al., 1994). None of these sequences have been detected in *P. minutus*, a feature shared with other gobies (Chen et al., 1998).

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