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Mitochondrial DNA STR analysis as a tool for studying the green sea turtle (*Chelonia mydas*) populations: The Mediterranean Sea case study

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ABSTRACT

The Mediterranean population of the green sea turtle *Chelonia mydas* is critically endangered. Genetic analysis of this population using the ordinary haplotyping system, based on sequence analysis of a segment of the mitochondrial DNA (mtDNA) D-loop (control region), revealed very little variation. The most common haplotype, CM-A13, was observed in all but three individuals in hundreds of samples in previous studies. In search for a more informative marker we sequenced the 3′ of the mitochondrial control region which contains an AT-rich microsatellite. We found a unique pattern that consists of four AT short tandem repeats (STRs) with varying copy numbers. This allowed us to construct a new haplotyping system composed of four different STR sizes for each mtDNA sequence. Our new mitochondrial STR (mtSTR) haplotyping approach revealed 33 different haplotypes within the nesting and stranded sea turtles along the Mediterranean Israeli seashore. The Israeli coast nesting females had 10 different haplotypes that can be used for monitoring and conservation purposes. The mtSTR haplotyping system can clearly assist in fingerprinting of individual turtles. Moreover, it can be used for estimating phylogenetic distances within populations. This case study shows that the mtSTR haplotyping is applicable for the study of global green sea turtle populations and could also be considered as markers of genetic variability in other species.

1. Introduction

The green sea turtle *Chelonia mydas* has been listed as endangered worldwide and critically endangered in the Mediterranean Sea (Baillie et al., 2004). Broderick et al. (2002) have estimated that there are 340–360 green turtles nesting annually along the eastern coasts of the Mediterranean, most of them in Turkey and Cyprus and only a few in other locations including Israel (Bjorndal et al., 1995; Kuller, 1999). Hornell (1935) reported that about 2000 green turtles were hunted annually along the coastline of Palestine (Israel), when a large population still existed there. According to the Israeli Authority of Nature and Parks' recent review (Levy, 2010), the current Israeli green turtle population consists of around ten nesting females.

Green turtles are philopatric and nesting females return to their natal regions (Allard et al., 1994; Peare and Parker, 1996; Lee et al., 2007; Nishizawa et al., 2011). They are promiscuous breeders and have polyandric reproduction with the capability of sperm storage (FitzSimmons, 1998; Pearse and Avise, 2001; Ireland et al., 2003; Bell et al., 2010).

Molecular techniques and DNA analysis in particular, proved to be effective in understanding breeding habits, migration and nesting patterns in sea turtles (Karl et al., 1992; Allard et al., 1994; Bowen, 1995; Ireland et al., 2003; Bowen and Karl, 2007). Genetic variation of the mitochondrial DNA (mtDNA) has been the most popular tool for phylogenetic studies of sea turtles (Bowen and Karl, 2007; Naro Maciel et al., 2010). Analysing mtDNA from six species of Testudines, including green turtles, has revealed a dramatic slowdown in the mutation rate. While the "conventional" mtDNA clock calibration between higher animal lineages is about 2%/Myr sequence divergence, the Testudines' divergence is eightfold lower (Avise et al., 1992). Therefore, genetic analysis of sea turtles has been mainly limited to sequence comparisons of the more variable D-loop (control region) of the mtDNA. The control region is the only non-coding region of the mtDNA and ease of selective pressure results in higher population variability in this region (Moritz et al., 1987). Sequencing the control region's 5′ end has set the basis for defining the current mtDNA haplotyping that revealed about 200 different haplotypes worldwide.

The Mediterranean population of the green turtles was first analyzed using mtDNA control region sequences by Encalada et al. (1996) who described the common Mediterranean haplotype, CM-A13. This haplotype was found in all of the samples (n = 147) excluding one turtle that had the CM-A14 haplotype following a single C → A transversion. Kaska (2000) found no polymorphism in his study of a population from Cyprus (n = 17). Levin (2008) studied the Israeli shore population (n = 149) and described two more haplotypes: CM-A60 (EU491956)

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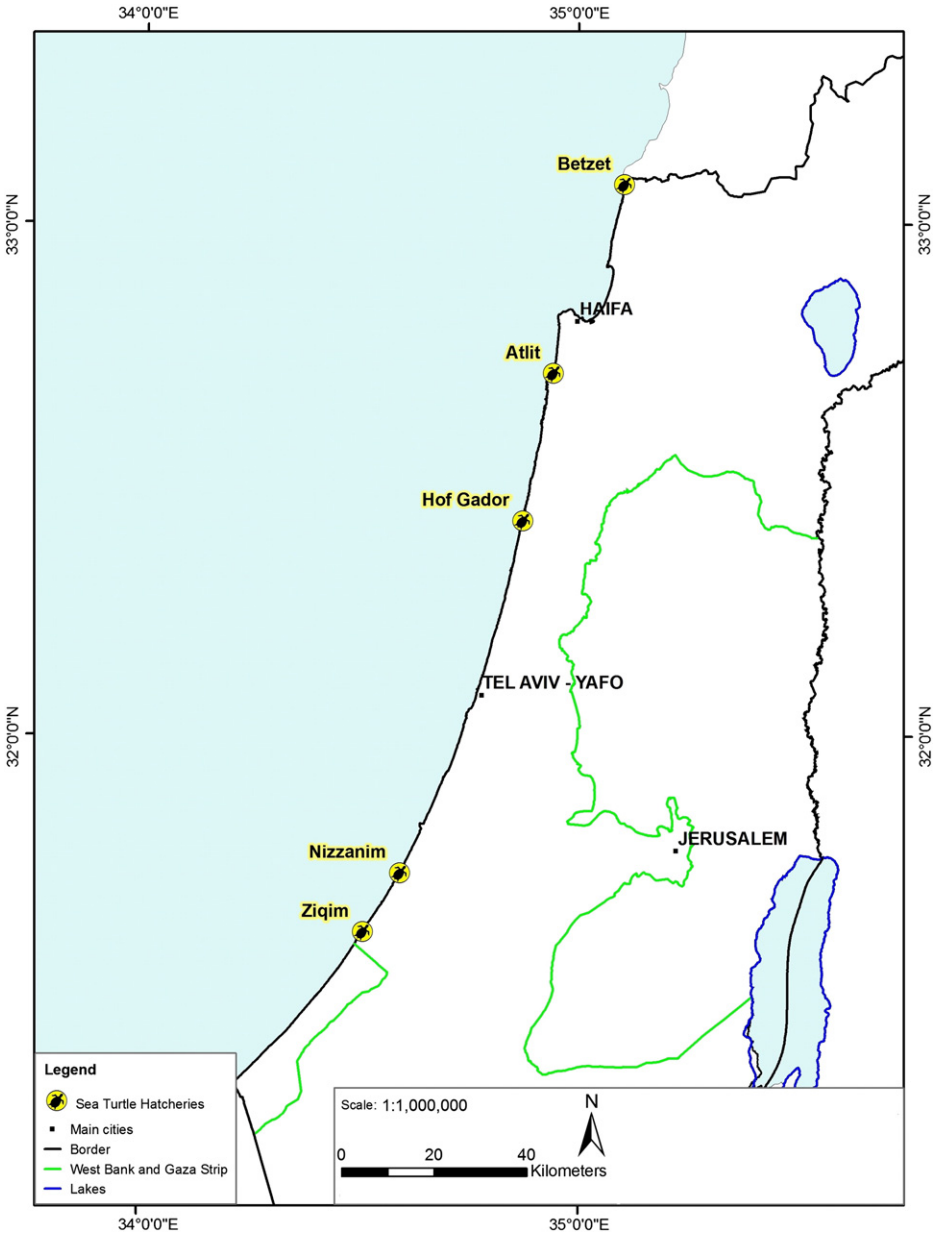

Fig. 1. Sea turtle hatcheries along the Israeli coastline (Modified from Israeli Nature and Parks Authority (INPA) GIS unit, using WGS 1984 coordinate system).

and CM-A59 (EU491957) both contained an A→G transition from CM-A13. Altogether, out of 313 Mediterranean samples analyzed, only 3 differed from the common CM-A13 haplotype. This common haplotype, also found in the Atlantic green turtle population, implies on the linkage between the two populations. Further analysis based on such a high degree of homogeneity renders it practically impossible to achieve an in-depth understanding of the Mediterranean population, its variability, and its spatial or temporal behavior.

Nuclear DNA microsatellite (or STR—short tandem repeat) studies of sea turtles revealed high polymorphism and allowed researchers to evaluate the male genetic contribution (Roberts et al., 2004; Jensen et al., 2006; Lee et al., 2007). STRs exposed turtle reproduction patterns and provided an estimate of multiple paternities in the range of 30–90% across sea turtle clutches (Moore and Ball, 2002; Bowen and Karl, 2007; Zbinden et al., 2007). The main limitation of this method is the difficulty to genotype nesting females through DNA analysis of dead hatchlings inside nests. All hatchlings from a given nest share a common maternal, but not necessarily a paternal origin. Therefore, it is often impossible to determine the mothers' genotypes for many microsatellites, limiting STR usage to studies comparing allele frequencies between different populations (Roberts et al., 2004).

Many species have different types of repetitive sequences in their mtDNA control region near the 5′ end (Arnason and Rand, 1992; Hoelzel, 1993; Faber and Stepien, 1998; Ludwig et al., 2000; Stärner et al., 2004), near the 3′ end (Broughton and Dowling, 1994; Wilkinson et al., 1997; Mundy and Helbig, 2004), or at both ends (White and Martin, 2009). These repeats are characterized by varying copy numbers because of their high mutation rate, and therefore can be used for evolutionary and population studies (Wilkinson and Chapman, 1991; Hoelzel et al., 1994; Lunt et al., 1998; Lo Brutto et al., 2004; Stärner et al., 2004; White and Martin, 2009). Phylogenetic and geographic patterns of variation of control region repeat number were demonstrated in several studies (White and Martin, 2009; Munwes et al., 2011). Green turtles are known to have an AT-rich mtDNA STR at the 3′ end of the control region (Kumazawa and Nishida, 1999; GenBank GI: 5836002).