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RESEARCH NOTE

A new record and molecular characterization of the Green Turtle *Chelonia mydas* (Testudines, Cheloniidae) in Sicilian coastal waters

Nuevo registro y caracterización molecular de la tortuga verde *Chelonia mydas* (Testudines, Cheloniidae) en las aguas costeras sicilianas

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Abstract.- A juvenile specimen of the Green Turtle, *Chelonia mydas*, was recovered in Sicilian coastal waters close to Avola (Syracuse province, south-eastern Sicily, Italy). Before being released, the specimen was measured and inspected for the presence of ingested hooks or other possible harms to its health. A fragment of the mtDNA marker COI was amplified and sequenced in order to provide the first molecular data for the species from the central Mediterranean area. The possible influence of climate change on the occurrence of this thermophilic species in the central Mediterranean area is briefly discussed.

Key words: Sea turtle, *Chelonia mydas*, COI mitochondrial DNA, Mediterranean Sea

INTRODUCTION

The green turtle, *Chelonia mydas* (Linnaeus, 1758), is one of the 4 Cheloniidae species known to occur in the Mediterranean Sea. It is included in the Habitats Directive 92/43/CEE (annex IV) as a priority species (European Economic Community 2007)[1] and, due to the past commercial exploitation (Sella 1995) and the current impact of fisheries (Casale & Margaritoulis 2010), the green turtle is classified as endangered according to the IUCN criteria (<www.iucnredlist.org>). To date, available knowledge on the species distribution in the central Mediterranean area is rather poor, and most of the information currently available deals with nesting sites, strandings, and satellite tracking activities in the Levantine basin (Kuller 1999, Kasparek *et al*. 2001, Russo *et al*. 2003, Rees *et al*. 2008, Türkecan & Yerli 2011, Türkozan *et al*. 2013, Stokes *et al*. 2015), and with some foraging grounds, mostly located along the southern regions of the Mediterranean basin (Karaa *et al*. 2012, Stokes *et al*. 2015).

The first record of a green turtle in Sicilian waters was reported by Basso (1992), although this record is considered dubious by Gianguzza *et al*. (2000). Later, Gianguzza *et al*. (2000) and the Centro Studi Cetacei (2000) provided sound reports on the presence of the species in Sicilian waters. However, the sightings of the species are quite rare in the central Mediterranean area and, according to Bentivegna *et al*. (2011), only 28 green turtles were recorded in Italian waters from 1986 to 2008.

Here we report on a new record of the green turtle in Sicilian waters, updating the list of records of this species in the Central Mediterranean area and providing a molecular characterization of the studied specimen.

MATERIALS AND METHODS

On 22nd February, 2016, a turtle specimen was found alive floating on the water surface close to the coastline of Avola (Syracuse province, Sicily, Italy; 36.888259 N; 15.143197 E). The individual was recovered by the local Coast Guard and conferred to the Regional Center of Recovery for sea turtles at the Veterinary Public Health Institute of Sicily (IZS Sicilia, <http://www.izssicilia.it>). The specimen was identified, weighed and measured in laboratory according to Wyneken (2001) guidelines. Before being released in the wild, the specimen was subjected to a general health check and to a radiographic investigation in order to check for the possible presence of fishing hooks or other allochthonous material in its digestive tract.

[1]European Economic Community (EEC). 2007. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Consolidated text. Official Journal L0043: 1-66

In the light of the paucity of molecular data currently available on Mediterranean populations of the species, and of the growing body of evidence regarding the high frequency of chelonian hybrids showing a decoupling between their morphology and mitochondrial genotypes (Lara-Ruiz *et al.* 2006, Bowen & Karl 2007, Vamberger *et al.* 2015), the presumptive *C. mydas* specimen was subjected to genetic analysis. Sequencing of a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was carried out using the universal primer pairs described by Folmer *et al.* (1994) and a standard PCR protocol (Marrone *et al.* 2016). The obtained COI sequence was deposited in GenBank (Accession Number: KU958179). Available *C. mydas* sequences from Atlantic, Pacific and Mediterranean sites, and a single sequence from each species belonging to the family Cheloniidae and Dermochelyidae, were downloaded from GenBank to be included in the phylogenetic analysis.

Bayesian inference (BI) of phylogeny was performed as implemented by MRBAYES 3.2.1 (Ronquist *et al.* 2012). BI was performed using a General Time Reversible model of sequence evolution with a gamma distributed rate variation among sites and a proportion of invariable sites (GTR+I+G). The best evolutionary models was selected by the Akaike information criterion in MrModeltest 2.2 (Nylander 2004). Node supports were evaluated by their posterior probabilities. The BI analysis was performed with two independent runs of 1,000,000 generations and four Markov chains using default heating values. Trees and parameter values were sampled every 100 generations resulting in 10000 saved trees per analysis. An initial fraction of 2,000 trees (20%) was conservatively discarded as 'burn-in'. Standard deviation of split frequencies reached values lower than 0.008767, and values of the potential scale reduction factor (PSRF) were between 1.000 and 1.001 for all parameters, indicating convergence of the runs.

Results and discussion

The specimen showed 1 pair of prefrontal scales, 4 postorbital scales, 4 (left and right) costal scutes, 5 vertebral scutes, 11 (left and right) marginal scutes, 4 inframarginal scutes without pores. All these morphological characteristics are those typical of the green turtle *Chelonia mydas* (Fig. 1). Studied specimen weighed 7 kg and showed the following measurements: curved carapace length (CCL) of 32 cm, straight carapace length (SCL) of 28 cm, curved carapace width (CCW) of 30 cm, straight carapace width (SCW) of 25 cm, plastron length of 25 cm, plastron width of 23 cm, head length of 6.5 cm, head width of 3.5 cm, body circumference of 56 cm, tail length of 2.5 cm and vent-to-tip of 1 cm. Due to its small size, it was not possible to ascertain its sex without endoscopic inspection. The general conditions of the specimen were good and carapacial and plastron were clean and without epibionts (Fig. 1).

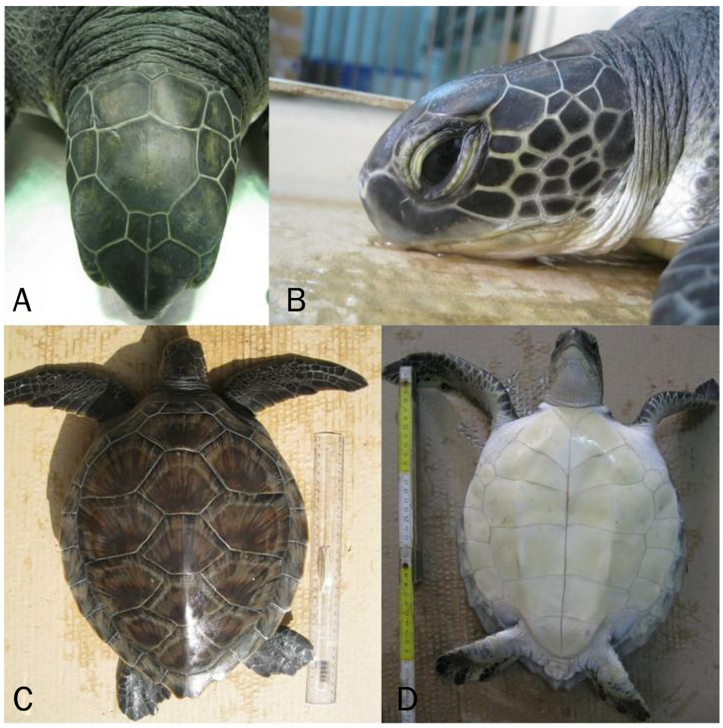

Figure 1. *Chelonia mydas*. Morphological characters of the studied specimen. A) frontal view of the head, B) lateral view of the head, C) dorsal view of the carapace and D) ventral view of the plastron / *Chelonia mydas.* Caracteres morfológicos del espécimen estudiado. A) vista frontal de la cabeza, B) vista lateral de la cabeza, C) vista dorsal del caparazón y D) vista ventral del plastron

Figure 2. Radiographical investigation of the studied specimen highlighting the presence of some foreign bodies compatible with mineralized debris in the distal portion of the digestive tract (white arrow) / Investigación radiográfica del espécimen estudiado resaltando la presencia de algunos cuerpos extraños compatibles con restos mineralizados en la porción distal del tracto digestivo (flecha blanca)

The performed radiographic investigation showed the presence of some foreign roundish bodies in the intestinal tract of the turtle (Fig. 2). These may be likely attributed to mineralized debris. The presence of allochthonous material in the digestive tract of the specimen is noteworthy since the danger that this may constitutes for the green turtle is well-known (Schuyler *et al*. 2013).

The nucleotide sequence of the studied turtle was nearly identical to the Mediterranean and western Caribbean sequences available in GenBank: an uncorrected p-distance of 0.3% was scored between our sample and the single Atlantic-Mediterranean haplotype reported by Naro-Maciel *et al*. (2009). The phylogenetic tree grouped the haplotypes observed in the Mediterranean Sea and Atlantic Ocean together in a well-supported clade (Fig. 3), thus confirming the strict relationships occurring between Mediterranean and Atlantic populations of the species (Naro-Maciel *et al*. 2009).

Most of the catches or sightings of this species in Italian waters occur during summer months (Russo *et al*. 2003, Bentivegna *et al*. 2011, Jesu & Doria 2011); conversely, the specimen here presented was found in winter. This can probably be ascribed to the random erratic activity of the studied specimen and/or to the environmental temperature anomalies experienced in Sicily during the first months of 2016, which influenced the surface water temperature, keeping it above average annual

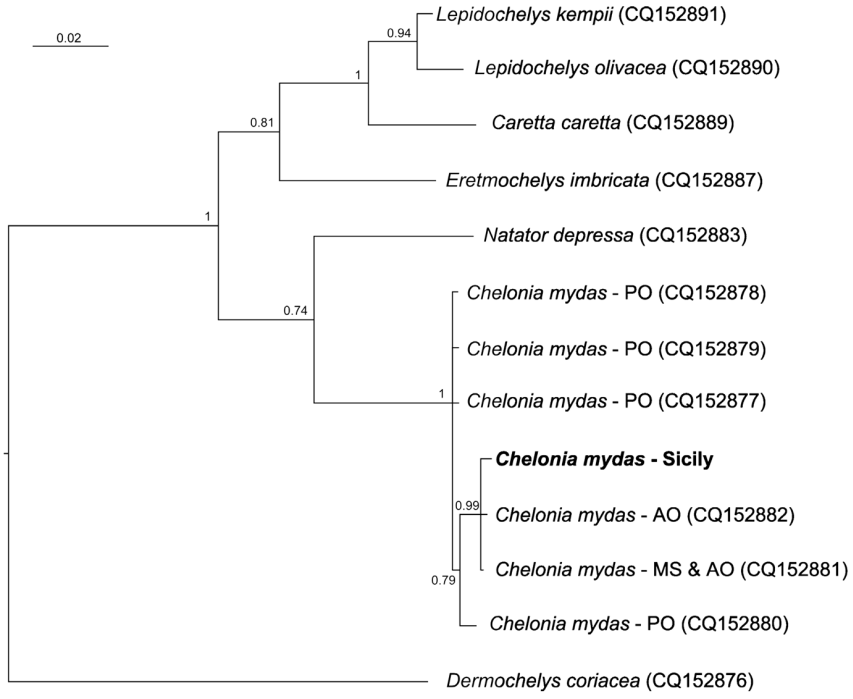

Figure 3. Bayesian phylogram based on a 624 bp fragment of the mtDNA COI. The phylogram is rooted on the dermochelyid *D. coriacea*. Node support is reported as nodal posterior probabilities. Accession numbers of sequences derived from GenBank are shown in brackets. AO: Atlantic Ocean; MS: Mediterranean Sea; PO: Pacific Ocean. The studied specimen is reported in bold / Filograma bayesiano basado en un fragment de 624 bp del mtADN COI. La raíz del filograma está basado en el dermochélido *D. coriacea*. El sustento del nodo es reportado como posteriores probabilidades nodales. Los números de acceso de las secuencias derivadas de GenBank se muestran en corchetes. AO: Océano Atlántico; MS: Mar Mediterráneo; PO: Océano Pacífico. El espécimen estudiado se reporta en negrita

values. In fact, the ongoing increase of surface water temperatures in the Mediterranean Sea is a well-known phenomenon (*e.g.*, Shaltout & Omstedt 2014, and references therein). In the study area, the surface temperatures detected between January-February 2015 and 2016 showed a slight increase [January-15 16.24 (±0.61)°C and February-15 15.22 (±0.52)°C; January-16 16.48 (±0.65)°C and February-16 15.82 (±0.57)°C - data from <http://marine.copernicus.eu>], which probably influenced the Mediterranean migratory activity of this thermophilic species to date mostly limited to the warmer waters of the Levantine basin.

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