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First report of an olive ridley (*Lepidochelys olivacea*) in the Mediterranean Sea

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Abstract

We report the first confirmed occurrence of a *Lepidochelys olivacea* in the Mediterranean Sea based on the study of an individual stranded on a beach in May 2014 in the town of Oropesa del Mar (40º05′32″N, 0º08′02″E), province of Castellón, East Spain. Morphological and genetic analyses were used to confirm species identification. The individual had a sequence that matched the 470-bp *Lepidochelys olivacea* haplotype F (Genbank accession number: AF051773) found in several Atlantic populations. This is one of the northernmost known occurrences of olive ridleys in the world and is the first report of this species in the Mediterranean Sea.

Keywords: *Lepidochelys olivacea*, species designation, distribution range, Mediterranean.

Introduction

The olive ridley sea turtle (*Lepidochelys olivacea*) is considered the most abundant sea turtle species in the world (Márquez, 1990; Marcovaldi, 2001; Abreu-Grobois & Plotkin, 2008). According to the IUCN Red List (IUCN, 2014), this species is categorised as vulnerable and is listed in Appendix I of the Convention on International Trade in Endangered Species of Flora and Fauna (Pritchard, 1997). *Lepidochelys olivacea* has a circumtropical distribution, which occurs regularly in the Pacific and Indian oceans, and less frequently in the South Atlantic (Reichart, 1993; Fretey, 2001; Abreu-Grobois & Plotkin, 2008). The main nesting grounds of this species are in the eastern Pacific and northeast India where 'arribada' nesting happens. In the Atlantic Ocean, the nesting grounds of olive ridley turtles are found in the western hemisphere, mainly in northeast Brazil, Suriname and French Guiana (Fretey, 1999; da Silva *et al*., 2007). At sea, the species has been reported in northern waters in Florida, USA (Foley *et al*., 2003). To date, the northernmost report for the species in the western Atlantic was a subadult that was incidentally captured by longline fishery at 43ºN (Stokes & Epperly, 2006). Data on olive ridleys in the eastern Atlantic are sparse. The main nesting areas are located along the west coast of Africa, between Guinea Bissau and Angola, and include many islands in the region (Godgender *et al*., 2009; Tomás *et al*., 2010; Maxwell *et al*., 2011). According to current knowledge, olive ridleys' northern limit of distribution is in North Senegal, West Africa (Fretey *et al*., 2012). However, occurrence of the species have also been reported in the waters of Madeira and the Canary Islands (Fretey, 2001; Carrillo & Alcántara, 2014), but it has never been reported in the Mediterranean Sea.

The stranding of a *Lepidochelys*-like sea turtle in the Gulf of Cadiz (South Spain), on the Atlantic side of the Strait of Gibraltar, has been reported as *L. olivacea* based on extra scute partitioning of the carapace (Rojo-Nieto *et al*., 2011). However, posterior genetic analyses identified this individual as *Lepidochelys kempii* (Carreras *et al*., 2014). Here we report the first confirmed occurrence of *Lepidochelys olivacea* in the Mediterranean Sea.

Material and Methods

On 8 May 2014, the carcass of a stranded sea turtle was found in the town of Oropesa del Mar (40º05′32″N, 0º08′02″E) on the Mediterranean coast, East Spain (Fig. 1). Sex was confirmed by a histological examination of Hematoxylin-Eosine stained sections of the gonad, based on the differentiation of the gonadal medulla and cortex (Yntema & Mrosovsky, 1980). The pattern of scutes on the head, the number of costal scutes and the presence of inframarginal pores, which are diagnostic for *Lepidochelys* species, were used as the initial identification criteria (Pritchard & Mortimer, 1999).

To confirm species designation, genetic analyses were also carried out. DNA was extracted from the sample with the QIAamp extraction kit (QIAGEN®) following the manufacturer's instructions. A ~800-bp fragment of the mitochondrial DNA (mtDNA) control region was amplified with primers LCM15382 (5'-GCTTAAC-

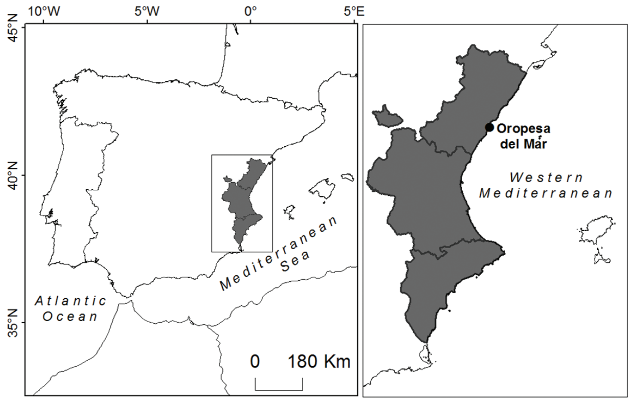

Fig. 1: Map of the western coast of the Mediterranean Sea showing the location where the *L. olivacea* carcass was stranded in Oropesa del Mar (Valencian Community: shaded area).

CCTAAAGCATTGG-3') and H950 (5'GTCTCGGATTTAGGGGTTT-3') (Abreu-Grobois *et al*., 2006) following the protocols of Carreras *et al*. 2014. Positive (a previously analysed loggerhead turtle (*Caretta caretta*) sample) and negative controls were also included. Sequences were aligned manually by the BioEdit programme, version 5.0.9 (Hall, 1999), and were compared with the haplotypes described for both species of the genus *Lepidochelys*, as found in the literature (Bowen *et al*., 1998; Shanker *et al*., 2004; López instead of Lopez-Castro & Rocha-Olivares, 2005; Reis *et al*., 2010; Jensen *et al*., 2013; Frey *et al*., 2014) and in the GenBank database (National Center for Biotechnology Information, USA: NCBI Home page http://www.ncbi.nlm.nih.gov) by a BLAST search. When sequences were not available in Genbank, they were extracted from the polymorphic tables found in the references that describe them. Information for all the sequences used is summarised in Suppl. Table 1, which is available as an electronic publication.

Results and Discussion

The *L. olivacea* turtle was identified based on the number of prefrontal (2 pairs), costal (7 pairs) and vertebral scales (7), and also on the presence of pores in the inframarginal scutes (Fig. 2A, 2B, 2C and 2D). The standard biometric variables of the turtle were: curved carapace length (CCL-notch to tip) = 65.7 cm and maximum curved carapace width = 64.9 cm. The gonad histology results confirmed that the stranded specimen was a female. The CCL of the specimen fell within the CCL range for nesting females in nesting rookeries around the world (60-70 cm, NOAA, 2014 and references therein).

The specimen had a sequence that matched the 470 bp *L. olivacea* haplotype F (Genbank accession number: AF051773) found in the Atlantic populations of Guinea Bissau (Orango National Park), Suriname (Elianti beach) and Brazil (Sergipe, Bowen *et al*., 1998). This haplotype differs in one single transition from the other Atlantic haplotype (E), and in another transition from Indo-Pacific haplotype J (Bowen *et al*., 1998; Shanker *et al*., 2004). The sequence also matched the 627-bp fragment found in 10 individuals from the same Brazilian population (Lara-Ruiz *et al*., 2006) in several loggerhead (*Caretta caretta*) and hawksbill (*Eretmochelys imbricata*) turtles from the same site that had a *Lepidochelys olivacea* mtDNA introgression (Genbank accession numbers: EU365972.1 for the wild *L. olivacea* haplotype and EU365971.1 for the *C. caretta* introgressed haplotype; Reis *et al*., 2010). The full sequence did not match any of the available ~800-bp sequences for the species as no recent study that used the same set of markers included any individual from the populations of Brazil, Suriname or Guinea Bissau. Our full sequence differed in one single nucleotide from haplotype Lo1 (Genbank accession number: JN391445.1) found in Australia (Jensen *et al*., 2013), which is the extended sequence of haplotype J. This indicates that these two sequences differ only in the short 470-bp section given the above-described transition, which differentiates haplotypes F and J. For these reasons, our full sequence was submitted to GenBank (accession number: KP117262).

Based on our results, we confirm that this turtle stranded in East Spain was a *L. olivacea* and we, therefore, state that this is the first confirmed report of the species in the Mediterranean Sea.

The Atlantic origin of the specimen described herein raises two migration possibilities depending on the exact population origin. The first possibility is a northeastward migratory route from the nesting areas or foraging grounds of Suriname or northeast Brazil to enter the Mediterranean Sea through the Strait of Gibraltar, as described for other sea turtle species (Monzón-Argüello *et al*., 2010; Carreras *et al*., 2014). This would match the nomadic oceanic migratory behaviours described for *L. olivacea* in other regions (Plotkin, 2010). The second possibility is of West African origin, which would result in a northward migration. Nevertheless, the movements involved are still not completely understood (Pikesley *et al*., 2013), although the south-north African route has already been described for green and loggerhead turtles (Carreras *et al*., 2011, 2014; Clusa *et al*., 2014).

Our case represents another example of the increasing number of reports of the olive ridley species at latitudes beyond its typical circumtropical range of distribution (Hodge & Wing, 2000; Stokes & Epperly, 2006; Kelez *et al*., 2009; Varo-Cruz *et al*., 2011). These reports could reflect changes in the species' migratory patterns and range. However, we consider it more feasible to make more research and rescue efforts, which would explain why the olive ridley turtle has not been reported in the area to date, as proposed for other turtle species (Carreras *et al*., 2014). The report of a new sea turtle species in the Mediterranean Sea raises new questions about the possible interactions with other sea turtle species (e.g., Reis *et al.* 2010) and anthropogenic threats, such as fisheries interaction, in this sea.

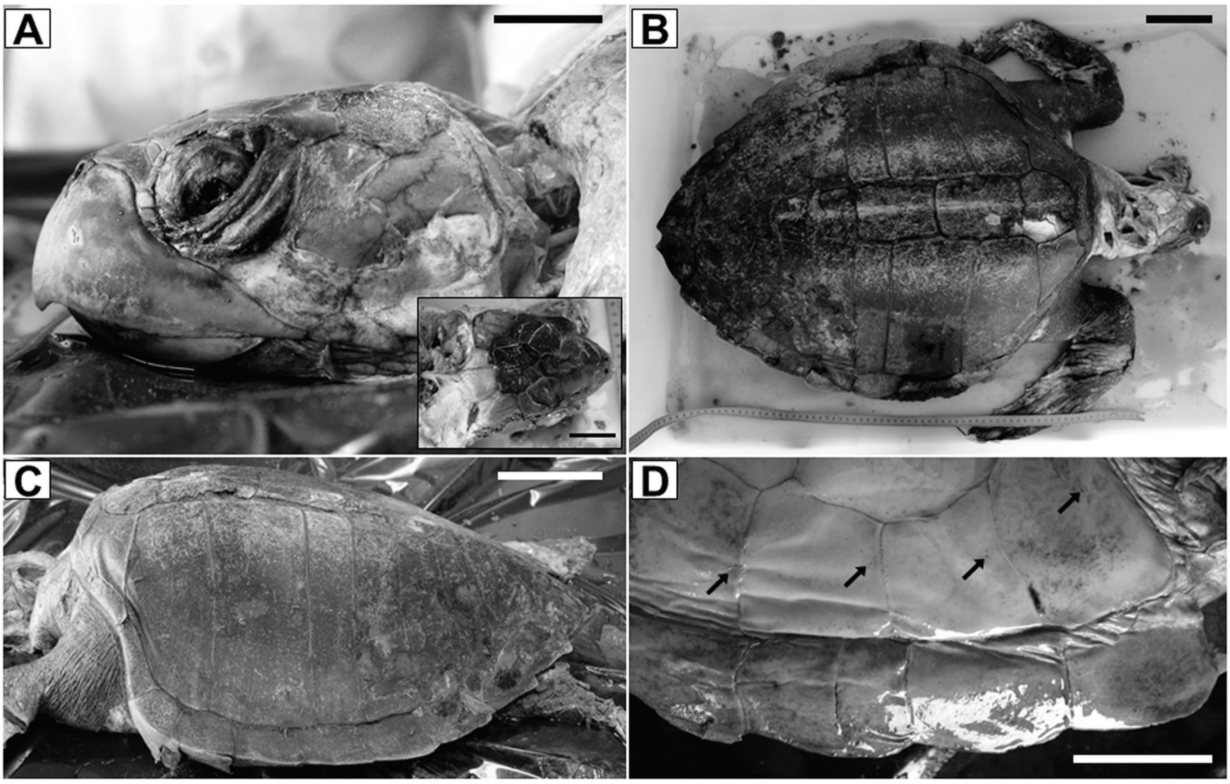

Fig. 2: A: Head and detail of the prefrontal scales of *Lepidochelys olivacea* stranded in eastern Spain. B and C: Costal and vertebral scutes. D: Detail of the pores (arrows) of the inframarginal scutes. Scale bars 5 cm (A and B) and 10 cm (C and D).

This first recording of an olive ridley in the Mediterranean Sea demonstrates the importance of correctly identifying future sea turtle strandings. The general similarity and variability of scute patterns of *L. olivacea*, *L. kempii* and *C. caretta*, caused by alterations in development, trauma or hybridisation, can result in the misidentification of single individuals when a traditional classification based on morphological characters is employed. Hence, we strongly recommend using genetic markers to avoid the misidentification of individuals with mixed or rare characteristics.

Acknowledgements

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Appendix

Table 1. Information on the sequences used for comparisons with our sample, including the GenBank Accession Number (GAN) when available, and the references that found them.

Species	Name	Other names	Site	GAN	Reference
L. kempii	A	Lk1.1	West Atlantic	KF385935§	(Bowen *et al.* 1998; Frey *et al.* 2014)
	B	Lk2.1	West Atlantic	KF385936§	(Bowen *et al.* 1998; Frey *et al.* 2014)
	C	Lk3.1	West Atlantic	KF385937§	(Bowen *et al.* 1998; Frey *et al.* 2014)
	D	Lk4.1	West Atlantic	KF385938§	(Bowen *et al.* 1998; Frey *et al.* 2014)
	X	Lk6.1, Lk6.2	West Atlantic, Mediterranean†	KC609750§, KF385940§, KF385941§	(Barber *et al.* 2003; Carreras *et al.* 2014; Frey *et al.* 2014)
	Lk5.1		West Atlantic	KF385939§	(Frey *et al.* 2014)
	Lk1.1		West Atlantic	KF385942§	(Frey *et al.* 2014)
L.olivacea	E		West Atlantic	AY920522.1§	(Bowen *et al.* 1998)
	F	EimBr2, CCxLO, LO	West Atlantic, East Atlantic, Mediterranean†	EU365971§, EU365972.1§ KP117262	(Bowen *et al.* 1998; Lara-Ruiz *et al.* 2006, Present Study; Reis *et al.* 2010)
	G	Lo2	West Pacific	JN391446.1§	(Bowen *et al.* 1998; Jensen *et al.* 2013)
	H	Lo4	West Pacific, Indic	JN391448§,	(Bowen *et al.* 1998; Jensen *et al.* 2013)
	I		Indic		(Bowen *et al.* 1998)
	J*	Lo1, Lo15	West Pacific, Indic	JN391445.1§, JN391459.1§	(Bowen *et al.* 1998; Jensen *et al.* 2013; Shanker *et al.* 2004)
	K		Indic	AY920519.1§ AF513539.1#, AF513540.1#	(Bowen *et al.* 1998; Shanker *et al.* 2004)
	K-1		Indic	AF513542.1#, AF314653.1#	(Shanker *et al.* 2004)
	K-2		Indic	AF314654.1#, AF513547.1#	(Shanker *et al.* 2004)
	K-3		Indic	AF513543.1#, AF314655.1#	(Shanker *et al.* 2004)
	K-4		Indic	AF513545.1#	(Shanker *et al.* 2004)
	K-4		Indic	AF513546.1#	(Shanker *et al.* 2004)
	L		East Pacific		(Bowen *et al.* 1998)
	M	Lo27	West Pacific, East Pacific	AY920520.1§, KC207830.1§	(Bowen *et al.* 1998; Jensen *et al.* 2013)
	N		East Pacific, Indic	AF051776.1, AY920521.1§ AF514311.1#	(Bowen *et al.* 1998; Shanker *et al.* 2004)
	O		East Pacific	AY920523.1§	(Bowen *et al.* 1998)
	P		East Pacific		(Bowen *et al.* 1998)
	Q		East Pacific		(Lopez-Castro & Rocha-Olivares 2005)
	R		East Pacific		(Lopez-Castro & Rocha-Olivares 2005)
	S		East Pacific		(Lopez-Castro & Rocha-Olivares 2005)
	T		East Pacific		(Lopez-Castro & Rocha-Olivares 2005)
	U		East Pacific		(Lopez-Castro & Rocha-Olivares 2005)
	W		East Pacific		(Lopez-Castro & Rocha-Olivares 2005)
	Lo3		West Pacific	JN391447.1§	(Jensen *et al.* 2013)
	Lo5		West Pacific	JN391449.1§	(Jensen *et al.* 2013)
	Lo6*	Lo10,Lo13	West Pacific	JN391450.1§, JN391454.1§, JN391457.1§	(Jensen *et al.* 2013)

(continued)

Table 1 *(continued)*

Species	Name	Other names	Site	GAN	Reference
	Lo7*	Lo9	West Pacific	JN391451.1§, JN391453.1§	(Jensen *et al.* 2013)
	Lo8*	Lo14,Lo18,Lo22	West Pacific	JN391452.1§, JN391458.1§, JN391462.1§, KC207828.1§	(Jensen *et al.* 2013)
	Lo11*	Lo17	West Pacific	JN391455.1§, JN391461.1§	(Jensen *et al.* 2013)
	Lo12		West Pacific	JN391456.1§	(Jensen *et al.* 2013)
	Lo16		West Pacific	JN391460.1§	(Jensen *et al.* 2013)
	Lo19		West Pacific	JN391463.1§	(Jensen *et al.* 2013)
	Lo20		West Pacific	JN391464.1§	(Jensen *et al.* 2013)
	Lo21		West Pacific	JN391465.1§	(Jensen *et al.* 2013)
	Lo23		West Pacific	KC207829.1§	(Jensen *et al.* 2013)

†Mediterranean individuals that are stranded animals *Different names denote the haplotypes that are identical for the analysed 470-bp fragment, but present differences when analysing longer sequences. §Sequences available at GenBank longer than 470 bp. # Sequences available at GenBank shorter than 470 bp.