

Haplotypic characterization of the olive ridley turtle (*Lepidochelys olivacea*) in northwest Mexico: the northernmost limit of its distribution

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Abstract

*Haplotypic characterization of the olive ridley turtle (*Lepidochelys olivacea*) in northwest Mexico: the northernmost limit of its distribution.* The olive ridley sea turtle (*L. olivacea*) has a pantropical distribution. In the Eastern Pacific, the official limits of its reproduction area are south of the Baja California peninsula and south of Sinaloa, Mexico. Ceuta beach in Elota, Sinaloa, Mexico, has served as a protection site for *L. olivacea* for over three decades. In this study, the *L. olivacea* population from Ceuta beach was genetically characterized. Specifically, a 712-bp fragment from the control region of mtDNA was amplified from 32 olive ridley turtles. Eight haplotypes (seven after cutting to ~468 bp) were identified, and these included two novel haplotypes (Lo-T7 and Lo-T8) and five haplotypes that were previously identified in other nesting beaches. The Lo-T2 haplotype was dominant (~60%) in the samples: $h = 0.6048 (\pm 0.0974)$ and $\pi = 0.002212 (\pm 0.001504)$. Although this study was conducted in the northernmost limit of the olive ridley turtle nesting distribution in the eastern Pacific, the sampled group presents moderate genetic diversity and belongs to a population that, on an evolutionary scale, only recently underwent demographic expansion. Because the olive ridley turtle in the eastern Pacific is considered resilient to environmental variation, nesting area studies in northwest Mexico are necessary.

Key words: Endangered species, mtDNA, Control region (D-loop), Haplotypic and nucleotidic diversity, Olive ridley turtle

Resumen

*Caracterización haplotípica de la tortuga golfina (*Lepidochelys olivacea*) en el noroeste de México: el límite septentrional de su distribución.* La tortuga golfina (*L. olivacea*) tiene una distribución pantropical. En el Pacífico oriental, los límites oficiales de su zona de reproducción son la península de Baja California y el sur de Sinaloa, en México. La playa de Ceuta en Elota, Sinaloa, México ha servido de sitio de protección para *L. olivacea* durante más de tres decenios. En este estudio, se caracterizó genéticamente la población de *L. olivacea* de la playa de Ceuta. Concretamente, se amplificó un fragmento de 712 pb de la región de control del ADNmt de 32 tortugas golfinas. Se identificaron ocho haplotipos (siete tras reducir a ~468 pb) y se incluyeron dos haplotipos nuevos (Lo-T7 y Lo-T8) y cinco haplotipos que se habían identificado anteriormente en otras playas de anidación. El haplotipo Lo-T2 era dominante (~60%) en las muestras: $h = 0,6048 (\pm 0,0974)$ y $\pi = 0,002212 (\pm 0,001504)$. Si bien este estudio se realizó en el límite septentrional de la zona de anidación de la tortuga golfina en el Pacífico oriental, el grupo estudiado presenta una diversidad genética moderada y pertenece a una población que, en la escala evolutiva, ha pasado recientemente por una expansión demográfica. Debido a que la tortuga golfina del Pacífico oriental se considera resiliente a la variación ambiental, es necesario estudiar las zonas de anidación en el noroeste de México.

Palabras clave: Especie amenazada, ADNmt, Región de control (bucle-D), Diversidad haplotípica y nucleotídica, Tortuga golfina

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Introduction

The olive ridley turtle (*Lepidochelys olivacea*) is the most abundant sea turtle in the world and has a pantropical distribution. Its main nesting beaches are located on the east coast of India and in the eastern Pacific (Abreu–Grobois and Plotkin, 2008). In the Mexican Pacific, olive ridley turtles nest from the peninsula of Baja California Sur to the state of Chiapas (Márquez, 1990). The nesting beach El Verde Camacho in Sinaloa is considered the northern limit of its nesting range in the mainland portion of the eastern Pacific (Abreu–Grobois and Plotkin, 2008), with nests observed since 1974 (Ríos–Olmeda, 2005). Although some sporadic nesting has been reported in regions with greater latitude, such as the Upper Gulf of California, El Verde Camacho is by far the most common nesting site in the Gulf of California (Seminoff and Nichols, 2007).

The olive ridley turtle has been observed in the Gulf of California, mainly for feeding, but fishermen and residents of the high and middle regions of the Gulf of California have reported that nests have been commonly observed for 50 years ago (Rodríguez–Valencia et al., 2005). The first reports date back to 1961 (Seminoff and Nichols, 2007), and other reports indicate observations of nests in 1995 and 1996 in Puerto Peñasco, Sonora; in 2004 in San Carlos, Guaymas, Sonora; and more recently in El Desemboque, Sonora (CEDO, 1995; COMCAAC, 2013; Navarro, 1996; Rodríguez–Valencia et al., 2005; Seminoff and Nichols, 2007) (fig. 1). However, due to a lack of documentation, it is difficult to assess how nesting numbers have changed in these regions. Moreover, it is unclear if the nesting observed here is a result of recolonization due to management and conservation strategies implemented in the last decade or whether environmental variations have induced changes in the nesting behavior of this species.

The olive ridley turtle is currently listed as a 'Vulnerable' species according to the IUCN Red List of Threatened Species (Abreu–Grobois and Plotkin, 2008) and as 'In danger of extinction' according to Mexican law (SEMARNAT, 2010). Conservation biology serves as a tool in decision-making regarding management of endangered species and focuses on the maintenance of genetic diversity at different levels, a major component of biodiversity (Hunter and Gibbs, 2007). Bottlenecks are an indicator of a loss of genetic diversity and thus constitute a threat to the conservation of many species (Mills, 2006). Genetic markers differ at the molecular level, and special attributes make these markers suitable for examining the life history and evolution of sea turtles (Bowen and Karl, 1996). One of these markers is mitochondrial DNA (mtDNA) from the maternal lineage. A rapidly evolving segment of this marker is the control region, called the D-loop (Awise, 1995), which is the replication start site of the mtDNA. The high mutation rate of this region allows fine-scale identification of populations.

Phylogenetic studies of the olive ridley turtle using mtDNA have revealed four lineages at a global level: east Pacific, Indo–west Pacific, Atlantic, and east coast of India (Bowen et al., 1998; Shanker et al., 2004). Bowen et al. (1998) suggested that the haplotypic

diversity of the olive ridley turtle is classified globally between 'moderate' and 'low' in comparison with that of other species of sea turtles. Briseño–Dueñas (1998) indicated that the genetic heterozygosity of the olive ridley turtle population in the Mexican Eastern Pacific showed no erosion due to a bottleneck effect despite its overexploitation in the second half of the twentieth century, and proposed that this species forms a panmictic population, characterized by no genetic differentiation among nesting colonies. However, Briseño–Dueñas (1998) did not include samples of the nesting area in Baja California Sur. In contrast, López–Castro and Rocha–Olivares (2005) argued that the rookery of Baja California Sur shows low genetic differences that are nevertheless significant with respect to turtles from nesting beaches in mainland Mexico ($\Phi_{ST} = 0.048$; $p = 0.006$). Rodríguez–Zárate et al. (2013) analyzed 10 microsatellite loci in 18 nesting sites along Mexico's Pacific coast (including the Baja California peninsula and mainland) and identified a clear signature of recent bottlenecks associated with changes in allelic diversity, very low levels of differentiation, and no clear geographical pattern in the population structure. An analysis of molecular variance (AMOVA) did not indicate a significant structure between rookeries of the Baja California peninsula and those of the mainland ($F_{ST} = 0.0004$, $P = 0.595$) (Rodríguez–Zárate et al., 2013).

The available reports of genetic diversity in the northern nesting limits of the species originated from the nesting beach El Verde Camacho ($n = 15$), where four haplotypes have been identified and haplotype and nucleotide diversities appeared to be higher than those in other Mexican Eastern Pacific rookeries ($h = 0.6190 \pm 0.1196$ and $\pi = 0.0022 \pm 0.0017$, respectively) (Briseño–Dueñas, 1998; López–Castro and Rocha–Olivares, 2005). In contrast, at the northern limits of Baja California Sur, López–Castro and Rocha–Olivares (2005) identified five haplotypes and lower haplotype and nucleotide diversities than those reported for other nesting beaches in the Mexican Pacific ($h = 0.1613 \pm 0.0715$ and $\pi = 0.0005 \pm 0.0007$).

For conservation purposes, management units (MUs) have been defined for populations showing significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles (Moritz, 1994). In addition, multi-scale regional management units (RMUs) have been developed to evaluate threats, identify areas of high diversity, highlight data gaps and assess the conservation status of sea turtles above the level of nesting populations but below the level of species (Wallace et al., 2010). On the basis of mtDNA and nDNA information, eight RMUs have been defined for olive ridley turtles worldwide (Wallace et al., 2010). The solitary olive ridley nesting turtles from the eastern Pacific belong to a single RMU distinct from that of 'arribada' (Wallace et al., 2010), and are highly resilient to environmental variations (according to traits such as rookery vulnerability, population trends and genetic diversity) in comparison to the olive ridley turtles from RMU belonging to the Indian Ocean regions and to the RMUs of other species, such as the Kemp's ridley turtle (*Lepidochelys kempii*) from the Gulf of Mexico (Fuentes et al., 2013).

The nesting beaches of olive ridley turtles in north-western Mexico can be classified as follows: (a) beaches with protection activities under a legal framework; (b) beaches with protection activities but without a legally protected status; and (c) potential nesting areas that require evaluation (Márquez et al., 2004) (fig. 1). Ceuta beach, Sinaloa, has legally defined protection activities and is cataloged as a sanctuary for olive ridley nesting areas (DOF, 2002). This 37-km-long beach is located between the Cospita River (24° 10' N and 107° 20' W) and the Elota River (23° 52' N and 106° 57' W) in the central region of the State of Sinaloa, Mexico (fig. 1). According to records dating back to 1976 (Sosa et al., 2012), Ceuta beach is far north of the limit of the nesting range for olive ridley turtles. In the present study, the olive ridley turtle population from Ceuta beach was genetically characterized based on the control region of mtDNA.

Material and methods

Sample collection

During the 2014 and 2015 seasons, 32 samples were collected from olive ridley turtles in the nesting sanctuary at Ceuta beach: five blood samples were extracted from the dorsal cervical sinus of nesting females (Owens and Ruiz, 1980), and 27 skin samples were taken from dead hatchlings. The blood samples were collected in Vacutainer tubes containing 7.2 mg of K₂EDTA and stored at -20°C until analysis, and the skin samples were preserved in 97% ethanol.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted using the Wizard® SV Genomic DNA Purification System (Promega, USA) according to the manufacturer's instructions. An ~800-bp fragment was amplified from the mtDNA control region using the primers H950 (5'GTC TCG GAT TTA GGG GTT T3') and LTEi9 (5'GAA TAA TCA AAA GAG AAG G3') designed by Abreu-Grobois et al. (2006). Amplification was performed in a thermocycler (T100TM Thermal Cycler, BioRad, USA) with an initial step of 94°C for 2 min followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1 min and a final extension step at 72°C for 7 min (modified from Abreu-Grobois et al. 2006). The amplified product was analyzed via electrophoresis in a 1.9% agarose gel stained with GelRed (BIOTIUM) and displayed on an Imaging system (Digi Doc-It, UVP, USA). The PCR products obtained were gel extracted, purified with the Wizard® SV Gel and PCR Clean-up System (Promega, USA) and sent for sequencing in both directions to MacroGen Inc®.

Data analysis

An exhaustive search for *L. olivacea* mtDNA control region haplotypes identified a total of 72 haplotypes of varying sizes. Of these, 52 were previously published or were included in the GenBank database, 13 were identified by Briseño-Deñás in 1998 (unpublished)

and the other seven were haplotypes investigated in this study. The sequences were trimmed to a common length of ~468 bp (Bowen et al., 1998) and then aligned and compared using the web-based program Multalin (Corpet, 1988) for haplotype identification. The haplotypes reported by Shanker et al. (2004), which presented a shorter length (~399 bp), were analyzed only in the mutated nucleotide positions. To analyze the grouping of the haplotypes, the DnaSP program was used (Librado and Rozas, 2009).

The haplotype (h) and nucleotide (π) diversities were calculated using Arlequin ver. 3.5.1.2 (Excoffier and Lischer, 2010). Historical demographics (colonization or bottlenecks) were analyzed based on mismatch distributions using DnaSP (Librado and Rozas, 2009), and the parameters of the sudden expansion model were calculated as follows: $\tau = 2\mu t$, $\theta_0 = 2\mu N_0$ before expansion and $\theta_1 = 2\mu N_1$ after expansion, where μ is the fragment-specific mutation rate, t is the time since expansion in generations, and N is the effective population size (Rogers and Harpending, 1992).

The evolutionary relationships of the identified haplotypes were identified using the maximum likelihood and neighbor-joining method for phylogenetic reconstruction based on the model suggested by the Model test module of MEGA ver. 7 (Kumar et al., 2016); node support for both analyses was assessed through nonparametric bootstrap analysis (1,000 replicates). The phylogenetic tree was constructed with 20 previously reported haplotypes representative of each region of the world and those identified in the present study. The *L. kempii* mitochondrial control region sequence was included as an outgroup (GenBank accession AF051777). Additionally, a minimum spanning network (MSN) was constructed using the median joining option in the Network ver. 4.6.1.1 program (Bandelt et al., 1999) to compare the evolutionary relationships between the identified haplotypes.

Results

A 712 bp mtDNA control region sequence was obtained from 32 olive ridley turtles from Ceuta beach. Comparison of these sequences revealed seven variable sites, namely, five transitions, one transversion and one indel (table 1). Eight haplotypes were identified (labeled Lo-T1 to Lo-T8) and submitted to GenBank (accession numbers KX768696 to KX768698 and KX812518 to KX812522). The dominant haplotype was Lo-T2, which was present in 62.5% of the samples, followed by Lo-T4, which was present in 9.4% of the samples (table 1).

All haplotypes were trimmed to ~468 bp fragments. After trimming, the 65 haplotypes identified previously (published or present in GenBank) decreased to 38, and the haplotypes identified in this study were reduced to seven because Lo-T1 and Lo-T5 were identical in the resulting segment (table 2; these haplotypes were plotted on a map, shown in fig. 2). Two of the seven haplotypes were identified as novel (Lo-T7 and Lo-T8) and were identified in 6.3 and 3.1%, respectively, of the samples, whereas the other five were reported

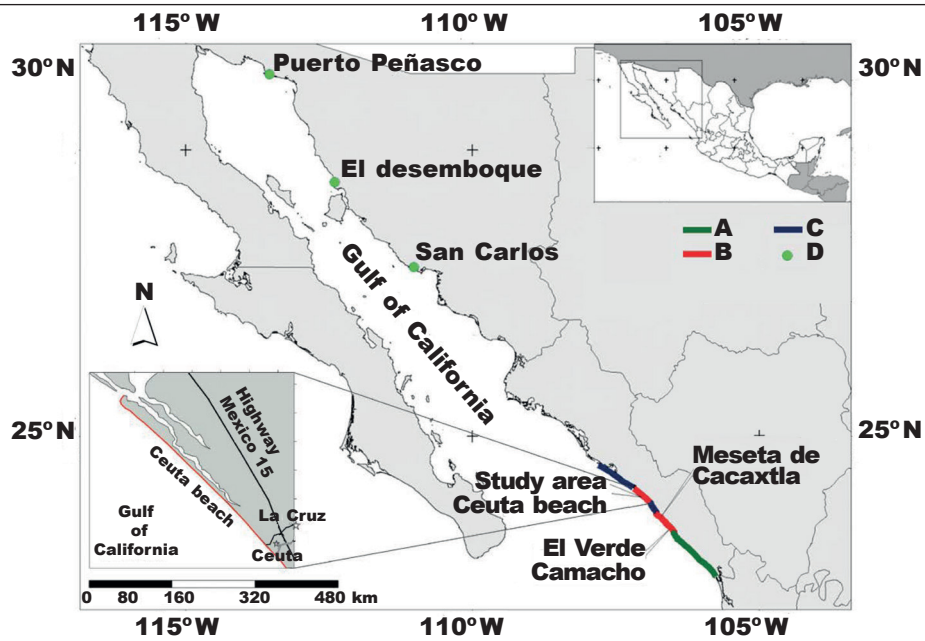


Fig. 1. Study area and main nesting beaches for the olive ridley turtle in northwestern Mexico (adapted from Márquez et al. (2004) and digitized Google Earth images): A, protected without a legal framework; B, protected under a legal framework; C, potential; D, sporadic nesting.

Fig. 1. Zona de estudio y principales playas de anidación de la tortuga golfina en el noroeste de México (adaptado de Márquez et al. (2004) e imágenes de Google Earth digitalizadas): A, protegida sin un marco legal; B, protegida bajo un marco legal; C, potencial; D, anidación esporádica.

Table 1. Variable sites in the mtDNA control region and frequencies of the eight haplotypes of olive ridley turtles identified in Ceuta beach, Sinaloa. The upper numbers represent the position of the base, and the first nucleotide corresponds to site 15,554 of the mitochondrial genome of the olive ridley turtle (GenBank accession number: AM258984): – indels (the novel haplotypes are marked in bold).

Tabla 1. Sitios variables en la región de control del ADNmt y frecuencias de los ocho haplotipos de tortuga golfina identificados en la playa de Ceuta, en Sinaloa. Los números de la fila superior representan la posición de la base y el primer nucleótido corresponde al sitio 15.554 del genoma mitocondrial de la tortuga golfina (número de accesoión de GenBank: AM258984): – mutaciones indel (los nuevos haplotipos se marcan en negrita).

Haplotype	Nucleotide position							Freq	%
	179	202	240	351	420	426	543		
Lo-T2	C	A	C	C	A	–	T	20	62.5
Lo-T4	.	.	T	.	.	–	.	3	9.4
Lo-T6	.	G	.	.	.	A	.	2	6.3
Lo-T7	A	.	T	T	G	–	.	2	6.3
Lo-T1	A	.	T	.	G	–	C	2	6.3
Lo-T3	A	.	1	3.1
Lo-T5	A	.	T	.	G	–	.	1	3.1
Lo-T8	.	.	.	T	.	A	.	1	3.1
								32	100

Table 2. (Cont.)

This study	UnP	Published or listed in GenBank							
		1	2	3	4	5	6*	7	8
	G								
	I								
	P								
			K-2 (AF314654)						
			K-3 (AF314655)						
			K-4 (AF513545)						
			K-5 (AF513546)						
					Lo-03 (JN391447)				
					Lo-05 (JN391449)				
					Lo-06/Lo-10/Lo-13 (JN391450)				
					Lo-07/Lo-09 (JN391451)				
					Lo-08/Lo-14/Lo-18 (JN391452)	Lo-1 (KM357629)			
					Lo-11/Lo-17 (JN391455)	Lo-3 (KM357631)			
					Lo-12 (JN391456)				
					Lo-16 (JN391460)				
					Lo-19 (JN391463)				
					Lo-20 (JN391464)				
					Lo-21 (JN391465)				
					Lo-23 (KC207829)				
						Lo-2 (KM357630)			
						Lo-5 (KM357633)			
					Lo-T7 (KX768697)				
					Lo-T8 (KX768698)				
7	13	15	8	5	16	5	2	1	

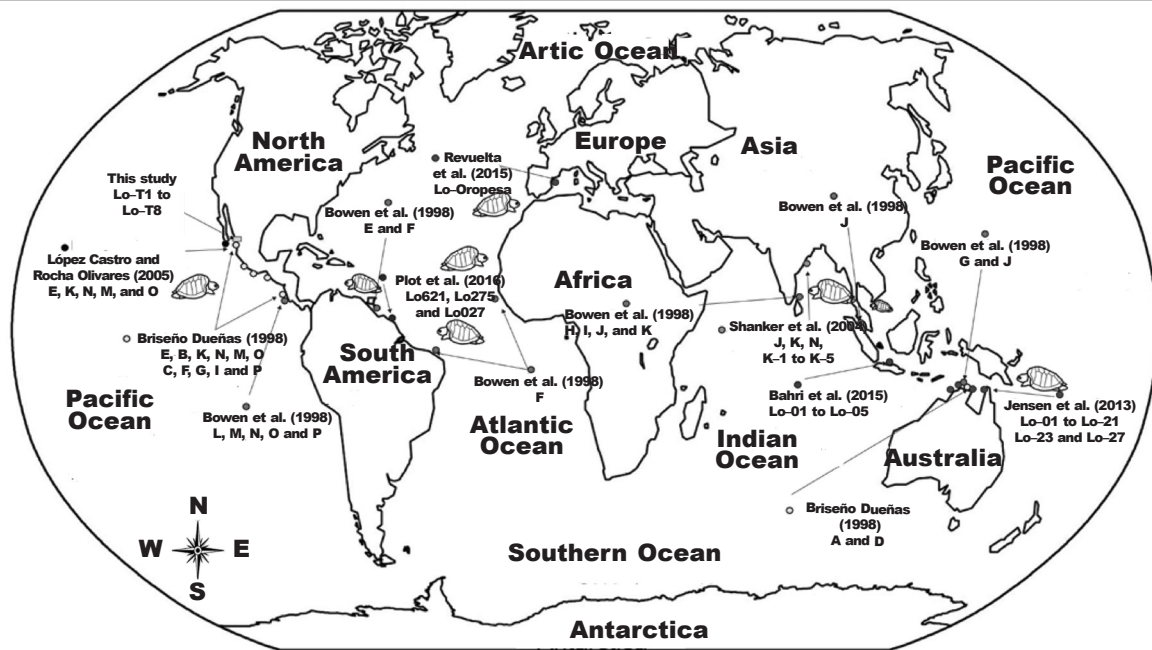


Fig. 2. World map showing the principal haplotypes of the mtDNA control region from olive ridley turtles identified globally (obtained from the GenBank database and unpublished data).

Fig. 2. Mapa mundial en el que se muestran los principales haplotipos de la región de control del ADNmt de las tortugas golfinas identificadas a escala mundial (obtenido de la base de datos GenBank y de datos sin publicar).

previously (Bowen et al., 1998; López–Castro and Rocha–Olivares, 2005) (table 2) and represent 90.6% of the samples. Three of the haplotypes were previously observed in the northern region of the Mexican Pacific. Specifically, Lo–T2 (haplotype N GenBank AF051776 by Bowen et al., 1998 and K, GenBank AY920519 by López–Castro and Rocha–Olivares, 2005) and Lo–T3 (haplotype N, GenBank AY920521 by López–Castro and Rocha–Olivares, 2005) were found in El Verde Camacho and represented 62.5 and 3.1% of the samples from Ceuta beach, respectively.

The third haplotype (Lo–T6) was previously reported for the nesting colony of BCS (Haplotype O GenBank AY920523 by López–Castro and Rocha–Olivares, 2005) and found at low frequencies (6.3%) in this study. The two dominant haplotypes in Ceuta beach (Lo–T2 and Lo–T4) have been reported outside the region: Lo–T2 in Madras, India (N GenBank AF514311 by Shanker et al., 2004) and Lo–T4 in Flinders Beach, Australia, in the western Pacific (Lo27 GenBank KC207830 by Jensen et al., 2013) (table 2). The remaining haplotypes (Lo–T1/Lo–T5) have been observed in other nesting regions of the eastern Pacific (Guerrero, México) (Briseno–Dueñas, 1998).

The haplotype diversity was $h = 0.6048 (\pm 0.0974)$, and the nucleotide diversity was $\pi = 0.002212 (\pm 0.001504)$. A mismatch distribution analysis indi-

cated that the historical demography of turtles nesting in Ceuta beach fit the sudden expansion model ($P > 0.05$) (Rogers and Harpending, 1992): $\tau = 0.258$, $\theta_0 = 1091$ and $\theta_1 = \text{infinite}$.

Of the models suggested by the Modeltest module of MEGA ver. 7 (Kumar et al., 2016), the evolutionary model that best fitted the data based on maximum likelihood phylogenetic reconstruction was T92, also known as the Tamura 3–parameter model. Under this model, the eight haplotypes identified (seven after cutting to ~468 bp) were found to belong to the lineage of the eastern Pacific (fig. 3). The MSN indicated that all other haplotypes present in the eastern Pacific descended from the dominant haplotype Lo–T2 (fig. 4).

Discussion

The olive ridley turtles that nest at Ceuta beach (the northern nesting limit in the eastern Pacific) had moderate genetic diversity compared with that found in other studies (table 3) (Bowen et al., 1998; López–Castro and Rocha–Olivares, 2005; Shanker et al., 2004). This rookery was characterized by a dominance of the haplotype Lo–T2, in agreement with the dominance of this haplotype (labeled as K; table 2) in rookeries from the Mexican Pacific, particularly from El Verde

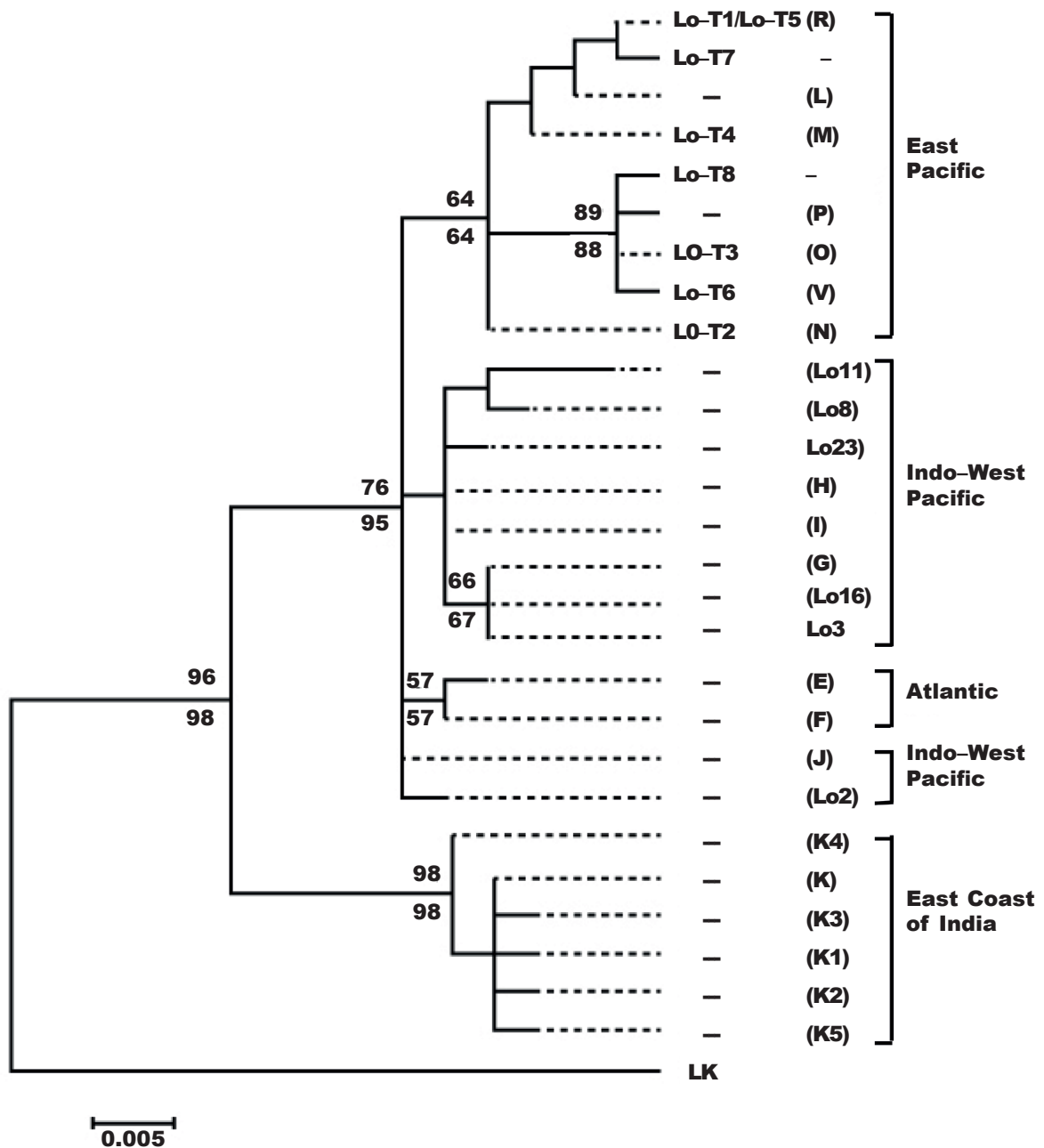


Fig. 3. Maximum likelihood tree obtained under the Tamura 3-parameter model of nucleotide substitution describing the relationships among olive ridley turtle haplotypes from the Ceuta nesting beach and olive ridley turtle haplotypes from other ocean basins. The nomenclature of previously published haplotypes is shown in brackets. The bootstrap values (1,000 replicates) for critical nodes were derived from the maximum likelihood (above the branch) and neighbor joining analyses (below the branch), and only values above 50% are shown. LK (*L. kempii*) was used as the outgroup (AF051777).

Fig. 3. Árbol de probabilidad máxima obtenido mediante el modelo Tamura de tres parámetros de sustitución de nucleótidos en el que se describen las relaciones entre los haplotipos de la tortuga golfina de la playa de anidación de Ceuta y los haplotipos de la tortuga golfina de otras cuencas oceánicas. La nomenclatura de los haplotipos publicados previamente se muestra entre paréntesis. Los valores de bootstrap (1.000 réplicas) de los nodos críticos se obtuvieron a partir de la probabilidad máxima (encima de la rama) y los análisis de unión de vecinos (debajo de la rama). Solo se muestran los valores superiores al 50%. LK (*L. kempii*) se empleó como grupo externo (AF051777).

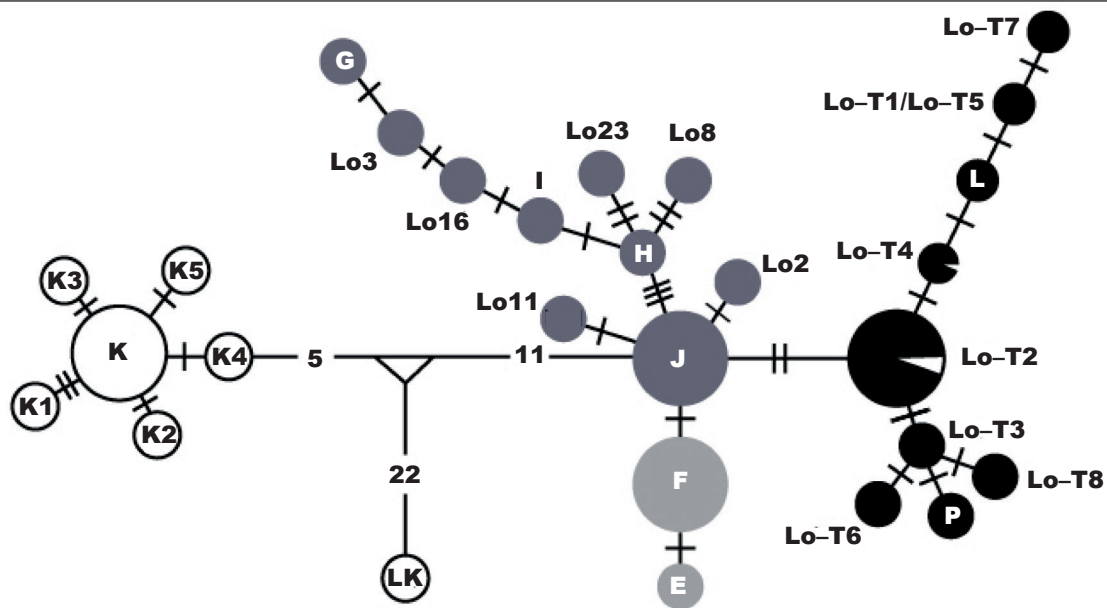


Fig. 4. Minimum spanning network (MSN) of haplotypes of the mtDNA control region of the olive ridley turtle. Each circle represents a haplotype, the largest circles represent the dominant haplotypes in each lineage, the lines show the connected haplotypes, and the numbers represent the number of nucleotide substitutions. Lineages: east Pacific (black), Indo-west Pacific (dark gray), Atlantic (light gray), and east coast of India (white): LK (*L. kempii*) was used as the outgroup (AF051777).

Fig. 4. Red de expansión mínima de los haplotipos de la región de control del ADNmt de la tortuga golfina. Cada círculo representa un haplotipo, los círculos más grandes representan los haplotipos dominantes en cada linaje, las líneas muestran los haplotipos conectados y los números representan la cantidad de sustituciones nucleotídicas. Linajes: Pacífico oriental (negro), Pacífico indooccidental (gris oscuro), Atlántico (gris claro) y costa oriental de la India (blanco): LK (*L. kempii*) se empleó como grupo externo (AF051777).

Camacho nesting beach, as reported by Briseño-Dueñas (1998). The evolutionary relationships of the identified haplotypes were grouped in the east Pacific lineage (fig. 3). Starting from the dominant haplotype (Lo-T2), all other haplotypes descend from and bind to the lineage of the Indo-west Pacific by haplotype J (AF051774 by Bowen et al., 1998), with only two variable sites. In turn, haplotype J joins the lineage of the east coast of India with 16 variable sites (fig. 4). Bowen et al. (1998) proposed that the lineage of olive ridley turtles in the eastern Pacific derives from turtles from India. These turtles colonized the eastern Pacific mainland, part of which is included in this study, approximately 300,000 years ago and more recently on an evolutionary timescale, Baja California Sur (López-Castro and Rocha-Olivares, 2005).

It is possible that the haplotypes identified as novel in this study (Lo-T7 and Lo-T8) have not been previously identified in the region due to the number of samples analyzed ($n = 15$; Briseño-Dueñas, 1998) or perhaps due to the low philopatry that characterizes the olive ridley turtle, which has unique and complex post-reproductive migrations that vary

annually (Morreale et al., 2007; Plotkin, 2010). The exchange of nesting beaches might form part of a complex phenomenon that the olive ridley turtle uses to colonize new areas or even completely change its nesting site (Tripathy and Pandav, 2008). A recent and novel report describes a female stranded in the Mediterranean Sea in the province of Castellon, Spain (KP117262; Revuelta et al., 2015), a region where the olive ridley turtle does not usually nest, and the haplotype identified for this individual turtle matches reports of haplotype F from the Atlantic (AF051773; Bowen et al., 1998). According to the MSN topology and a mismatch distribution analysis, the rookery of Ceuta beach belongs to a population that recently (on the evolutionary scale) expanded demographically (Grant and Bowen, 1998), and this notion agrees with that proposed by López-Castro and Rocha-Olivares (2005) for Mexican rookeries of olive ridley turtle nesting.

Although this study was conducted within the northernmost nesting limits of olive ridley turtles in the eastern Pacific, the findings revealed a moderate genetic diversity ($h = 0.6048$), very similar to that

Table 3. Levels of genetic diversity in the mtDNA control region of the olive ridley turtle: n, sample size; H, number of haplotypes; h, haplotype diversity; π , nucleotide diversity; and bp, base pairs.

Tabla 3. Grados de diversidad genética en la región de control del ADMmt de la tortuga golfina: n, tamaño de la muestra; H, número de haplotipos; h, diversidad haplotípica; π , diversidad nucleotídica; bp, pares de bases.

Site	n	H	h	π	bp	Bibliographic source
Ceuta beach, Sinaloa, México	32	8	0.6048	0.0022	712	This study
El Verde Camacho, Sinaloa, México	15	4	0.6190	0.0023	488	Briseño–Dueñas (1998) López–Castro and Rocha–Olivares (2005)
Baja California Sur, México	48	5	0.1613	0.0005	829	López–Castro and Rocha–Olivares (2005)
Ixtapilla, Michoacán, México	27	8	0.6800	0.0029	750	Rojas–Cortés et al. (2015)
Global	80	12	0.8100	0.0108	470	Bowen et al. (1998)
East coast of India	81	8	0.2700	0.0030	399	Shanker et al. (2004)
Flinders beach, Australia	27	5	0.7493	0.0033	780	Jensen et al. (2013)

reported by Briseño–Dueñas (1998) for the El Verde Camacho nesting beach ($h = 0.6190$) (reported for Sinaloa by López–Castro and Rocha–Olivares, 2005; table 3) and that described in similar reports from other areas of the Mexican Pacific, such as Ixtapilla, Michoacán (Rojas–Cortés et al., 2015). In contrast, the haplotype diversity of olive ridley turtles within the nesting limits in the peninsular area of Baja California Sur was low ($h = 0.1613$), either due to rare genetic flows with other nesting colonies or recent colonization of these beaches (López–Castro and Rocha–Olivares, 2005). Recent studies in western Pacific rookeries reported haplotypic diversities similar to those found in the present study (Jensen et al., 2013; table 3). The genetic diversity obtained in this study was greater with respect to the ancestral lineage (Indian Ocean) ($h = 0.2700$) that gave rise to the turtles in the eastern Pacific (Bowen et al., 1998; Shanker et al., 2004), and this increase was likely due to genetic drift events that favor certain haplotypes (Shanker et al., 2004). The haplotype diversity identified is congruent with respect to the only worldwide study of the species (table 3; Bowen et al., 1998), which included seven colonies in three ocean basins (Pacific, Atlantic and Indian) and identified a haplotype diversity of $h = 0.8100$ (table 3); this haplotype diversity is greater than that found in any study conducted in any specific area of the world. The nucleotide diversity was estimated to equal $\pi = 0.0022$ (table 3), which falls within the range recognized for this parameter in a typical population (0.0005 to 0.020) (Avice, 1994) and coincides with the range reported for El Verde Camacho ($\pi = 0.0023$, the official northern nesting limit in the eastern Pacific). The low nucleotide diversity could be due to the

recent origin of some haplotypes, which might have resulted from a recent colonization by a few individuals, resulting in the elapsed time being too short to allow the accumulation of mutations that increase nucleotide diversity (Bowen et al., 1998), as in the rookery of Baja California Sur (table 3; López–Castro and Rocha–Olivares, 2005).

The olive ridley turtles that nest in Ceuta beach have moderate genetic diversity with respect to other turtles studied in the eastern Pacific and worldwide. This trait is common to resilient species facing environmental changes because it can facilitate adaptations to variable conditions (Fuentes et al., 2013; Wallace et al., 2010). According to Wallace et al. (2010), although the geographical boundaries of the RMU for arribada and solitary nesters of olive ridley turtles in the eastern Pacific show some overlap, there are differences in the abundances of populations and trends between the two behaviors. Information based on arribada events (Fuentes et al., 2013; Wallace et al., 2011) indicates that the olive ridley turtle that nests in the eastern Pacific is a species with high resilience to environmental variations. Nests have been observed in regions located north of the current study area (Seminoff and Nichols, 2007). Rodríguez–Valencia et al. (2005) suggested that most of these nests must face and overcome adverse environmental conditions, such as changes in temperature, humidity or extreme storms to reach the hatching stage. Because sea turtles have adapted by redistributing their nesting sites (Hamann et al., 2007), it is quite possible that olive ridley turtles alter the range of their latitudinal distribution depending on spatiotemporal environmental variations. It is therefore necessary to continue the maintenance and protection

of important nesting regional beaches as well as the identification and legal protection of areas that will continue to provide suitable environments for nesting areas in the future (Fuentes et al., 2012). Currently, three zones in the state of Sinaloa have been legally recognized for the protection of olive ridley turtles: El Verde Camacho (DOF, 2002), Ceuta beach (DOF, 2002), and Meseta de Cacaxtla (DOF, 2003) (fig. 1). However, some protected areas, such as Caimanero beach, Rosario, Sinaloa, where the number of nests has increased at least fourfold in the past ten years (UAS, 2015), have not been legally recognized. Additionally, other areas with sporadic nests that can potentially be used in various types of studies (e.g., with available data regarding genetic information, indicators of abundance, and records of environmental factors) have been identified (San Carlos, Guaymas, Sonora; El Desemboque, Sonora) (Seminoff and Nichols, 2007) (fig. 1). Area in which nesting occurs, even if only sporadically, should therefore be studied and continuously surveyed.

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