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Olive ridley arribada on Gahirmatha beach, Odisha, India, with the nearby Maipura river delta in the background. See pages 1-2. Photo: M. Muralidharan.

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Lepidochelys olivacea in Puerto Rico: Occurrence and Confirmed Nesting

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Lepidochelys olivacea (Eschscholtz 1829) is known as the olive ridley turtle, or "golfina" in Spanish, and belongs to one of the six genera of the family Cheloniidae (Reichart 1993). This species is considered the most abundant marine turtle in the world with a near-circumtropical distribution (Reichart 1993). According to the most recent update from the IUCN Red List (2008), the species has been decreasing worldwide and it is listed as a Vulnerable Species (www.redlist.org). Despite the population declines, positive trends in abundance in individual nesting sites within regional management units have been observed in the Western Atlantic and Northeast Indian Ocean (Mazaris *et al*. 2017). Olive ridleys are widely distributed along the Pacific coast of Central and South America, but they are not abundant in the Western Atlantic basin (Reichart 1993; Marcovaldi 2001). In the Western Atlantic, the highest nest densities have been reported from beaches in Suriname, French Guiana, and Brazil (Marcovaldi 2001; da Silva *et al.* 2007; Plot *et al*. 2012). The northernmost published observations of olive ridley turtles in the Western North Atlantic were in Floridian waters (Foley *et al.* 2003), where they occurred as strandings.

Although the occurrence of *L. olivacea* in the Greater Caribbean region is rare, there have been some sightings in Cuba, the U.S. Virgin Islands, and the Dominican Republic (Varona 1974; Bacon 1981; Carr *et al*. 1982; Fretey 1999; Moncada *et al.* 2000; Eckert & Eckert 2019). Eckert & Eckert (2019) report that all known nesting sites for *L. olivacea* around the wider Caribbean are found along the northeastern coast of South America and Trinidad. The presence of *L. olivacea* in Puerto Rico is infrequent (Dow Piniak & Eckert 2011; Eckert & Eckert 2019). The first recorded and officially published sighting of an olive ridley turtle in Puerto Rico waters was near the San Juan harbor in 1967 (Caldwell & Erdman 1969). The specimen had a straight carapace length of 48 cm with a width of 51.5 cm; the specimen was determined to be a subadult (Reichart 1993). Rivero (1998) mentioned two other sightings of *L. olivacea* at Toa Baja in 1967 and at San Juan in 1976, all in northern Puerto Rico. In 1997, a female was found incidentally entangled in a net two miles offshore between Aguadilla and Rincón (Horta *et al.* 2000).

The coasts of Puerto Rico are used as nesting sites for three other species of marine turtles: *Dermochelys coriacea* (Vandelli 1761), *Eretmochelys imbricata* (Linnaeus 1766) and *Chelonia mydas* (Linnaeus 1758) (Eckert & Eckert 2019). Monitoring efforts by the Committee for Marine Turtle Conservation "Yo amo el Tinglar," a local grassroots group, have documented 10 possible nesting events by *L. olivacea*. Of these 10 nests, five dead hatchlings recovered from four different nests were preserved. Based on morphological traits (color of the hatchling, number of scutes), the specimens collected from nests laid in Arecibo are considered *L. olivacea.* This study summarizes all available nest data of the olive ridley turtle in Puerto Rico between 2017-2019 and confirms the presence of *L. olivacea* through molecular and morphological analysis. In addition, this year (2020), a female *L. olivacea* was observed nesting by a local fisher on one of the beaches, where other nests have been previously reported. However, data from the hatchlings from this nest have not been analyzed nor presented in this study (Figs. 1 and 2). The impetus of presenting the data of 2017-2019 is to contribute to the knowledge of the biology and distribution of this species, in order to improve population assessments of the olive ridley turtle locally in Puerto Rico and in the Wider Caribbean.

The beaches with olive ridley nests were Playa Abacoa

Figure 1. Female *Lepidochelys olivacea* before laying a clutch in Playa Abacoa, January 2020.

Figure 2. One of the live hatchings of *Lepidochelys olivacea* found in the nest in Playa Abacoa, March 2020.

(18.474028 °N, -66.703056 °W) and Playa Grande (18.491056 °N, -66.610472 °W) along the north coast of Arecibo, Puerto Rico. Regular monitoring for freshly laid nests on these beaches is common because two other marine turtle species, the leatherback turtle and the hawksbill turtle are present in these areas (Eckert & Eckert 2019). Because the nest patrols are normally conducted in the morning, there were no visual confirmations of a nesting *L. olivacea*, although the size and characteristics of the nesting crawl raised doubts about the identification of the species for each nest. After the nests hatched, they were evaluated following the protocol of Miller (1999) and the number of emerged hatchlings from each nest, the number of eggs, the percentage of those that hatched and the emergence success were recorded. Dead hatchlings were preserved in 95% ethanol. The morphological characters of the specimens were then measured (Table 1). Total genomic DNA was extracted from tissue taken from the carapace or posterior right flipper, using a Qiagen DNeasy 96 Blood & Tissue Kit (Qiagen, Germany) and following the manufacturer's protocol. A ~800 bp fragment of the mtDNA control region, or D-loop, was amplified with the primers H950g (5'- GTCTCGGATTTAGGGGTTT-3') and LTEi9 (5'- GAATAATCAAAAGAGAAGG -3') (Abreu-Grobois *et al.* 2006). Amplification was carried in a MyCycler™ Thermal Cycler (BioRad), using the PCR conditions in Campista-Leon *et al.* (2019). The quality and quantity of all PCR products was estimated with 1% agarose gel electrophoresis and the NanoDrop™ spectrophotometer. The PCR products from five hatchlings were sent to the McLAB facility (San Francisco, CA, USA) for Sanger sequencing in both directions. Quality control, end trimming and sequence editing was done with CodonCode Aligner 9.0.1. No mutations were observed among the sequences of the five specimens (GenBank Accession Numbers MT501679- MT501683). Sequences were submitted with BLASTn (Altschul *et al*. 1997), in GenBank and the top hits results were recorded.

The north coast of Puerto Rico has dynamic sandy beaches with variable widths, shaped by storms, hurricanes and high wave energy (Morelock *et al.* 2000; Barreto-Orto *et al.* 2019). Turtle hatchling L came from a nest in Playa Grande, a sandy beach that is well covered by vegetation (Morelock *et al.* 2010). Specimens M3 and M4 also came from Playa Grande. The other two specimens were sampled from two nests of Playa Abacoa, located next to the river mouth of Río Grande de Arecibo. Open sandy beaches near river mouths appear to be the typical nesting beach profile for *L. olivacea* (Pritchard & Mortimer 1999).

Pritchard (1969) described the carapace of the Western Atlantic olive ridley turtles as having 5-7 central scutes. The common number of marginal scutes is 12 on the left and 12 on the right, but there are cases where the marginals scutes are 13 (Pritchard 1969). According to the description from Pritchard & Mortimer (1999), olive ridley turtles have five to nine costal scutes with asymmetrical configuration with a typical carapace length ranging from 38-50 mm. In a morphometric study by Michel-Morfin *et al.* (2001), the maximum curved carapace length (CCL) in hatchlings was 50 mm, while the maximum curved carapace width (CCW) was 52 mm.

All hatchlings collected (Table 1) exhibited the aforementioned described carapace scute characteristics; however, the length of the carapaces varied from that of the original descriptions. This could be due to slight carapace deformations associated with the nest conditions and/or the preservation process. The curved

	Carapace Scutes					Carapace size (mm)	
		Costal		Marginal			
ID	Central	Left	Right	Left	Right	Width	Length
L	7	8	9	13	13	55	57
M1	5	6	6	-	-	43	55
M2	5	7	6	13	13	47	50
M3	7	7	7	13	13	56	64
M4	5	7	8	13	13	55	61

Table 1. Morphological characters of the carapace of each olive ridley specimen hatchling. (-): Specimen M1 had damaged marginal scutes, therefore, no measurements were recorded.

ID	Site	Date laid	Date emerged	CS	HS	ES
L	Playa Grande	1/18/2017	3/21/2017	105	95	92
M1	Playa Abacoa	1/4/2019	3/20/2019	108	67	62
M2	Playa Abacoa	1/25/2019	4/9/2019	120	83	68
M3 M4	Playa Grande	7/6/2019	8/25/2019	89	47	39

Table 2. Data from individual confirmed *Lepidochelys olivacea* nests laid in Arecibo, Puerto Rico. CS = clutch size, HS = hatching success (%) and ES = emergence success (%).

Figure 3. Carapace from the dead female olive ridley found in Isla Cabra, San Juan, Puerto Rico. Numbers 1-7 indicate costal carapace scutes.

Figure 4. Skull from the dead female olive ridley found in Isla Cabra, San Juan, Puerto Rico.

carapace measurements and the manner in which the specimens were preserved, either with a straight carapace or curved carapace following the egg circumference, might have caused a small increase in the length of the curved carapace measure.

In the nests we examined, the hatching success ranged from 47 - 95% and the emergence success ranged from 39 - 92% (Table 2). The small number of inspected nests (n = 4) limits our inferences on the hatching and emergence success of *L. olivacea* in Puerto Rico, however they are similar to values reported in other studies. In the western Atlantic, the average duration of incubation ranged from 41 - 72 days and the clutch size of *L. olivacea* nests ranged between 4 and 182 eggs, with an average of 100 eggs (SD = 0.29) (da Silva *et al.* 2007). In the East Pacific, Barrientos-Muñoz *et al.* (2014) reported a range of 45 - 100% and 45.8 - 93.4% for hatching and emergence success, respectively.

Our five D-loop DNA sequences (625 bp length, after quality check and end-trimming) were identical to several publicly available sequences in GenBank, found in olive ridley turtles from the western Atlantic Ocean (*e.g.*, Genbank accession numbers FJ795429-FJ795433; Plot *et al.* 2012), the Mediterranean (*e.g.*, KP117262; Revuelta *et al.* 2015), Indian Ocean (*e.g.*, MN342239; Stelfox *et al.* 2020) and the Pacific (*e.g.*, JX454987; Duchene *et al*. 2012). Our sequences were identical (69% coverage) to haplotype F (*e.g.*, AF051773; Bowen *et al.* 1998). This haplotype is present in 94% of olive ridleys from rookeries in Suriname and Brazil, which harbor low mtDNA diversity (Bowen *et al.* 1998). Finally, although the control region sequences in Foley *et al*. (2003) were not published in a database, they reported that all three specimens found in Florida waters matched haplotype F. Haplotype F appears to be widespread in areas as geographically separated as Florida (Foley *et al.* 2003), Puerto Rico (this study), Suriname and Brazil.

On 6 October 2020, a sexually mature female *L. olivacea* was found dead on the coast of Isla Cabra, San Juan (18.4699671 °N, -66.1353916 °W). By the decomposition state of the body, we estimated that the turtle was found a week after her death. Since eggs were observed in the oviduct, we assumed that the olive ridley was disoriented by the light of a lamppost when it came out for nesting and she then fell off a cliff. The female weighed 27.2 kg and the curved carapace length and width measurements were, respectively, 65 cm and 63 cm; a total of 7 costal carapace scutes were observed. The carapace and skull of the olive ridley turtle were preserved for evidence of this event (Figs. 3 and 4).

Until now, only three species of marine turtles have been confirmed nesting in Puerto Rico. Using morphological characteristics, descriptive data from the nests, and DNA data, we now confirm that *L. olivacea* is the fourth species of marine turtle that has used the beaches of Puerto Rico for nesting. Even though the nesting events have been sporadic in frequency and low in numbers, it is extremely important to continue monitoring and gathering data for olive ridley turtles in Puerto Rico for biodiversity and management purposes.

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