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# Molecular Identification and Novel Mitochondrial COI Gene Haplotypes of Nesting Kemp's Ridley Turtles (*Lepidochelys kempii*) in Rancho Nuevo Sanctuary, Mexico

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**Abstract:** One hundred new COI sequences of nesting female Kemp's ridley turtles (*Lepidochelys kempii*) were obtained in the Rancho Nuevo Sanctuary (RNS). The COI sequences were analyzed and contrasted with others retrieved from BOLD and GenBank with the aim of investigating genetic variability, genetic divergence, and haplotypes of the nesting female population at RNS. Four new COI haplotypes for Kemp's ridley were described; two are redundant with (LK-RN01) 97 and (LK-COI-01) 17 specimens belonging to the RNS and other localities, respectively. Nucleotide (0.00052) and haplotype (0.303) diversity showed low and conserved COI values The fixation index (FST) between these main redundant haplotypes showed a high degree of differentiation with ~1. Genetic divergence clearly demonstrated two different Kemp's ridley nesting populations, one from RNS and a second outside Mexico. Phylogenetic COI analysis was useful to differentiate these redundant (LK-COI-01 and RNS LK-RN01) haplotypes and, therefore, these different Kemp's ridley populations. In addition, phylogenetic COI analysis clearly separates Kemp's ridley turtles from other sea turtle species, supporting its use as a barcode marker.

**Keywords:** barcode analysis; COI gene; endangered species; Kemp's ridley turtle; *Lepidochelys kempii*; Mexico; Rancho Nuevo Sanctuary

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## 1. Introduction

Mexico is home to extremely important nesting and foraging habitats for six of the seven recognized species of sea turtles distributed worldwide, including leatherback (*Dermochelys coriacea*); green (*Chelonia mydas*); hawksbill (*Eretmochelys imbricata*); loggerhead (*Caretta caretta*); olive ridley (*Lepidochelys olivacea*); and Kemp's ridley (*Lepidochelys kempii*) turtles. All six species are classified as endangered by the Mexican Government and other civil international organizations [1,2]. Efforts for their conservation and management have expanded over more than five decades [3–5].

Kemp's ridley and olive ridley turtles are the two species that nest in 'arribadas,' an evolutionary strategy of synchronized massive turtle nesting events where tens of thousands of turtles converge on very specific beaches to lay millions of eggs simultaneously [6]. Kemp's ridley has the most restricted nesting range, with the primary nesting site in the Rancho Nuevo Sanctuary (RNS), Tamaulipas, Mexico, with few exceptions [7]. After 50 intense years of recovery actions [2–5], numbers have bounced to 12,000 to 13,000 turtles a year between the 2014 and 2021 nesting seasons [8–10].

Today, 80–90% of the global nesting for the species continues in the RNS [3,7,10]. Other documented nesting sites include Soto la Marina, Tepehuajes, Barra del Tordo, Altamira, and Miramar, all in Tamaulipas; a few nests have been identified outside of Mexico, in South Padre Island, Texas, USA [5,11,12].

DNA barcoding has been extensively used to study species diversity [13]. It can be implemented by the molecular analysis of mitochondrial or nuclear regions, strengthening results. For instance, mitochondrial DNA (mtDNA) is often used in animals as a global identification system to investigate phylogeographic history [14–17]. These genes include a 16s rDNA, cytochrome C oxidase subunit 1 (COI), and D-loop regions [18]. These genes are highly conserved but can present changes in their sequence that distinguish between specific populations within a species [19]. The D-loop region has been widely used in variability genetic studies in different sea turtle populations [20,21]. However, when the efforts focus on distinguishing between different sea turtle species, COI analysis becomes useful as a molecular barcode [22,23]. COI sequences have also been used to construct haplotype networks and phylogenetic trees to investigate DNA divergence and population genetic parameters [19,24–27]. This gene is a common barcoding technique used to perform high-throughput taxonomic assignments [19,28]. It provides an important tool to support taxonomic studies in different species. Therefore, COI sequences have opened an opportunity to study sea turtles that arrive on Mexican coasts. These molecular techniques provide a better understanding of the ancestral demographic connections across ocean basins in sea turtles [19,24,25].

Traditional morphometric and genetic studies have been previously described for Kemp's ridley turtles [1,11,29–31]. However, few studies have used COI as a molecular marker to evaluate the genetic parameters of the species. Some specimens [2–10] collected in the United States have been evaluated by COI [22,26]; however, no specimens have been tested at RNS. The main objective of this study was to use the COI gene to generate Kemp's ridley DNA barcodes from nesting females and to evaluate their usefulness in accessing the genetic variability and divergence of this endangered species at RNS.

## 2. Materials and Methods

### *2.1. Field Specimens and DNA Biobank*

Blood and biopsy samples were collected at the following locations: RNS, 23.181675, −97.766996; Playa Miramar (MIR) 22.311026, −97.814562; Playa Tepehuajes (TEP) 23.4975501, −97.7613688; Barra del Tordo (BdT) 23.024637, −97.761465; and Altamira (ALT) 22.499617, −97.855637, all in the State of Tamaulipas, Mexico (Figure 1). Whole blood was collected from the occipital sinus of females using sterile heparin tubes (Vacutainer, Bristol Circle, Oakville, ON, Canada). Skin biopsies were collected from the rear flipper using a human-grade 6-mm dermal biopsy punch (Surgical Design Inc., Lorton, VA, USA). All procedures were performed after nesting as previously described [32]. One to three cm biopsies were collected from the hind flippers of dead hatchlings. Blood specimens were preserved at 4 °C before total DNA extraction [33], and biopsies were immediately fixed in 70% ethanol. All turtles were released unharmed after sampling.

| Country | State | Location | Latitude | Longitude |
|---|---|---|---|---|
| Mexico | Tamaulipas | Playa Tepehuajes (TEP) | 23.4975501 | −97.761369 |
| Mexico | Tamaulipas | Racho Nuevo Sanctuary (RNS) | 23.181675 | −97.766996 |
| Mexico | Tamaulipas | Barra del Tordo (BdT) | 23.024637 | −97.761465 |
| Mexico | Tamaulipas | Altamira (ALT) | 22.499617 | −97.855637 |
| Mexico | Tamaulipas | Playa Miramar (MIR) | 22.311026 | −97.814562 |

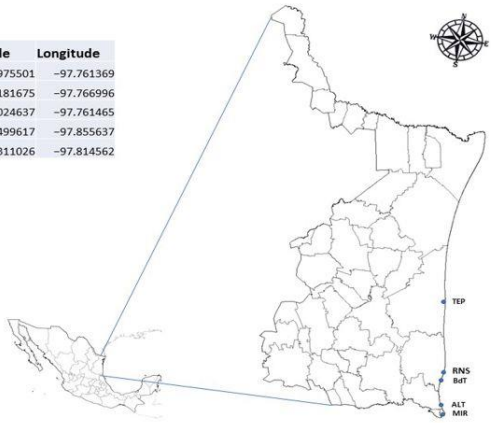


**Figure 1.** Location of the main Kemp's ridley nesting beaches in Tamaulipas, Mexico. The figure shows the name and abbreviations of the nesting beaches and their respective Latitude and Longitude.

### *2.2. Amplification and Sequence of Gene Segments*

Genomic DNA was extracted with the Qiagen DNeasy® blood and tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. All DNA samples were stored at 4 °C until use. The primers used to amplify the COI gene were VF 5′-TCAACCAACCAC AAAGACATTGGCAC-3′ y VR 5′-TAGACTTCTGGGTGGCCAAAGAATCA -3′ [13,34–36]. A 25 µL PCR reaction containing 17.05 µL PCR water, 1 µL of 10 ng/µL DNA, 0.1 µL of 5 U of Taq polymerase (Bioline), 1.25 µL of 25 mM of MgCl2, 0.2 µL of 10 µM of each primer, 0.2 µL of 10 mM of dNTPs, and 5 µL of 5X Tris-KCl as a regulator was performed. PCR conditions were 95 °C for 5 min; 30–35 cycles of 95 °C for 45 s, 54 °C for 45 s, 72 °C for 35 s, 42 °C for 45 s; and 72 °C for 6 min, followed by storage at 4 °C until use. PCR products obtained were purified by standard ethanol purification and resuspended in sterile water. The samples were then sent to Eurofins Scientific (Eurofins Genomics LLC., Louisville, KY, USA) for sequencing.

### *2.3. Data and Quality Sequence Analysis*

All retrieved sequences were analyzed using FASTQC software (Babraham Bioinformatics, Cambridge, UK) for quality control and BioEdit v7.2.5 software [37] to analyze each sequence. Manual editions of the sequence were performed when necessary. Analysis was complemented with 17 Kemp's ridley sequences retrieved from BOLD or GenBank. These accessions came from the United States, two from the east coast, five from the New York area, and 10 from South Padre Island (SPI). In addition, the full COI gene sequence from GenBank (ID: MN136061) was used as the control sequence. This analysis also included available sequences of other turtle species and a sequence of a tortoise (GenBank ID: MW996700) as an outgroup. All sequences were transformed, aligned, and analyzed in FASTA format using BioEdit v7.2.5 for Clustal W software [38]. Sequence information and access numbers from BOLD were annotated. All public sequences from BOLD and GenBank were loaded. New sequences documented herein were deposited in BOLD with access numbers LKRNC002-20–LKRNC100-21.

### *2.4. Haplotype Analysis*

A parsimonious network of unrooted haplotypes was created with PopART v. 1.7 software [39] using the TCS method [40] (Figure 2, Table 1). Following all sequence alignments using Clustal X, a full consensus sequence of the COI gene was obtained, approximately 1547 bp of Kemp's ridley tagged as GBMTG044-16.COI-5P or NC_000886 from BOLD ID or GenBank [20], respectively. The consensus sequence from this work was 624 bp, which is useful for considering the nucleotide variations in these sequences. Divergence and p-distance [23] among these sequences were determined with MEGA X v.10.2.4 using Kimura 2-Parameters and a K2P nucleotide distance model [41,42].

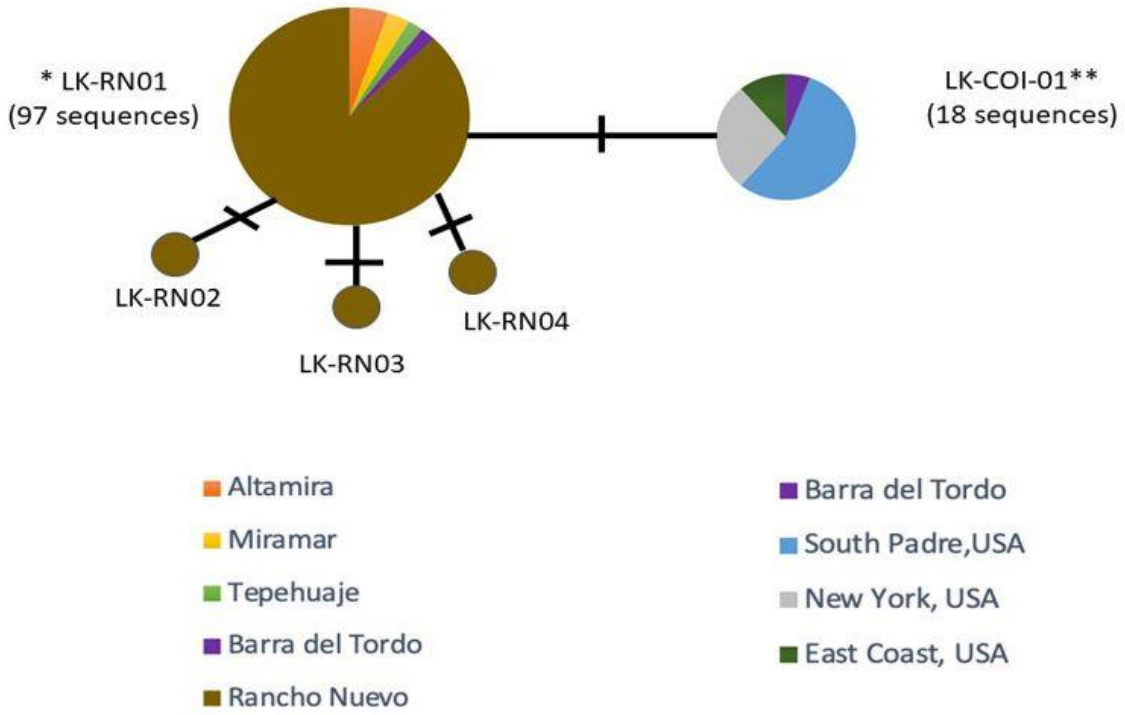


**Figure 2.** Haplotype network of the 5 haplotypes found for Kemp's ridley (*Lepidochelys kempii*) using COI sequences obtained from BOLD and specimens collected in Mexico, 2010, 2016, 2018. The size of the circle indicates the number of individuals that share the same haplotype, and the colors correspond to different sampling locations. The small line indicates a mutation step. * New Haplotypes. ** Previously reported haplotype.

**Table 1.** Mitochondrial haplotypes were obtained for 118 COI sequences from Kemp's ridley (*Lepidochelys kempii*) in BOLD and field samples collected in Mexico, 2018–2019; Nucleotides change positions related to the consensus sequence (113 or 716, GenBank accession, respectively), number of sequences (n), number of haplotypes (*H*), nucleotide (Pi), and haplotype (h) diversities, and G + C.

| ID Sequence | Position | | | | Number of Sequences (n) | Number of Haplotypes (*H*) | h | Pi | G + C |
|---|---|---|---|---|---|---|---|---|---|
| | 71 | 550 | 590 | 604 | | | | | |
| Population (LK-COI-01 */LK-RN01) | NA | NA | NA | NA | 118 | 2 | 0.303 | 0.00052 | 0.422 |
| LK-COI-01 * | C | T | T | A | 97 | 1 | 0 | 0 | ND |
| LK-RN01 | | | | C | 18 | 1 | 0 | 0 | ND |
| LK-RN02 | | C | | C | 1 | 1 | ND | ND | ND |
| LK-RN03 | | | C | C | 1 | 1 | ND | ND | ND |
| LK-RN04 | T | | | C | 1 | 1 | ND | ND | ND |

* Control sequence. NA: Not Applicable. ND: Not Determined.

### *2.5. Tree-Building for COI*

IQ-Tree v. 1.6.12 software [43] was used to infer phylogenetic trees of the COI gene sequences (Figure 1) using the best-fit model by ModelFinder [44,45], according to Bayesian Information Criteria—BIC [43]. In addition, a complementary Maximum Likelihood tree (ML) was built using IQ-Tree with 1000 ultrafast bootstrap (BS) repetitions [44]. MEGA X was used to visualize the phylogenetic tree.

### *2.6. Population Gene Parameters*

DNA Sequence Polymorphism (DnaSP v6.12.03) software (Barcelona, Spain) by Rosas [46] was used to determine the fixation index or Fst [47] and to measure haplotype and nucleotide diversity. Then, multiple DNA sequence variation values were determined, for example, the number of variable sites (S), the G + C content, and the haplotype/nucleotide diversity. The analysis using DnaSP was divided into three parts: a) analysis of all Kemp's ridley sequences by MEGA; b) grouping sequences by haplotype, then a comparative analysis between 2 groups (haplotype diversity values higher than 0); and c) haplotype grouping based on the location. Two main groups, one from the RNS (n = 97) sequences, and another with all sequences (n = 17) from outside Mexico (excluding the RNS) and only one sequence from BdT. Also, the number of segregating and parsimony-informative sites was determined usingPopART. The levels of haplotype variation for Kemp's ridley archived in BOLD were analyzed using an R logarithm called HACSim—Haplotype Accumulation Curve Simulator [48].

## 3. Results

### *3.1. Haplotype Diversity*

A total of 101 field specimens from different locations were analyzed and sequenced, 91 from the RNS and 10 from other field sites, including TEP, BdT, MIR, and ALT (Figure 1). COI gene size was 1547 bp [20].

Positions 1 and 604 of the analyzed sequences corresponded to positions 113 and 716 of the reference sequence (ID: MN136061). The haplotype network shows five different haplotypes, of which four have not been previously described. The different sequences analyzed were grouped by assigned haplotype based on their sequence. Likewise, those sequences were correlated with other features such as their ID, previous haplotype, BOLD ID, GenBank accession, locations and reference (Table S1).

The analysis of all sequences using PopART (Figure 2, Table 1) showed changes between the aligned sequences and their potential haplotypes. The nomenclature used during this study correlated to the one established by DNA barcodes for globally threatened marine turtles [26] and the Archie Carr Center for Sea Turtle Research (ACCSTR) of the University of Florida [26,49] with some modifications. For instance, the first letters for the genus and species remained the same, *Lepidochelys kempii* (Lk); however, the changing variable established by ACCSTR was the use of the nesting beach where the sample was obtained, i.e., Rancho Nuevo (RN) Sanctuary, instead of the COI gene. Finally, we listed the number of identified haplotypes (01, 02, etc.). The nomenclature proposed is summarized as Lk-RN-01, Lk-RN-02, etc.

Four out of five haplotypes were assigned to the RNS as those sequences originated from this location. One of them, haplotype LK-RN01, grouped sequences from five localities, RNS, MIR, ALT, TEP, and BdT, as they share the same haplotype, regardless of the beach they were nesting. The haplotypes LK-RN02 to LK-RN04 were non-redundant, including single sequences. Here, it is important to mention that the polymorphism of these single sequences was confirmed by sequence double-checking. These non-redundant haplotypes were specific to the RNS location (Figure 3; Table 2).

**Table 2.** The number of *Lepidochelys kempii* individuals for each haplotype found in each locality.

| HAPLOTYPES | Altamira MEX | Miramar MEX | Tepehuajes MEX | Barra del Tordo MEX | Rancho Nuevo MEX | South Padre USA | New York USA | East Coast USA | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| LK-RN01 | 5 | 3 | 2 | 2 | 85 | 0 | 0 | 0 | 97 |
| LK-COI-01 | 0 | 0 | 0 | 1 | 0 | 10 | 5 | 2 | 18 |
| LK-RN02 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| LK-RN03 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| LK-RN04 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| TOTAL | 5 | 3 | 2 | 3 | 88 | 10 | 5 | 2 | 118 |

Additionally, Table 2 shows the number of sequences analyzed in this study by location and correlated 100% with the number of field samples obtained. Previous COI genes retrieved from databases were grouped in the redundant LK-COI-01 haplotype previously reported [26]. Sequences belonging to this haplotype originated primarily from different USA locations, except for a Mexican sequence belonging to the BdT location.

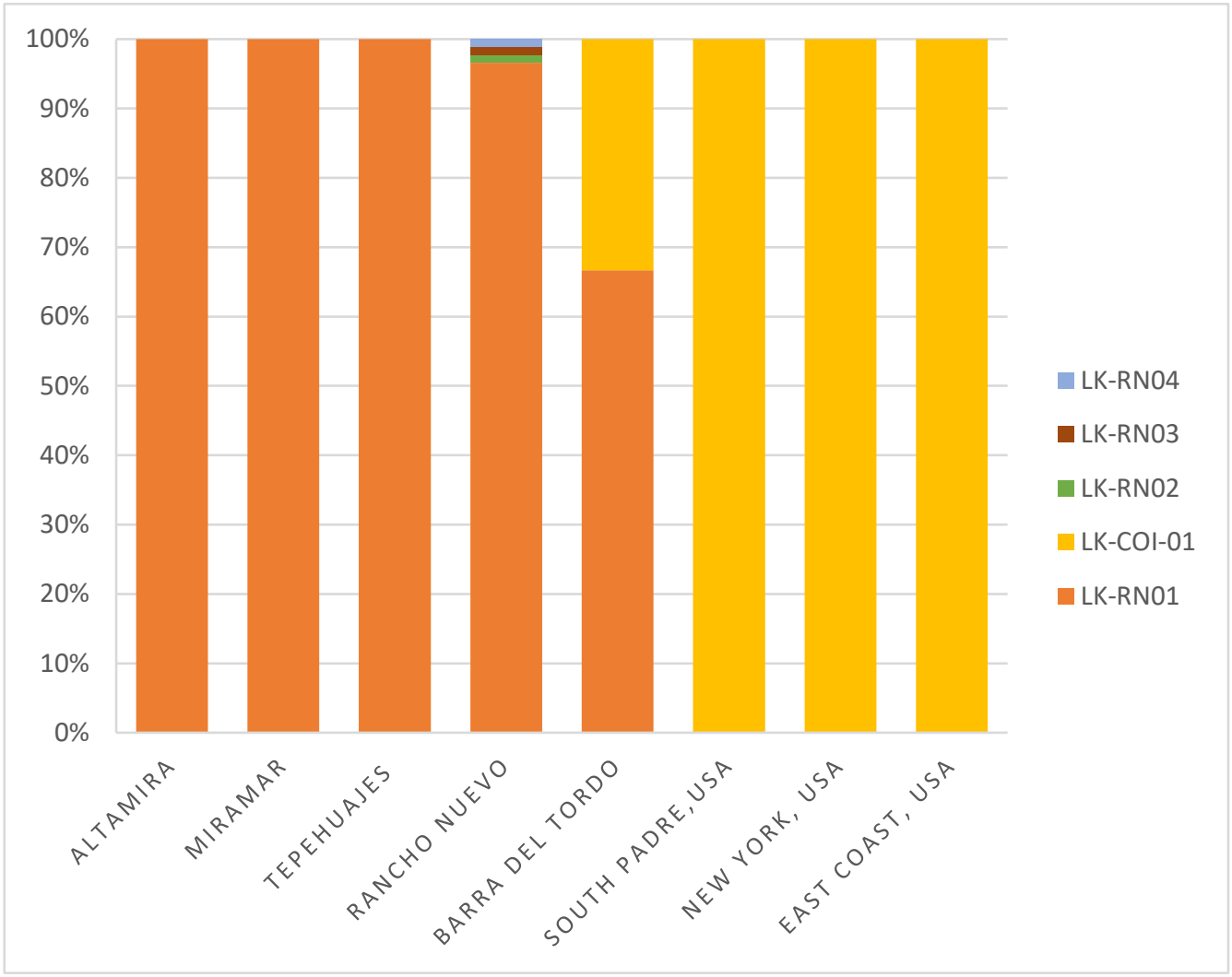


**Figure 3.** Distribution of the 5 *Lepidochelys kempii* haplotypes based on the mitochondrial COI gene.

### 3.2. Genetic Divergence

Genetic divergence values (K2P and *p*-distance) among the species are summarized in Table 3. Kemp's ridley sequence values (K2P) ranged between 2% to 9% within the same turtle's family and 12% when compared to leatherback turtles. Divergence values (*p*-distance) among sea turtles and a tortoise were 14% to 17% and 14% to 16%, respectively (Table 3).

In general, the population genetic parameters demonstrated a high G + C content (0.422) in all COI sequences. Haplotype diversity (H) was 0.303 ± 0.049, the nucleotide diversity (Pi) was 0.00052 ± 0.00009, and the mean value of nucleotide differences (k) was 0.31160. FST values were only determined among the main redundant haplotypes (LK-RN01 and LK-COI-01). An interspecific sample size for the haplotype accumulation curve was calculated (Figure 4) to determine genetic diversity with an initial sample size of N = 118 individuals; the initial estimate for sampling sufficiency was N = 18 individuals. The algorithm converged after eight iterations.

**Table 3.** Interspecific distance established between COI gene sequences among Kemp's ridley (*Lepidochelys kempii*) collected in Mexico and sequences described in BOLDSystem from other sea turtle species, 2010, 2018–2019. Mean pairwise divergences between species are below (K2P) and above (*p*-distance) the diagonal.

| Species | *Lk* | *Lo* | *Cc* | *CmP* | *CmA* | *Ei* | *Dc* | *Nd* | *Cs* |
|---|---|---|---|---|---|---|---|---|---|
| *Lepidochelys kempii (Lk)* | | 0.02 | 0.05 | 0.08 | 0.08 | 0.07 | 0.10 | 0.08 | 0.14 |
| *Lepidochelys olivacea (Lo)* | 0.02 | | 0.05 | 0.08 | 0.08 | 0.07 | 0.11 | 0.08 | 0.15 |
| *Caretta caretta (Cc)* | 0.05 | 0.06 | | 0.09 | 0.08 | 0.07 | 0.11 | 0.08 | 0.14 |
| *Chelonia mydas (CmP)* | 0.08 | 0.08 | 0.10 | | 0.01 | 0.08 | 0.12 | 0.06 | 0.14 |
| *Chelonia mydas (CmA)* | 0.09 | 0.08 | 0.09 | 0.01 | | 0.08 | 0.11 | 0.06 | 0.13 |
| *Eretmochelys imbricata (Ei)* | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 | | 0.10 | 0.08 | 0.13 |
| *Dermochelys coriacea (Dc)* | 0.12 | 0.12 | 0.13 | 0.13 | 0.13 | 0.11 | | 0.11 | 0.15 |
| *Natator depressus (Nd)* | 0.09 | 0.09 | 0.08 | 0.08 | 0.07 | 0.09 | 0.12 | | 0.13 |
| *Centrochelys sulcata (Cs)* | 0.16 | 0.17 | 0.16 | 0.16 | 0.15 | 0.15 | 0.17 | 0.14 | |

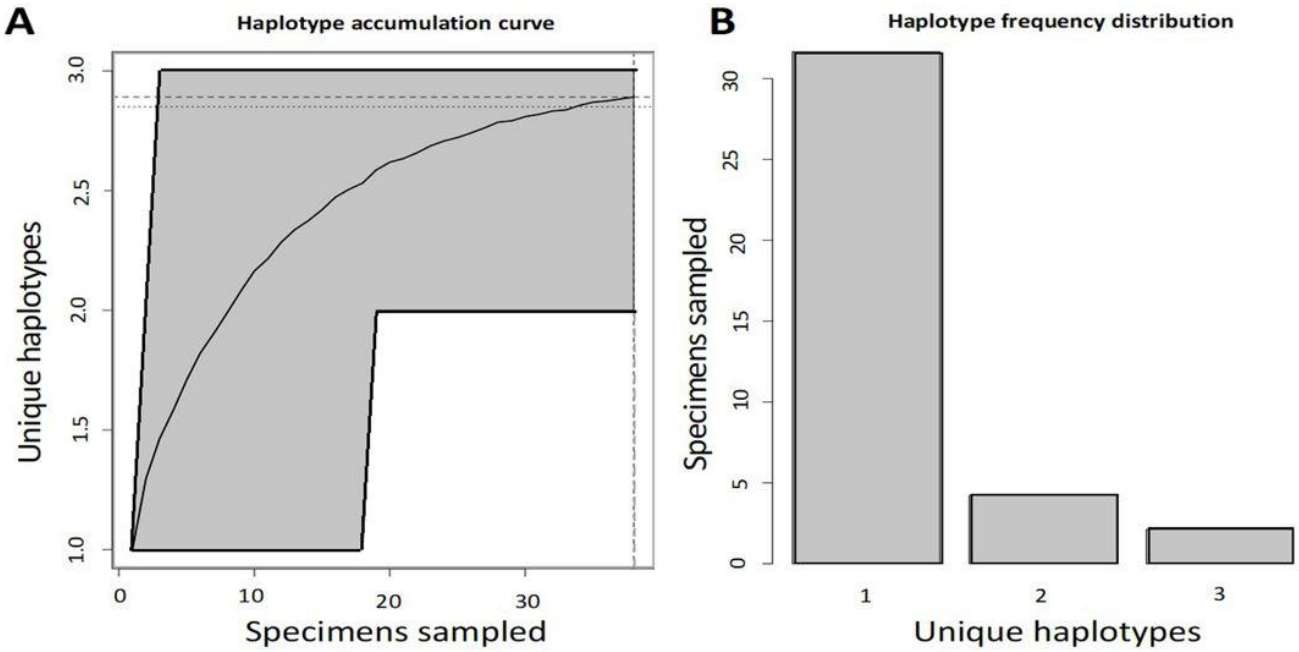


**Figure 4.** Haplotype accumulation curve. (**A**) Haplotype accumulation curve; (**B**) Haplotype frequency distribution. The data obtained were made using the HACSim function in the R package with 1000 permutations (Phillips et al., 2020).

The estimate of sampling sufficiency for $p$ = 95% haplotype recovery was N * = 38 individuals (95% CI: 36.6068–39.3932). The number of additional specimens required to be sampled for $p$ = 95% haplotype recovery was N * − N = 20 individuals.

Finally, a phylogenetic ML tree (Figure 5) with the wide nucleotide substitution model (TN + F + G4) highlighted the sequence divergence among species. All the Kemp's ridley sequences belonged to the main clade, whereas other sea turtle sequences exhibited different clades (distance > 0.7) and the tortoise sequence a distant (>5) specific clade.

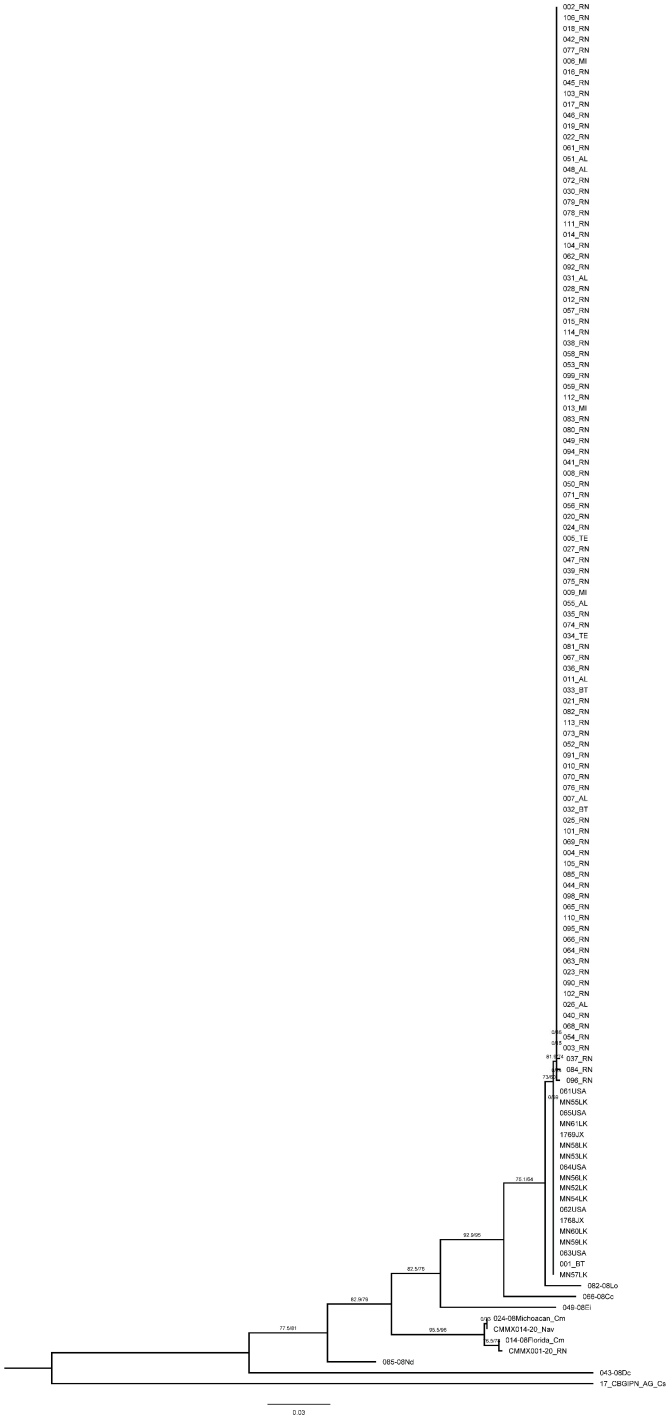


**Figure 5.** The maximum likelihood phylogenetic tree for Kemp's ridley representing the phylogenetic relationships for the 624 bp COI sequence with the marine turtle outer cluster.

## 4. Discussion

Molecular gene markers can be used to identify sea turtle species [22,23]. The COI gene can be used as a universal molecular marker [50] to generate Barcodes or can be used in divergence studies to determine population structure and gene flow in other animal species [19,24,26]. Specifically, in Kemp's ridley turtles, there are 7 BOLD records and a full mitochondrial genome (GenBank: MN136061.1) with assembled sequences from South Padre Island, TX [51].

Kemp's ridley turtles are considered native to the Gulf of Mexico, where essential feeding, growing, and nesting sites are located, demonstrating high fidelity (82%) to this

region, specifically in the RNS location [52,53]. In the current study, 101 COI DNA barcodes mainly from nesting females in the RNS were added to the BOLD system. This contribution has increased the usefulness of this gene marker in the complementary analysis of previous data [22,26] to strengthen molecular studies and provide a better understanding of genetic divergence. Considering the redundant haplotypes, the LK-RN01 haplotype was highly represented and is distributed in specimens from all Tamaulipas locations. In contrast, the LK-COI-01 haplotype was limited to specimens from SPI and New York [26,51] and a BT specimen, indicating differences among nesting beaches far from the primary nesting beach in the RNS. Three new non-redundant haplotypes are described for the RNS in addition to the redundant LK-RN01 haplotype.

Sequences analyzed using DnaSP software [46], $F_{ST}$ [47], and nucleotide diversity demonstrated a polymorphic region compared to the reference collected in SPI [51] with 4 segregating sites and 1 parsimony-informative site. In general, the COI gene tends to be a more conserved sequence showing low intraspecific divergence and a low evolutionary rate, regardless of the multiple sequences provided [26]. Still, in the current study, the number of polymorphic sites is relatively low. This outcome may be a consequence of the limited distance and the kinship between samples for one population and among specimens since the RNS is near other nesting beaches in Tamaulipas, Mexico.

The dispersion of pelagic juvenile Kemp's ridley turtles has been observed in northeast Florida, the Mississippi river basin, SPI, Terminos Lagoon in Campeche, and sporadically in the Yucatan peninsula [54]. Recently, rare events were documented in the Atlantic Ocean and Spanish Mediterranean waters [8,55]. Strandings of Kemp's ridleys linked to cold-stunning events have been frequently documented in Cape Cod, Massachusetts [56]. Finally, due to the dramatic population decline of Kemp's ridleys after 1947 [9], a Mexico-USA program was vigorously established to protect the nesting population [57]. A massive number of eggs were transplanted to SPI [10], followed by a population recovery from 700 to approximately 13,000 nesting females per year by 2020 [58,59]. The artificial or natural occurrence of other nesting sites, even with few specimens, might contribute to the occurrence of two redundant haplotypes suggesting the establishment of two apparent Kemp's ridley turtle populations. Nevertheless, it is important to emphasize that the nucleotide differences remain very low (0.31160) compared to other sea turtle species [26]. In fact, during this study, interspecific divergence was identified between the two turtle families, and the divergence values split all six species of the Cheloniidae family.

Genetic species divergence is supported by the ML tree, where there are clear separations among the primary species clades. All Kemp's ridley sequences are grouped within a clade with two slightly separated subclades, corresponding to the redundant haplotypes. COI haplotype variability is reduced compared with wide haplotype variability identified in the CR region [51]. In fact, haplotype curve accumulation shows that asymptotic curves reached saturation, suggesting that new individuals sampled would not contribute to newly discovered haplotypes. In addition, the $F_{ST}$ value between the subclades was high (~1), suggesting low levels of interbreeding between these populations.

COI gene analysis allows the identification of new haplotypes and the early establishment of a new Kemp's ridley population outside of the RNS. However, the low number of haplotypes, the low nucleotide difference, and the low number of polymorphic sites in this mitochondrial marker demonstrates the need to combine their use with other mitochondrial genes such as CR (the D-loop region) for the nuclear microsatellites to support any further genetic analysis [18,60]. As previously mentioned, there are other molecular markers with potential use in population genetics in various species, but not as DNA barcode markers. COI was successfully used in freshwater turtles as a DNA barcode and genetic divergence [61], although for various species of sea turtles, it was not very informative [22,23,26]. However, it is worth mentioning that for the Kemp's ridley study, there is no robust study (limited number of sequences or works in this species of sea turtles) using the COI as a barcode marker in nesting females in any main camp or even in the RNS that shows its usefulness nor which shows the opposite. This work tries for the first

time to establish the barcode to confirm the usefulness of the COI for the study of genetic divergence within and between sea turtle species, and between Kemp's ridley and tortoise. For example, COI haplotype diversity showed approximately half (0.303) of the CR value obtained in other Kemp's ridley studies [51,62]; therefore, a larger research effort is needed to increase the CR database in the female nesting population of the RNS. Nevertheless, the COI gene was useful for identifying separate Kemp's ridley populations.

## 5. Conclusions

The 101 sequences of nesting Kemp's ridleys in the RNS substantially enriched the BOLD database. Two redundant COI haplotypes (LK-RN01 and LK-COI-01) were identified. The first corresponds exclusively to the RNS specimens and the second to specimens of other locations. FST values (~1) between these groups suggest low levels of interbreeding between these locations. Nucleotide variation and informative sites were low in COI sequences. Therefore, COI genetic analysis in combination with other mitochondrial or nuclear gene markers is widely desirable. Finally, the COI gene as a DNA barcode is highly useful for identification and discrimination studies of sea turtle populations.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14050390/s1, Table S1: Relationship of COI sequences for haplotypes, ID, previous haplotypes, BOLD ID, GenBank accession, and countries for Kemp's ridley (*Lepidochelys kempii*) in Mexico, 2018–2019 and other previously published sequences.

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**Institutional Review Board Statement:** The research meets all applicable standards and Mexican laws regarding the ethics of experimentation and research integrity, and the following is being certified/declared true. All turtle specimens were managed following laws and regulations of Mexican authorities under permits issued by SEMARNAT: SGPA/DGVS/04674/10 and SGPA/DGVS/003769/18.

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