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Using a COI mini-barcode and real-time PCR (qPCR) for sea turtle identification in processed food

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Abstract

The worldwide regulations protect sea turtles, nevertheless, the illicit consumption of their by-products keeps them as endangered species. In Mexico, its meat is used illegally to prepare food and in recent years this has caused a huge problem for control agencies. Analytical methods used for species detection in food are affected due to changes in the protein structure or degradation of DNA after heat treatment. The present work aimed to use a mini-barcode based on the COI gene (MBCOI) and real-time polymerase chain reaction (qPCR) for sea turtle identification in processed foods. The MBCOI-qPCR showed high specificity without any cross-reactions, the limit of detection was 1 pg using processed turtle DNA with a PCR efficiency of 101.9%. This study demonstrated the ability to estimate up to 1% of processed sea turtles, which could be considered as a tool to stop the illegal trade and consumption of sea turtles.

Novelty impact statement: Sea turtles are endangered species however their meat is consumed illegally in Mexico in a dish known as sea turtle soup. This study presents the development of a novel method combining a mini-barcode and real-time polymerase chain reaction capable to detect and estimate sea turtles in processed foods. Our results suggest that the method described herein could be an important tool to detect those involved in the illegal consumption and protect sea turtle species.

1 | INTRODUCTION

The illicit commercialization of sea turtle products has been a determining factor in the decline of their populations (Hancock et al., 2017). For this reason, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (https://cites.org/eng) currently lists all sea turtles in its Appendix I. Additionally, different organizations developed conservation programs in which they are looking to mitigate the impact caused by the exploitation of these chelonians (Gaona & Barragán, 2016). However, despite the efforts of these organizations the obtaining of sea turtle by-products such as meat, eggs, and oil is still carried out and derived from these acts. Currently, six species are on the red list of the International Union for the Conservation of Nature (IUCN) (https://www.iucn.org/).

Particularly México hosts the second greatest number of sea turtle nesting sites globally with a total of 205 sites of which only 32% are protected (Mazaris et al., 2014). Therefore, several government efforts have been carried out in the last few years to improve the protection of sea turtle species (PROFEPA, 2019). Even so illegally commercialized meat is used mainly in coastal areas to cook a dish known as "Sea turtle soup" or "Caguama", which is prepared by prolonged cooking of the meat adding various ingredients and combining it with other marine species (Mancini & Koch, 2009). Therefore,

TABLE 1 Composition of the model systems for the simulation of the sea turtle soup

Model system	Meat composition	Other ingredients
100%	500 g - sea turtle 0 g - black ray, white shrimp, and black bass	200 g of tomato, 150 g of onions, 100 g of celery, salt, pepper, 30 g of margarine, and 500 ml of canola oil
50%	250 g - sea turtle 250 g - black ray, white shrimp, and black bass	
12%	60 g - sea turtle 440 g - black ray, white shrimp, and black bass	
10%	50 g - sea turtle 450 g - black ray, white shrimp, and black bass	
1%	5 g - sea turtle 495 g - black ray, white shrimp, and black bass	

the corresponding authorities constantly execute surveillance operations to detect those involved in the trade and consumption of sea turtles, for this generally techniques based on morphological characters are used. However, after sea turtle soup preparation morphological characters are not usable since this species cannot be discerned (Rak et al., 2014; Vasconcellos et al., 2018),

For years, different methods have been developed for species identification in food applying analytical techniques such as electrophoretic, immunological, or chromatographic (Boyaci et al., 2014; Hong et al., 2017). These methods generally use lipids or proteins as target analytes reducing their capacity for the analysis of processed foods due to the low stability of these molecules at high-temperature conditions (Hong et al., 2017). On the other hand, DNA has been widely used for the identification of species mainly due to its stability to different handling and processing conditions, resulting in an excellent alternative to overcome the limitations during the analysis of processed foods (Amaral et al., 2017; Meira et al., 2017).

Regarding DNA-based methods, the recent barcode methodology applied to the study of mitochondrial genes such as cytochrome b, 16S rRNA, and cytochrome c oxidase subunit I (COI) has shown great potential for species identification (Shi et al., 2020; Vasconcellos et al., 2018). In recent years, the COI gene has been designated as the consensus region used as a barcode for animal species identification (Hebert et al., 2003; Hellberg et al., 2017), in the specific case of sea turtles, the barcode of 815 bp is characterized by a high resolution in the identification of its species (Naro-Maciel et al., 2010).

However, DNA barcoding for species identification could present certain disadvantages because it requires sequencing of relatively large PCR amplicons, thus being of limited application to processed samples due to DNA fragmentation (Wozney & Wilson, 2012). As an alternative, several authors have reported the use of regions between 100 and 300 bp called mini-barcodes coupled with qPCR for the identification of meat (Sakadiris et al., 2013; Wu et al., 2019) and seafood (Fernandes et al., 2018; Isaacs & Hellberg, 2020; Shi et al., 2020) in processed products. These assays report high specificity, sensitivity, allowing species detection even in very low content levels. On the other hand, some studies based on the same principle of mini-barcode and qPCR have been used to identify and discriminate exotic or endangered species in various types of samples (Buddhachat et al., 2021; Cardeñosa et al., 2017; Kitpipit et al., 2016).

Despite the above, to this date, the mini-barcode in combination with qPCR has not been focused to identify species in danger of extension that are consumed for different reasons such as the sea turtle. Therefore, this study aimed at using a mini-barcode based on the COI gene (MBCOI) and qPCR to identify sea turtles in processed foods to provide tools that reinforce the protection and surveillance systems of these species.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Sea turtle meat sample (*Chelonia mydas*) was collected in coordination with the federal attorney for environmental protection (PROFEPA) and following the guidelines indicated in the Official Letter No. SGPA/DGVS/05709/17. For the sea turtle species *Lepidochelys kempi*, *Lepidochelys olivacea*, *Eretmochelys imbricata*, and *Dermochelys coriacea*, DNA was donated by the Genomic Biotechnology Center of the National Polytechnic Institute. Meat samples from the species of black manta ray (*Dasyatis violacea*), black bass (*Micropterus salmoides*), and white shrimp (*Litopenaeus vannamei*) were obtained in the local market. All samples were kept at −20 °C until further analysis.

2.2 | Preparation of simulated sea turtle soup

Model systems were made to simulate the preparation conditions of the dish known in Mexico as "sea turtle soup". Sea turtle meat was mixed in different proportions 100%, 50%, 12%, 10%, and 1% (w/w) with black manta rays, white shrimp, and black bass (Table 1). The model systems were submitted to a temperature of 120 °C for 1 hr at 1.1 kg cm^{-2} (units of pressure) in a pressure cooker, subsequently, the meat was minced and placed in another container with 500 ml of canola oil, 30 g of margarine, 200 g of tomatoes, 150 g of onions, and 100 g of celery and fried at 100 °C for 10 min. Finally, the mixture was transferred to a pressure cooker adding seasonings (salt

and pepper) for second equal thermal processing for 30 min. After preparation, the soup model systems were stored in hermetic recipients at −20 °C until analysis.

2.3 | DNA extraction

For DNA extraction 100 g of sample was taken to carry out a homogenization by mincing the sample with a sterile knife to reduce the particle size. After that 100 mg of the homogenate was placed in a 1.5 ml microcentrifuge tube to perform the extraction using chloroform: isoamyl alcohol method with some modification as described by Green and Sambrook (2012). The sample was incubated at 65 °C for 30 min in 400 µl of buffer (25 mM EDTA, 100 mM NaCl, 1% SDS in 10 mM Tris-HCl), with 10 µl of proteinase K (20 mg ml^{-1}) (Invitrogen). Subsequently 400 µl of chloroform:isoamyl alcohol (24:1) (Sigma-Aldrich) was added and centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to a new 1.5 ml microcentrifuge tube, two washes were carried out at 13,000 rpm for 5 min with 400 µl of isopropanol and 400 µl of cold 70% ethanol (Merck), the supernatant was decanted, and the pellet was dried for 1 min. Finally, the extractions were resuspended in 50 µl of Milli-Q sterile water and stores at −20 °C for further analysis.

2.4 | DNA quality and concentration

DNA was quantified using AccuBlue Broad Range dsDNA Quantitation Kit with DNA Standards (Biotium), following the manufacturer's protocol. Purity was assessed by UV Multiskan Go spectrophotometer (Thermo Fisher Scientific) using 2 µl of nuclease-free water as a blank and 2 µl of each DNA sample and applying the ratio 260/280.

2.5 | Identification of conserved regions

Barcode sequences ($n = 25$) of seven species of sea turtle, eight species of reptiles, and ten marine species of edible animal origin were retrieved from the Barcode of life data system (BOLD), these sequences were aligned using the program Mega 7 to locate more conserved regions for sea turtle species within COI barcode fragment.

2.6 | Primer design and in silico analysis of DNA mini-barcode

The conserved regions of the sea turtle barcode were used for primer design TortMaFw (CATCATCAGGAATTGAAGC) and TortMaRv (GGTGTTTGGTATTGTGATA) to target a short DNA fragment (MBCOI) ~205 bp. During primer design parameters such as dissociation temperature (Tm), guanine-cytosine percentage (GC%), and formation of secondary structures were reviewed using the Primer 3 Plus program (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Once the primers were selected, they were synthesized by IDT (Integrated DNA Technologies, Inc., Iowa).

To demonstrate the identity of the sequences of the TortMaFw and TortMaRv primers in the hybridization sites of the COI gene, these sequences were queried using the Basic local alignment search tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) available in the National Center for Biotechnology Information (NCBI). Also, an in silico PCR was performed using the primers designed using the web tool Sequence Manipulation Suite: (http://www.bioinformatics.org/sms2/pcr_products.html).

2.7 | PCR amplification

The PCR assays were carried out in a total reaction volume of 12.5 µl, containing 6.25 µl (1×) of the GoTaq G2 colorless master mix kit (Promega, USA), 0.625 µl (250 nM) of primers (TortMa), 5 ng of DNA (1 µl), and 4,625 µl of sterile MiliiQ grade water. The reactions were performed under the following thermal conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 6 min. The amplified PCR products were observed on 2% agarose gel electrophoresis at 90V-250A for 50 min and capture using the MiniBIS Pro system and GelCapture Software.

2.8 | qPCR amplification

The qPCR reactions were carried out in a StepOne Real-Time PCR System (Applied Biosystems), in a volume of 20 µl containing 10 µl (1×) of Fast Plus Eva Green Master Mix with High ROX (Biotium, USA), 2 µl (500 nM) of TortMaFw and TortMaRv primers, 7 µl of sterile MilliQ grade water, and 5 ng (1 µl) of gDNA. The amplification conditions were initial denaturation 95 °C for 2 min, 40 cycles at 95 °C for 10 s, 54 °C for 15 s (fluorescence uptake), 72 °C for 30 s, with a final extension at 72 °C for 2 min. For melting curve analysis PCR products were denatured from 54 to 95 °C with temperature increments of 0.3 °C and the fluorescence measurements were acquired by the end of each melting temperature. The data and graphs obtained were analyzed with StepOne Software v 2.2. The minimum criteria for quantitative experiments by real-time PCR mentioned in the guidelines of Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) (Bustin et al., 2009) were carefully considered during the development of this work.

The specificity of the assay was determined by analyzing DNA corresponding to non-target species (black manta ray, white shrimp, and black bass) and DNA from sea turtle species, considering the qPCR conditions mentioned above. On the other hand, the amplification efficiency, and the limit of detection (LOD) of the MBCOI-qPCR were performed by analysis of 10-fold serial dilutions of DNA from the 100% model system ranging from 10 ng μl^{-1} to 0.1 pg μl^{-1} each dilution was analyzed in triplicate. The amplification efficiency (E) was calculated by the slope of the curve using

the equation: $E = [10 (-1/\text{slope}) - 1] \times 100$. The LOD was calculated by the lowest concentration of the analyte in a sample which can be detected with a level of confidence of 95% (Bustin et al., 2009).

2.9 | Construction of curve for sea turtle estimation in food

The model systems (100%, 50%, 10%, and 1%) were used as standards for the construction of the sea turtle estimation curve. The DNA was isolated from these model systems according to the method described above and normalized to 5 ng μl^{-1}. The standards were assayed in triplicate by qPCR and a calibration curve was generated plotting the value of Cq versus the logarithm of the sea turtle content in the model system, to obtain a linear prediction equation ($y = mx + b$), the efficiency value, and the coefficient of determination (R^2). The limit of quantification (LOQ) can be lower or equal to the lowest amount or concentration included in the dynamic range of the assay (ENGL, 2015). The standard curve was constructed, and the prediction equation was used to estimate the sea turtle in a blind sample of turtle soup containing 12% (w/w), the analysis determined the trueness expressed in terms of bias using the formula: (mean value-real value)/real value × 100. Where the mean value is determined experimentally, and the real value corresponds to the known concentration or percentage.

3 | RESULTS

3.1 | DNA purity and in silico analysis

The protocol used for DNA extraction in samples of model systems evaluated during this study had a yield in the range of 38.5–46.8 ng μl^{-1} with purity values (260/280 nm) oscillating from 1.78 to 1.85. On the other hand, in the in silico analysis, the sequences of the TortMaFw/TortMaRv primers were queried in the NCBI database using the BLAST algorithm, where 100% identity was obtained regarding the hybridization sites in the barcodes sea turtles. In Figure 1, the complete sequences corresponding to the MBCOI are shown, which were obtained by in silico PCR using Sequence Manipulation Suite, this confirmed the specificity of the designed primers for the molecular identification of sea turtle species and theoretically discarded the cross-reactivity with the DNA of other non-specific marine species.

3.2 | Sea turtle identification by PCR

During the PCR assays, the ability of the TortMaFw/TortMaRv primers to amplify the sea turtle MBCOI was evaluated, Figure 2a shows an amplicon of ~205 bp for all species of a sea turtle. Additionally, in all the extractions of the model systems, the amplification of the MBCOI was also achieved (Figure 2b), the above confirms that all the extractions tested (raw or cooked) contain DNA that can detect the species of interest.

3.3 | Specificity, efficiency, and sensitivity of qPCR

The specificity evaluated by qPCR demonstrated no cross-reactivity because the non-target species (black manta ray, white shrimp, and black bass) did not produce a Cq value after the 40 qPCR reaction cycles also this assay showed amplification for the five species of sea turtle which had an average Cq value of 21.5, with different dissociation curves (Tm) for each MBCOI. Figure 3 shows the average values of Tm for the species of *C. mydas* (82.57 ± 0.02), *D. coriacea*

FIGURE 1 MBCOI sequences (~205 bp) were obtained by in silico PCR using the TortMaFw and TortMaRv primers

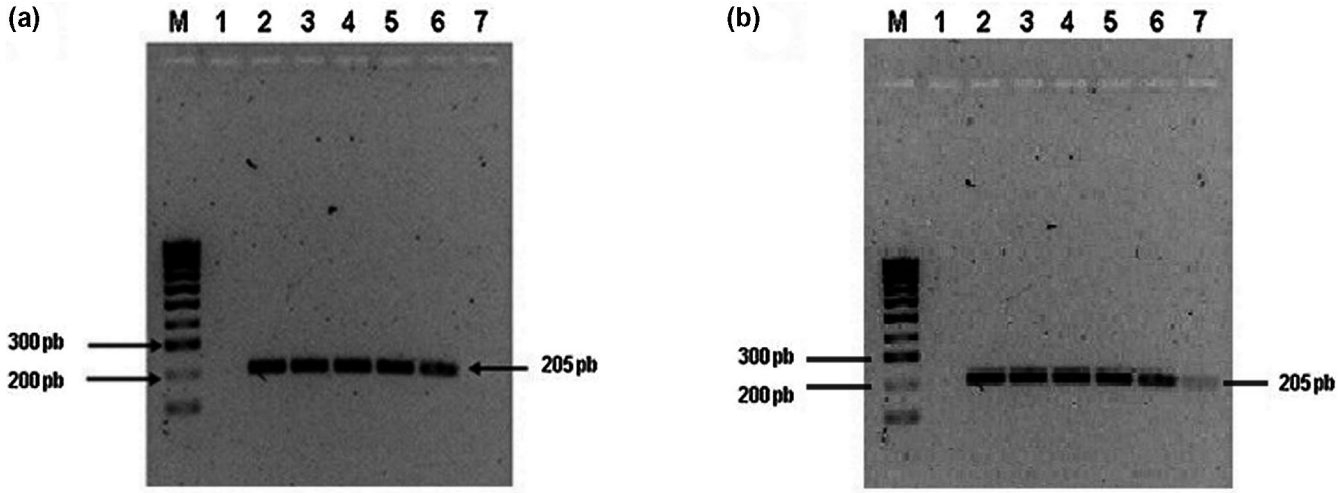

FIGURE 2 PCR assays using the primers TortMaFw and TortMaRv. (a) MBCOI (~205 bp) of sea turtle species in 2% agarose gel. M = molecular marker (100 bp DNA Ladder), line 1: Negative control, line 2: *Chelonia mydas*, line 3: *Dermochelys coricea*, line 4: *Lepidochelys kempii*, line 5: *Eretmochelys imbricata*, line 6: *Lepidochelys olivacea*, line 7: Control negative. (b) MBCOI (~205 bp) of different sea turtle soup model systems in 2% agarose gel. M = molecular marker (100 bp DNA Ladder), line 1: negative control, line 2: positive control (*C. mydas* DNA), line 3: 100%, line 4: 50%, line 5: 12%, line 6: 10%, line 7: 1%

(80.50 ± 0.04), *L. kempii* (81.98 ± 0.01), *E. imbricata* (81.68 ± 0.09), and *L. olivacea* (81.68 ± 0.02).

Figure 4b shows a calibration curve with PCR parameters in terms of slope (−3.277), PCR efficiency (101.9%), and R^2 value (.995). This calibration curve presented a linear dynamic range (R^2 = .995) in five orders of magnitude, hence the LOD reached was 1 pg where all the replicates were amplified with a precision expressed as coefficient of variation percentage (CV%) under 1.04% for each point in the dynamic range. On the contrary, 0.1 pg dilution did not produce a detectable fluorescence signal during the 40 cycles.

3.4 | Sea turtle estimation in food

The standard sea turtle estimation curve (Figure 5b) in the established dynamic range presented an R^2 = .993, a slope = −3.164, and PCR efficiency = 107.05% within four orders of magnitude. The method showed CV% values ≤ 0.53% expressed as precision. The LOQ was established at the concentration of 1% sea turtle due to this was the lowest percentage included in the dynamic range of the mixtures of the model systems. The 12% (w/w) blind sample of sea turtle soup resulted in an estimated average concentration value of 9.64% (w/w) for this blind test the calculated bias was 19.66% and considering the replicates (3) we obtained a CV% of 0.12%.

4 | DISCUSSION

Determining the concentration and purity of DNA extracts from foods and processed products is particularly important before PCR tests, due to the large number of inhibitors that different types of samples can present by nature. In the particular case of the simulated sea turtle soup analyzed in this work, the high content of proteins, lipids, seasonings in addition to the type of heat treatment in this kind of dishes could result in a contaminated DNA extract with subsequent inhibition of the PCR reaction (Lo & Shaw, 2018; Sultana et al., 2018). For this reason, when an extraction method is inefficient, molecular techniques may be limited due it would not be possible to achieve the amplification of the target DNA (Van Frankenhuyzen et al., 2011). In this study, the performance of the extraction method used was acceptable in terms of yield (38.5–46.8 ng μl^{-1}) and purity (1.78–1.85), since the amplification range for the sea turtle species was 100% with a ~205 bp MBCOI (Figure 2a), and an equal performance for the DNA extracted from the model systems (Figure 2b), allowing to discard the presence of inhibitors and the degradation of the evaluated DNA.

In this sense, it is important to point out that in this work a mitochondrial gene was used (COI), which is present in multiple copies for each cell, which increases the amplification range and sensitivity even when degraded and processed samples are tested that normally contain relatively low amounts of amplifiable DNA (Rojas et al., 2011). Besides, several authors agree that the use of short fragments (<300 bp) such as mini-barcodes contributes significantly to enhancing the amplification range, especially in highly processed sample processing (Cardeñosa et al., 2017; Motalib et al., 2019).

The MBCOI-qPCR method was selective because demonstrated no cross-reactivity with the marine species (black manta ray, white shrimp, and black bass) tested in the different model systems evaluated as mentioned in the results section, confirming the suitability for the specific detection of sea turtle species. This was demonstrated by the different dissociation curves corresponding to the MBCOI which presented Tm values >80 °C for all species of sea turtle (Figure 3). The variations in the Tm allowed obtaining a specific product for each species: *C. mydas* (82.57 ± 0.02°C), *D. coriacea* (80.50 ± 0.04 °C), and *L. kempii*

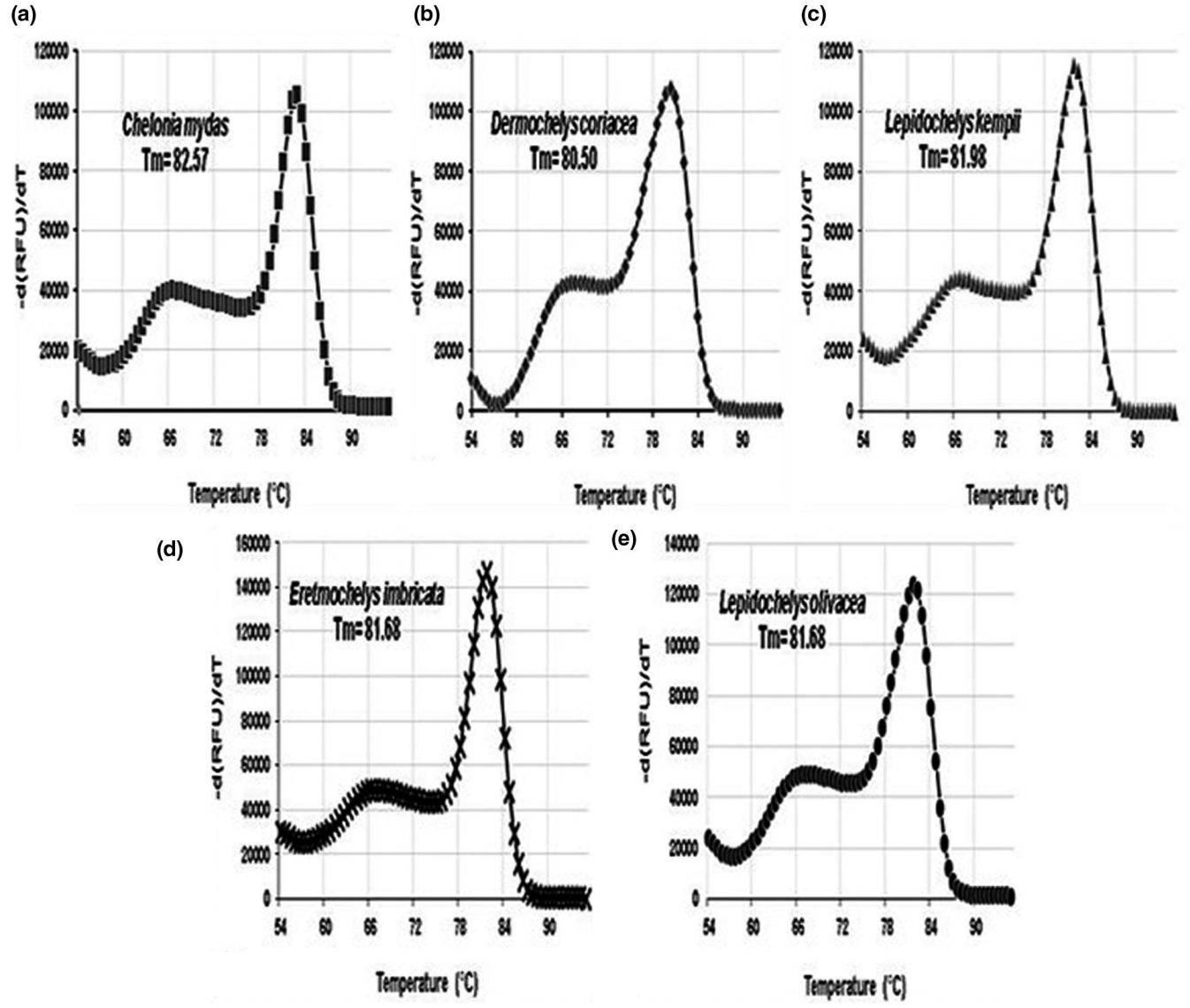

FIGURE 3 Dissociation curves obtained by MBCOI-qPCR for sea turtle species. (a) *Chelonia mydas* Tm = 82.57, (b) *Dermochelys coricea* Tm = 80.50, (c) *Lepidochelys kempii* Tm = 81.98, (d) *Eretmochelys imbricata* Tm = 81.68 and (e) *Lepidochelys olivacea* Tm = 81.68

(81.98 ± 0.01 °C) however, in the case of *E. imbricata* (81.68 ± 0.09 °C) and *L. olivacea* (81.68 ± 0.02 °C), there were no differences in the Tm values obtained despite the differences in its sequences as shown in the in silico analysis (Figure 1). This limitation of the method could be solved with the availability of tools to perform a high-resolution melt curve analysis (HRM) which has been used in some studies for the discrimination of closely related species or those with minimal differences in the sequences (Chen et al., 2019; Fernandes et al., 2018; Filipiak & Hasiów-Jaroszewska, 2016).

In qPCR assays, the efficiency is a very important parameter to consider because determines the number of products generated during each reaction cycle, accordingly, is recommended to have an efficiency value oscillating 90% and 110% (Bustin et al., 2009). Several reports using qPCR in processed foods have mentioned that the complex nature of the samples complicates reaching acceptable levels of efficiency, mainly due to a high degree of processing, the presence of inhibitors, and/or a poor optimization of the reaction (Camma et al., 2012; Kubista et al., 2006; Soares et al., 2013). Nevertheless, in this study using 10-fold serial dilutions of a heat-treated 100% model system, the efficiency (101%) showed high performance hence it could be concluded that the heat applying during the processing of sea turtle soup does not affect the response of the different DNA concentrations in the MBCOI-qPCR assay developed.

The same as qPCR efficiency, some studies have reported a reduced detection capacity of genes of interest in qPCR methods due to a strong heat treatment of the samples which reduces the fragment length and leads to higher LOD (López-Andreo et al., 2012; Meira et al., 2017). Following the MIQE guidelines, the concentration of 1 pg was established as LOD because all the replicates showed amplification resulting in a confidence level above 95%. The LOD obtained in the present work was quite satisfactory because the sensitivity was similar or higher than other studies that have reported LOD

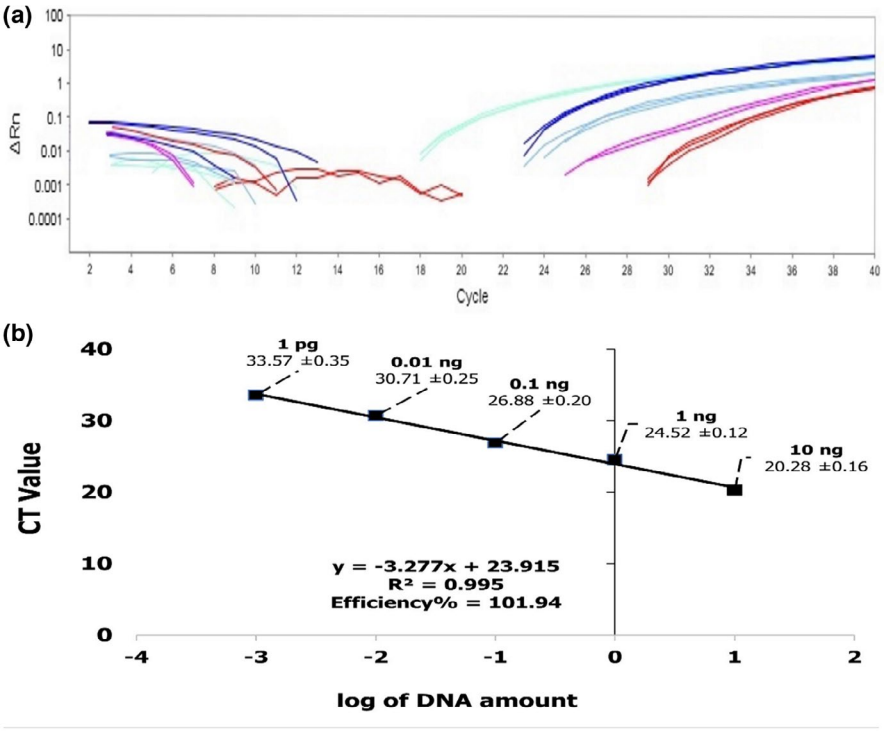

FIGURE 4 MBCOI qPCR assay using serial dilutions of 100% model system DNA (10 ng μl^{-1} to 1 pg μl^{-1}). (a) Amplification plots of qPCR. (b) Calibration curve ($n = 3$)

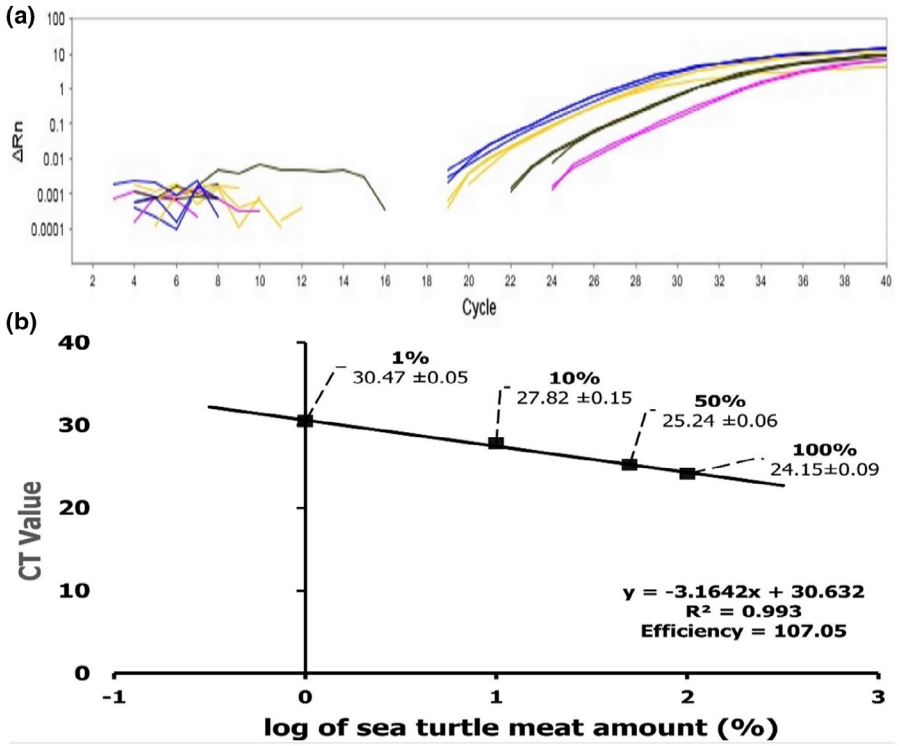

FIGURE 5 MBCOI qPCR assay using model systems in different percentages (100%, 50%, 10%, and 1%). (a) Amplification plots of qPCR. (b) Calibration curve for estimation of sea turtle ($n = 3$)

ranging 5–10 pg in heat-treated food samples (Liao et al., 2017; Meira et al., 2017; Soares et al., 2013). Also, the method can ensure a good precision in the detection of sea turtles due to the CV% value below 1.04% in the standard curve. Therefore, the method described is considered with a high potential for detection of the illegal consumption of sea turtle species owing to the high sensitivity and precision.

On the other hand, the estimation of target species in a food matrix is affected by the mixture of different species and ingredients, since the correlation of DNA and the content of the species is generally not consistent (Lo & Shaw, 2018). The use of standards or model systems with known proportions and high similarity to the sample matrix can reduce these limitations and provide reliability in a quantification assay (Eugster et al., 2008). In order to simulate the possible effects of cooking procedures as well as the presence of non-target marine species in sea turtle soup illegally consumed, the estimation curve used four calibrators based on the model systems proportions of 100%, 50%, 10%, 1% (Figure 5b). MIQE guidelines mention the dynamic range in a qPCR assay ideally should cover five or six concentrations, however, when using a highly complex or processing template to generating the calibrations curves such as model systems in this work the dynamic range could cover down to three orders of magnitude being sufficient for estimation of the target species (Bustin et al., 2009).

The estimation curve values of slope (−3.164), efficiency (107.05%) and coefficient R^2 (0.993) showed a linear response and complied with the criteria for quantitative qPCR assays (Bustin et al., 2009). The CV% ≤ 0.53% over the dynamic range was ≤25% showing a good precision (ENGL, 2015). Based on the above, 1% LOQ established using the linear dynamic range can be considered as a reliable value allowing an estimation of sea turtles in processed foods even if this is present in low content. Other works have used similar calibrators as in our study to construct calibration curves and estimate proportions of several meat species in foods, they report LOQ values for target species ranging from 1% to 0.01% (w/w) which is similar to the LOQ (1%) reported in our study (Amaral et al., 2017; Kang & Tanaka, 2018).

The determination of the trueness demonstrates the proximity of the mean value obtained and an accepted reference value, this measure is generally expressed in terms of bias. The bias value calculated for the 12% blind sample was −19.66% which is within the criteria of ±25% of the real value over the tested dynamic range (ENGL, 2015). Additionally, the coefficient variation of 0.12% indicates the good precision of the method and is also according to the criteria of the European Network of GMO Laboratories. Therefore, it can be inferred that the method is capable to indicate good proximity between the real value and the estimated value which could be especially important to provide information of the content of sea turtle in illegal food. The Mexican laws punish with up to nine years in prison to those involved in the commercialization and consumption of sea turtles or any of their by-products, hence the trueness in estimation capacity of the MBCOI-qPCR method could result in a value element to assign criminal punishment.

5 | CONCLUSION

In the present work, the MBCOI-qPCR method was able to specifically detect and estimate the content of sea turtles in processed food, demonstrating that the developed tool could be an alternative for regulatory authorities and control laboratories focused on actions to stop the illegal trade and consumption of sea turtles.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Jesus Daniel Villanueva-Zayas: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Writing-original draft; Writing-review & editing. **Roberto Rodríguez Ramírez:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Writing-original draft; Writing-review & editing. **Luz Angelica Ávila-Villa:** Conceptualization; Investigation; Methodology; Resources; Supervision; Writing-review & editing. **Aarón F. González-Córdova:** Conceptualization; Investigation; Software. **Miguel Angel Reyes-López:** Resources; Software; Visualization. **Daniel Hernández-Sierra:** Formal analysis; Methodology; Visualization. **Sergio De los Santos-Villalobos:** Methodology; Resources; Writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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