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Presence of chelonid herpesvirus 5 (ChHV5) in sea turtles in northern Sinaloa, Mexico

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ABSTRACT: The presence of fibropapilloma and its associated chelonid herpesvirus 5 (ChHV5) was assessed in 82 wild sea turtles. Olive ridley turtles *Lepidochelys olivacea* (n = 58) were caught in the pelagic Area of Marine Influence (AMI) (off the coast of Guasave, Sinaloa), and black turtles *Chelonia mydas agassizii* (n = 24) were captured in the Navachiste Lagoon System. The apparent physical condition was evaluated as 'good' or 'poor' by physical examination. The population structure and general health status was determined by condition index, hematocrit and total plasma protein. Detection of ChHV5 from skin samples was done by PCR. The overall physical condition of black turtles was good and all the individuals were tumor-free. Likewise, the physical condition of most olive ridley turtles was good, except for 10 individuals with poor condition. Of these, 4 had fibropapilloma-like tumors. PCR analyses showed that 3 tumors were ChHV5-positive. The DNA sequence showed 96% identity with ChHV5. All other skin samples from black or olive ridley turtles were ChHV5-negative. This is the first report of fibropapillomatosis–ChHV5 in foraging grounds off northern Sinaloa. The virus was present in a small proportion of *L. olivacea* individuals, a free-ranging species. It is suggested that infected turtles acquired the virus at a different location somewhere during their development before arriving in the AMI zone. This finding makes the case for setting up a health monitoring program for turtle populations in the area, enforcing sanitary measures to reduce the spread of the pathogen.

KEY WORDS: Chelonid herpesvirus 5 · Fibropapilloma · General health status · Northern Sinaloa · Foraging grounds · One-step PCR · *Lepidochelys olivacea* · *Chelonia mydas agassizii*

1. INTRODUCTION

Marine turtles are considered sentinel species of marine ecosystem health due to their longevity and life history (Aguirre & Lutz 2004). Sea turtle populations worldwide have sharply declined due to fisheries bycatch, predation, poaching and illegal human consumption (Mancini & Koch 2009). Habitat loss due to coastal urbanization, industrial and agricultural activities is perhaps one of the most pervasive impacts on their decline (Aguirre & Tabor 2008, Komoroske et al. 2011). Five of the 6 marine turtle species inhabiting the Pacific Ocean have been recorded off the Pacific coast of Mexico (Marquez 2002, Spotila 2004) where they are considered as species at risk of extinction (IUCN 2012, CITES 2015) and hence are protected under the Mexican law NOM-059-SEMARNAT-2010 (DOF 2010).

The ecology, population structure, general health and infectious diseases of marine turtles in foraging grounds have been poorly studied due to access limitations to their oceanic habitats (Whiting et al. 2007, Chaves et al. 2017, Labrada-Martagón et al. 2017).

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Assessing the body condition index of individuals is an important conservation goal (Stevenson & Woods 2006). Body condition index can be an indicator of well-being and health of individuals and populations, since it changes according to energy reserves, food availability, predation risk, ecological perturbation, health status and presence of emerging diseases (Stevenson & Woods 2006, Labrada-Martagón et al. 2010). Blood parameters are also useful as non-destructive diagnostic tools that can be used in the field for evaluating and monitoring wildlife health status (Aguirre & Balazs 2000). Hematocrit and total protein may provide valuable information on the health status of reptiles (Aguirre & Balazs 2000, Rossi et al. 2009, Sykes & Klaphake 2015). These parameters, along with body condition and the external physical assessment (visual examination) of the shape of the plastron and/or carapace, clinical signs of disease and presence of fibropapillomatosis (FP)-related tumors, are good indicators of general health status (Rossi et al. 2009, Thomson et al. 2009, Chaves et al. 2017).

FP is probably the most important infectious disease threatening sea turtles worldwide (Alfaro-Núñez et al. 2016). It is a debilitating neoplastic disease characterized by epithelial fibropapillomas and fibromas commonly found in the soft tissues of eyelids, mouth, cervical, axillary and inguinal regions (Foley et al. 2005, Ackermann et al. 2012). Tumor formation begins with proliferation of fibroblasts in the superficial dermis, followed by epidermal proliferation with acanthosis and orthokeratosis. As a nodule enlarges, the epidermis becomes verrucous. The continuous fibroblast proliferation thickens the epidermis, resulting in soft to firm masses (Herbst 1994, Oros et al. 1999). Morphological changes caused by FP can interfere with basic functions such as hydrodynamics, locomotion and feeding, resulting in weakening of the affected animal and ultimately causing its death (Rossi et al. 2009, Rodenbusch et al. 2014). The Chelonid herpesvirus 5 (ChHV5) has been commonly associated with FP (Quackenbush et al. 2001, Alfaro-Núñez et al. 2014) and has been proposed as one of the etiologic agents (Alfaro-Núñez et al. 2016). This virus may be transmitted from infected turtles to uninfected ones by direct contact, through vectors or fomites (Curry et al. 2000). It has been suggested that FP infection occurs in the feeding grounds where turtles recruit in neritic environments (da Silva et al. 2016). Infected animals may either develop active infection or remain latently infected (Alfaro-Núñez et al. 2014). The disease has been linked to degradation of the marine environment (Chaves et al. 2017).

The objectives of the present study were to determine the presence of ChHV5 in association with FP and to assess the general health condition of wild black turtles *Chelonia mydas agassizii* and olive ridley turtles *Lepidochelys olivace*a in foraging grounds off northern Sinaloa, Mexico.

2. MATERIALS AND METHODS

2.1. Study area

The San Ignacio-Navachiste-Macapule lagunar system is located within the coordinates 25° 15′ to 25° 35′ N and 108° 30′ to 109° 03′ W (Sanchez-Bon et al. 2010) in the Gulf of California, Mexico. It was declared a conservation-priority marine area in 2005 (Morgan et al. 2005). Here, 5 marine turtle species use this region as migration, foraging, breeding and nursing habitats (Aguilar-González et al. 2014). This area is considered the most important foraging ground off northern Sinaloa for black and olive ridley turtles (Zavala-Norzagaray et al. 2014). Two capture zones were set (Fig. 1): (1) the Area of Marine Influence (AMI) located in oceanic waters adjacent to the lagoon system and (2) the Navachiste Lagoon System (NLS). This is the main coastal lagoon in the Gulf of California and the biggest in Sinaloa (79 873 ha) with an area of 190 km^2 (Martinez-Lopez et al. 2007).

2.2. Field sampling

Olive ridley turtles were captured in the AMI zone at random using the rodeo technique between March 2015 and January 2016 (Whiting et al. 2007, Valverde et al. 2008). In the NLS zone, black turtles were captured using entanglement nets (100 × 6 m, stretched mesh size 50 cm), suspended in the water column. These nets were examined for entangled turtles every 2 h throughout a 24 h period. The nets were lightly fixed to a lead line, so trapped turtles could reach the surface to breathe and avoid drowning (Koch et al. 2007). All captured turtles were transported to the shore and processed for individual identification, morphometric measurements, general health assessment and collection of blood and tissue specimens. Turtles were tagged with Inconel tags (National Band and Tag) and released unharmed after processing.

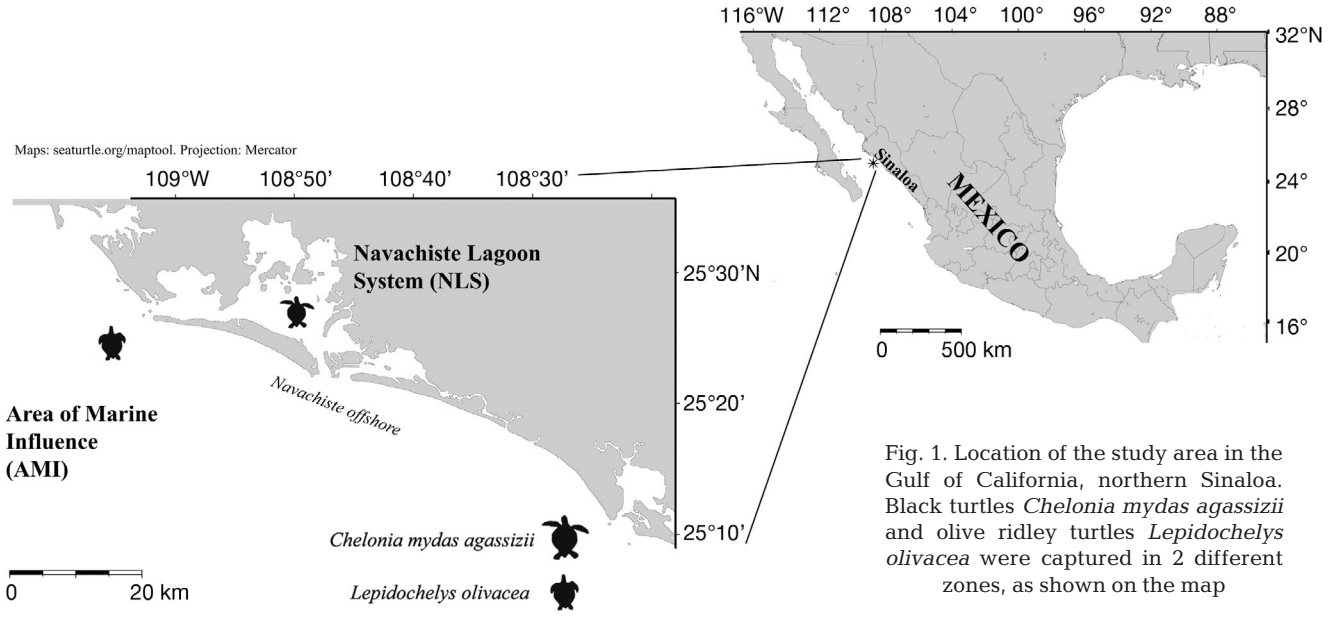

Fig. 1. Location of the study area in the Gulf of California, northern Sinaloa. Black turtles *Chelonia mydas agassizii* and olive ridley turtles *Lepidochelys olivacea* were captured in 2 different zones, as shown on the map

The weight (±1 kg) and the straight carapace length (SCL; ±0.1 cm) were used to determine the development stage of turtles (Bolten 2000). Black turtles with SCL between 65 and 77.3 cm were considered subadults and those with SCL ≥77.3 cm were considered adults, according to the mean size of nesting females in Colola, Michoacan (Lopez-Mendilaharsu et al. 2008). Likewise, olive ridley turtles were considered subadults (SCL between 46 and 60 cm) or adults (SCL ≥60 cm) based on the mean size of nesting females at La Escobilla, Oaxaca (Marquez 2002) and data from the feeding area of Bahia de Los Angeles, Baja California (Koch 2013). For both species, sexual dimorphism was used to determine mature animals, where adult males had an elongated and prominent tail extending beyond the carapace margin and adult females had a short tail that never exceeded the posterior margin of the carapace. Immature turtles were classified as undetermined (Wibbels 2000).

The apparent physical condition of each turtle was determined by visual inspection, paying special attention to the presence of tumors. Whenever present, tumors were described by morphology, location, size, texture and color. Physical condition had 2 categories: (1) good, when apparent disease was absent and lesions were small but did not impair mobility or physical functions, and (2) poor, when any abnormal signs were present (e.g. ectoparasites, flipper amputations, traumatic injuries, FP, deformed carapace, impaired movement or emaciation) (Thomson et al. 2009). The condition index (CI) was determined according to Labrada-Martagón et al. (2010). Three categories (very good, good and low) were determined according to Barrios-Garrido et al. (2015).

Blood (ca. 8 ml) was drawn by venipuncture from the dorsal postoccipital sinus using 10 ml sterile plastic disposable syringes with gauge 21 needles (Sykes & Klaphake 2015). Blood from each animal was placed into a Vacutainer® (BD) tube containing sodium heparin and stored on ice or refrigerated at 4°C until processing at the laboratory. The time elapsed between blood collection and analysis did not exceed 24 h.

Biopsies were taken from a posterior flipper using a sterile dermal punch. If the turtle had tumors, biopsies of them were collected with sterile surgical instruments. In all cases, tissues were stored dry in sterile cryovials, 1 per tube (Chaves et al. 2017). All samples were placed on ice and transported to the laboratory, where they were stored at −70°C until processing for DNA extraction (Rodenbusch et al. 2014).

During this study, an olive ridley turtle was captured that showed severe emaciation and weakness. It presented sunken eyes, damaged plastron and reduced muscular mass on head and neck, body discoloration, respiratory problems, foul smell, lethargy, a deformity in the anterior part of the head and traumas under the carapace. These health problems, along with the suspicion of FP infection, were diagnosed by a certified veterinarian during the clinical examination. It was decided that the licensed veterinarian should euthanize this turtle with an overdose of barbiturates (Pisa) and a necropsy was performed. All sampling, manipulation and analyses of turtles

was carried out under Mexican laws and a permit from SEMARNAT SGPA/DGVS/04478/15.

2.3. Laboratory analyses

Hematocrit (Ht) was determined using the microhematocrit technique (Rossi et al. 2009). Briefly, the blood sample was drawn into a capillary tube and centrifuged (5 min at 13 000 × *g*) to separate erythrocytes from plasma. The tube was compared to a reference scale to obtain a value. The Ht is the ratio (percentage) of the volume of packed red blood cells to the total blood volume. Total protein (TP) in plasma was obtained by centrifugation (10 min at 13 000 × *g*). An aliquot was drawn to read the protein concentration (g dl^{-1}) with a veterinary refractometer (Reichert) (Aguirre & Balazs 2000).

Total DNA extraction was performed by individually dicing each normal skin or tumor sample into small pieces, which were placed into a microtube (2 ml) containing 4 to 6 ball bearings (1/8 inch) and incubated in liquid nitrogen (1 min). Afterwards, tubes were placed in a Tissue Lyser II (Qiagen) at 1800 rpm for 90 s to grind tissues. Total DNA was extracted with 400 µl 3% cetyl trimethylammonium bromide buffer (100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, pH 8.0, added with 0.2% [v/v] β-mercaptoethanol) (Zhang et al. 1998). DNA was dried and resuspended in 70 µl ultrapure distilled water (Invitrogen). DNA concentration was determined by spectrophotometry (Nanodrop 2000c, Thermo Scientific). DNA integrity was confirmed by 1-step PCR amplification of mitochondrial (mt) DNA for each species. Primers TCR5 and TCR6 amplified a 428 base pairs (bp) fragment of *Chelonia mydas agassizii* mtDNA (Norman et al. 1994), while primers FDLo1 and RDLo1 amplified a 330 bp fragment of *Lepidochelys olivacea* mtDNA (Camacho-Mosquera et al. 2008).

All DNA samples from normal skin and tumors (1 µl) were used as templates for 1-step PCR analyses to detect ChHV5. Specific primers (GTHV2 and GTHV3) were used to amplify a 483 bp fragment of ChHV5 genome (Quackenbush et al. 2001). A negative control (ultrapure water) was included in every PCR amplification. The positive control was DNA from an FP tumor of *C. mydas* captured in Akumal, Mexico. This positive control was determined by PCR and sequencing. The PCR products were resolved in a 1% Tris–acetate–EDTA agarose gel electrophoresis and visualized with a Molecular Imager Gel Doc XR+ system (Biorad).

The resulting PCR products were purified using a ExoSAP-IT® PCR Product Cleanup kit (GE Healthcare) and sent for Sanger sequencing at Genewiz (New Jersey, USA). The sequence was used for multiple sequence alignment and phylogenetic analyses using MEGA version 6 software (Tamura et al. 2013) along with 29 other alphaherpesvirus DNA polymerase gene sequences available from GenBank using the Basic Local Alignment Search Tool (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (National Center for Biotechnology Information, Bethesda, MD, USA). The method used was Maximum Likelihood based on the Tamura-3 model with discrete Gamma distribution 5. Results were obtained after 1000 bootstrap replicates (Chaves et al. 2017).

Statistical analyses were performed using Minitab 17 (Minitab). Data for SCL (cm), weight (kg), Ht (%), TP (g dl^{-1}) and CI were expressed as mean ± standard deviation (SD). Pearson correlations between blood parameters and CI were calculated using a simple regression model ($p < 0.005$, $r^2 \geq 0.50$).

3. RESULTS

A total of 82 sea turtles were captured in the study area. Of these, 24 black turtles *Chelonia mydas agassizii* were captured in the NLS area, and 58 olive ridley turtles *Lepidochelys olivacea* were captured in the AMI zone.

All examined black turtles were immature individuals (SCL <77.3 cm), either juveniles (n = 11) or subadults (n = 13). Only 1 individual might be classified as male by sexual dimorphism. The overall apparent physical condition was categorized as 'Good' (n = 24). The ranges and mean values for the biometric data of black turtles are presented in Table 1. The mean CI was 1.22 ± 0.20, defined as 'Very Good'. No significant correlations were found between Ht and CI ($p < 0.05$, $r^2 = 0.0039$), or TP and CI ($p < 0.05$, $r^2 = 0.0345$). All black turtles were

Table 1. Meristic and blood data of black turtles *Chelonia mydas agassizii* from the Navachiste Lagoon System, Sinaloa, Mexico 2015–2016. SCL: straight carapace length

Parameter	Mean ± SD (n = 24)	Range
SCL (cm)	64.15 ± 8.01	45–74.50
Weight (kg)	34.35 ± 11.30	11–49
Condition Index	1.22 ± 0.20	0.62–1.66
Hematocrit (%)	29.33 ± 7.42	17–43
Total protein (g dl^{-1})	4.72 ± 0.93	2.80–6.80

Table 2. Meristic and blood data of olive ridley turtles *Lepidochelys olivacea* apparently healthy (without tumors) and with tumors from the Area of Marine Influence, Sinaloa, Mexico 2015–2016. SCL: straight carapace length

Parameter	Without tumors		With tumors	
	Mean ± SD (n = 54)	Range	Mean ± SD (n = 4)	Range
SCL (cm)	59.98 ± 3.37	50.5–68.8	61.07 ± 1.09	59.7–62.0
Weight (kg)	31.3 ± 4.7	17–44	33.0 ± 5.9	25–38
Condition Index	1.44 ± 0.16	0.95–2.03	1.44 ± 0.22	1.17–1.65
Hematocrit (%)	28.66 ± 5.94	13–40	26.25 ± 10.63	19–42
Total protein (g dl^{-1})	3.73 ± 0.77	2.5–6.4	3.7 ± 0.88	2.7–4.4

tumor-free, showed no traumas or other serious lesions. All skin samples from this species were ChHV5-negative by one-step PCR.

All examined olive ridley turtles were mature individuals, either adults (n = 56) or subadults (n = 2). The gender classification showed 27 males, 29 females and 2 undetermined. The ranges and mean values for the biometric and hematologic data of olive ridley turtles are presented in Table 2. Three CI categories occurred: 'Very Good' (n = 55), 'Good' (n = 2) and 'Low' (n = 1).

With respect to the blood parameters, no significant correlations were found between Ht and CI ($p < 0.05$, $r^2 = 0.004$) or between TP and CI ($p < 0.05$, $r^2 = 0.0023$). The apparent physical condition was categorized as 'Good' (n = 48) or 'Poor' (n = 10). Turtles with poor condition showed wounds from propellers or from fishing gear, shark bites, carapace malformations and copulation marks.

Four turtles presented tumors and one showed severe physical damage and emaciation: this turtle was euthanized. Hematological parameters of turtles without tumors (n = 54) and with tumors (n = 4) are presented in Table 2. The values from tumor-free turtles were compared with previous studies (Table 3). Tumor diameter varied between 1 and 3 cm. Tumors were concentrated on the eye conjunctiva, anterior fins and dorsal neck (Fig. 2). Tumor morphology varied from warty to papillary masses and color included light pink, white, black, grey and light yellow.

PCR analyses in tumor tissues showed ChHV5 DNA in 3 out of 4 (75%) tumors found in olive ridley turtles. The Ht and TP values from ChHV5-positive turtles (n = 3) were compared with those from other studies (Table 4). In contrast, all normal skin samples taken from the posterior left fins either from black or olive ridley turtles were ChHV5-negative. DNA sequence analysis of a 449 bp PCR product using the BLAST program showed 96% identity with the ChHV5 DNA polymerase gene (AF239684.2). The obtained sequence was deposited in GenBank with accession number MH450167. Phylogenetic analyses of a 388 bp fragment of the ChHV5 UL30 polymerase DNA gene was done to determine the relationship of the Sinaloa isolate to other ChHV5 isolates reported in the Americas. The analysis grouped the Sinaloa isolate in a cluster along with virus isolates from olive ridley turtles collected in the USA in 1998 and Nicaragua in 2010–2011, isolates from green and loggerhead turtles from the USA in 2004 and, more distantly, with a Mexican isolate from 2000 (Fig. 3).

The euthanized turtle was an olive ridley male diagnosed with severe emaciation and presented tumors related to FP in the inguinal zone. Several focal bacterial infections were identified in different organs in the form of granulomas or inflammation. This turtle had extensive injuries in the head and neck, absence of cranium and extensive scarred tissue in the brain and mandible. It showed scant mus-

Table 3. Hematocrit and total protein values from previous studies of healthy (without fibropapillomatosis tumors) free-ranging sea turtles. na: not analyzed

Parameter	N	Mean ± SD	Range	Reference
Total protein (g dl^{-1})				
Chelonia mydas	100	5.1 ± 0.8	2.6–6.9	Bolten & Bjorndal (1992)
Chelonia mydas	37[a]	5 ± 0.7	3.5–6.7	Aguirre & Balazs (2000)
	53[a]	4.2 ± 0.6	2.9–5.6	
Chelonia mydas	30	4.3 ± 0.34	na	Swimmer (2000)
Chelonia mydas	51	4.3 ± 1.04	2.0–6.1	Whiting et al. (2007)
Chelonia mydas	28	4.37 ± 0.70	3.1–6.0	Montilla et al. (2008)
Chelonia mydas	45	4.05 ± 0.35	na	Rossi et al. (2009)
Lepidochelys olivacea	24	2.95 ± 0.37	2.4–3.7	Brenes Chaves et al. (2013)
C. mydas agassizii	24	4.72 ± 0.93	2.8–6.8	This study
Lepidochelys olivacea	54	3.73 ± 0.76	2.5–6.4	This study
Hematocrit (%)				
Chelonia mydas	106	35.2 ± 3.2	26.4–42	Bolten & Bjorndal (1992)
Chelonia mydas	55	29.37 ± 7.63	10.2–43.4	Whiting et al. (2007)
Chelonia mydas	45	24.69 ± 2.63	na	Rossi et al. (2009)
Lepidochelys olivacea	24	28.58 ± 3.07	24.0–34.0	Brenes Chaves et al. (2013)
C. mydas agassizii	24	29.33 ± 7.42	17.0–43.0	This study
Lepidochelys olivacea	54	28.93 ± 6.19	13.0–42.0	This study

[a]Data from 2 separate subpopulations/areas in Hawaii

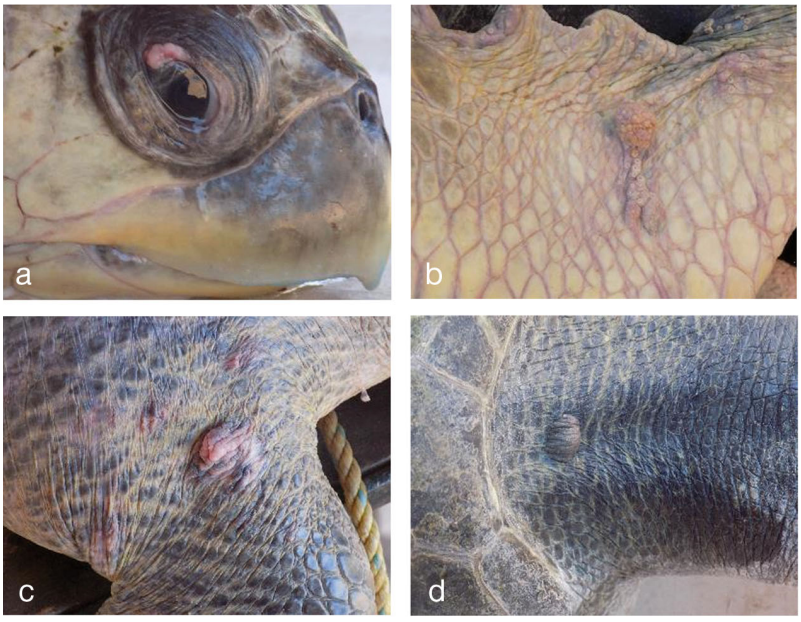

Fig. 2. Olive ridley turtles *Lepidochelys olivacea* with cutaneous tumors (a) on the eye conjunctiva region, (b) on the front of the fin, and (c,d) on the back of the neck of 2 different turtles

Table 4. Hematocrit and total protein values from previous studies of free-ranging sea turtles with fibropapillomatosis (FP) tumors. na: not analyzed

Parameter	N	Mean ± SD	Range	Reference
Total protein (g dl^{-1})				
Chelonia mydas	12[a]	4.9 ± 0.6	na	Aguirre & Balazs (2000)
	16[b]	4.5 ± 0.8	na	
	28[c]	3.5 ± 1.0	na	
Chelonia mydas	5[b]	6.0 ± 0.46	na	Rossi et al. (2009)
	10[a]	5.20 ± 0.21	na	
	12[c]	4.87 ± 0.28	na	
Lepidochelys olivacea	24	2.95 ± 0.37	2.4–3.7	Brenes Chaves et al. (2013)
Lepidochelys olivacea	4	3.5 ± 1.20	2.7–4.4	This study
Hematocrit (%)				
Chelonia mydas	5[b]	26.0 ± 6.5	na	Rossi et al. (2009)
	10[a]	24.6 ± 1.98	na	
	12[c]	22.75 ± 2.23	na	
Lepidochelys olivacea	24	28.58 ± 3.07	24.0–34.0	Brenes Chaves et al. (2013)
Lepidochelys olivacea	4	21.0 ± 2.0	19–23	This study

[a,b,c]Different FP severities: [a]slight; [b]moderate; [c]severe

cular body mass, a digestive system with esophageal edema and hemorrhagic gastritis, enlarged stomach and scarred gastric mucosa, and cysts in the duodenum and small intestine. The large intestine contained a possible tumor or fibroma. Both lungs showed necrosis and emphysema, and the right lung had multiple firm nodules (2 mm in diameter) and compacted parenquima. The heart was hypertrophied (3 times normal size) with extremely thin walls, extensive hydropericardium and petechiae at the base of the aortic arch. Severe auricular fibrosis with obstruction and fibrosis was present at the right atrioventricular valve. There was splenomegaly and hepatomegaly, with white nodules (1 to 3 mm) across the liver parenchyma and a plethoric gall bladder free of stones. Both kidneys had white nodules. The left kidney was atrophied (30% smaller than right) and the right kidney was enlarged with a cyst (5 cm) full of transparent fluid, and many fibrotic nodules (1 mm) were present throughout the organ. No other significant lesions were identified.

4. DISCUSSION

Very few studies have been carried out on the health status of marine turtle populations in foraging grounds. Moreover, studies on the health status of marine turtles in the study area are also limited. The present study provides information on population features of black and olive ridley turtles occurring in the study area, which serves as an important foraging zone. All the captured black turtles in NLS were juveniles and subadults. This is consistent with reports for the neritic zone in different foraging areas off Baja California, Mexico (Koch et al. 2007, Labrada-Martagón et al. 2010). Juveniles and subadults of this species spend most of their time inside lagoons and bays where few natural predators occur (Mancini & Koch 2009). The overall apparent physical condition of black turtles was healthy. The spatio-temporal fidelity to foraging grounds located in near-shore waters (Sampson et al. 2015) may prevent black turtles from becoming exposed to virus fomites and/or its vectors coming from other areas. These conditions may explain their healthy status and FP absence (Aguirre & Lutz 2004, Renan de Deus Santos et al. 2017). The average Ht and TP values from black turtles were very similar to those of healthy green turtles *Chelonia mydas* from other locations (Table 3). Based on the body condi-

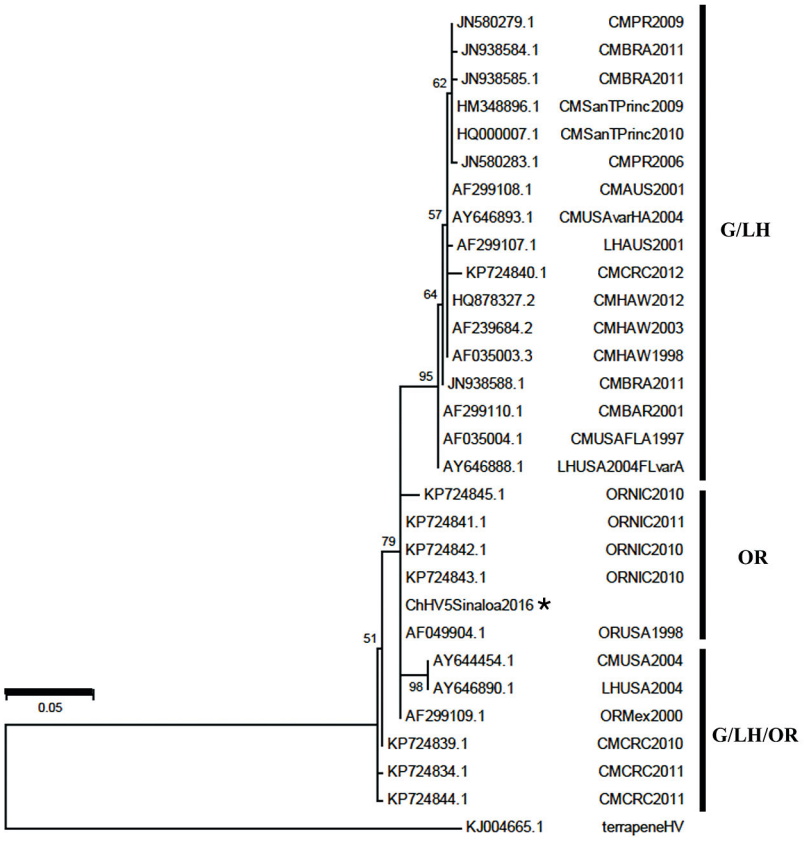

Fig. 3. Phylogenetic analyses of 29 sequences of the UL30 polymerase DNA gene from chelonid alphaherpesvirus 5 (ChHV5) with a length of 388 bp after gaps and missing data had been eliminated. A terrapene box turtle herpesvirus DNA polymerase (GenBank acc. no. KJ004665) was used as an outgroup sequence. The Sinaloa isolate (★) clustered with a USA isolate from 1998, Nicaraguan isolates from 2010–2011, and to a lesser extent, with American isolates from green and loggerhead turtles from 2004 and a Mexican isolate from 2000. The number at each branch indicates the percentage bootstraps after 1000 bootstrap evaluations. Abbreviations used are olive ridley turtle *Lepidochelys olivacea* (OR), green turtle (G), loggerhead turtle *Caretta caretta* (LH), *Chelonia mydas* (CM), herpesvirus (HV), Australia (AUS), Barbados (BAR), Brazil (BRA), Costa Rica (CRC), Florida (FLA, FL), Hawaii (HAW), Nicaragua (NIC), Puerto Rico (PR), United States of America (USA) and San Tome and Principe (SanTPrinc)

tion index, hematological values and lack of FP-related tumors, black turtles found in the study area were well fed, not anemic nor dehydrated and not infected with FP.

All captured olive ridley turtles were mature (mean SCL 60.05 cm) based on the mean SCL values from sites along the Pacific coast of Mexico (>60 cm, Marquez 2002; 63.15 cm, Zavala-Norzagaray et al. 2014). Olive ridley turtles have pelagic habits (Spotila 2004) and in this study, they were found in oceanic waters far offshore the Guasave coast. Adult females and males were captured in spring, when females approach the beaches to breed (Valverde et al. 2008). Most information on olive ridley turtles focus on nesting, since their pelagic habits makes it difficult to follow their life cycle at sea (Spotila 2004). In the present study, most adults had good physical condition (n = 48), whereas the rest showed poor condition due to severe injuries caused by fishing gear, predators or disease (Thomson et al. 2009). Although these turtles were evaluated with poor physical condition, their weight and blood parameters were similar to those with good condition. This may be explained by the fact that they were located in the foraging zone, so these turtles were more likely to be well fed, as indicated by the overall CI. This may be one reason why they did not show anemia or dehydration, in contrast to reports of turtles with poor condition from nesting areas (Brenes Chaves et al. 2013). Information on the general health status of olive ridley turtles in northwest Sinaloa is scarce, therefore the mean CI (1.44 ± 0.16) obtained in this study represents a reference for adults in this area. This result indicates that the olive ridley turtle population had a good feed intake (Labrada-Martagón et al. 2010).

No statistical correlation was found between CI, physical condition or blood parameters. Nonetheless, animals with tumors had somewhat lower average Ht than animals without tumors (Table 2). This result may reflect the impact caused by the disease which could reduce feeding or nutrient absorption, thus weakening the animal. A report from Ostional, Costa Rica showed differences in Ht and TP between mature olive ridley turtles with and without tumors (Brenes Chaves et al. 2013). Low TP and Ht values were associated with disease and low CI (Aguirre et al. 1995, Bjorndal et al. 2000). The Ht value determines the volume of red blood cells in an animal. It is important because a low value may indicate that an individual has a low amount of red cells, suggesting anemia or an ongoing infection due to the higher rate of white blood cells. Conversely, a high Ht value may suggest dehydration or organ disease (Weiss & Tvedten 2012).

Differences in hematological parameters between the present study and the one from Costa Rica may be influenced by the geographic area and physiological status of the populations (Brenes Chaves et al. 2013). Our study area is a known foraging zone which provides food and shelter to turtles, whereas Ostional is a nesting zone, where higher energy expenditures are required for nesting and food sources may be less abundant than in a foraging zone. These conditions may enhance the physical impact of FP disease in turtles from nesting grounds.

A previous study carried out in the Gulf of California off the Baja California Peninsula detected FP by physical observation, histopathology and transmission electron microscopy in *C. mydas* coming from a Hawaiian population (Resendiz et al. 2016). Although it was a new record of FP, that study did not provide molecular information on the virus. In the present study, the presence of FP and its associated virus ChHV5 was determined in tumor samples from olive ridley turtles in foraging grounds off northern Sinaloa using PCR and DNA sequencing. This represents a new location record for FP–ChHV5 in foraging grounds off northern Sinaloa in the free-ranging olive ridley turtle *Lepidochelys olivacea* and provides molecular information on the virus. Four olive ridley turtles presented at least 1 tumor consistent with FP and PCR analyses showed that 3 out of 4 (75%) tumor tissues were ChHV5-positive using the GTHV2 and GTHV3 primer pair (Quackenbush et al. 2001). In contrast, all skin biopsies from the posterior fins of olive ridley or black turtles were negative for ChHV5 DNA, including normal skin samples from the same turtles with tumors. Normal skin samples were taken at a long distance from where tumors were located (eye conjunctivae, neck, anterior fins and/or inguinal zone); therefore, it is possible that the virus was not present in skin from posterior fins (Quackenbush et al. 2001).

Latent ChHV5 infections have been determined in skin samples from green, loggerhead and olive ridley turtles without tumors (Quackenbush et al. 2001). Also, swab samples from green and olive ridley turtles in Costa Rica and Nicaragua have tested positive for ChHV5 DNA using real-time PCR (Chaves et al. 2017). Alfaro-Núñez & Gilbert (2014) designed singleplex primers and reported herpesvirus DNA in 100% of samples from normal skin of sea turtles without tumors using a set of 3 independent PCR primers. These primers recognized highly conserved regions of 3 different genes in the ChHV5 genome. The 1-step PCR used in the present study was sensitive enough to detect herpesvirus DNA in most tumor samples obtained. One tumor was ChHV5-negative, probably because the amount of virus DNA in the sample was under the detection limit of the PCR technique. Alfaro-Núñez & Gilbert (2014) used 1-step PCR with multiple DNA targets to increase the chance of detecting virus DNA. In contrast, in a study of *L. olivacea* in Nicaragua, only 78% of turtles displaying FP tumors were ChHV5-positive by PCR using the GTHV2 and GTHV3 primer pair (Chaves et al. 2017). There, the authors attributed the ChHV5-negative results to either tumor regression or the amount of viral DNA being under the sensitivity limit of the technique used to detect the virus. These possibilities may also explain why ChHV5 DNA was not detected in this tumor by 1-step PCR.

The results indicate that ChHV5 is still scarce in the foraging ground off northern Sinaloa as it was only detected in 3 *L. olivacea* turtles, a free-ranging species with pelagic habits. This in turn suggests that the turtles had acquired ChHV5 in a different location at some point during their development. Later, when the turtles were collected in the AMI zone, they already showed FP clinical signs. Another study (Resendiz et al. 2016) has recorded FP-infected *C. mydas* from Hawaii in a feeding ground off Baja California. It is possible that turtles testing FP-positive in the present study also became infected in an endemic zone away from the Gulf of California. The appearance of FP in immature turtles such as juveniles and subadults is related to unfavorable environmental conditions (Chaves et al. 2017). In the present study, most olive ridley turtles were mature when they were collected in the foraging ground off Sinaloa. Hence, they may have become infected when they were more susceptible to FP infection in other locations. The fact that FP-infected turtles were recorded in this area makes a case for setting up a health monitoring program to enforce sanitary measures and pathogen surveillance in turtle populations arriving in this zone.

The phylogenetic analyses indicate that the Sinaloa isolate is closely related to ChHV5 isolates from olive ridley turtles recorded in the USA (1998) and Nicaragua (2010–2011), isolates from green and loggerhead turtles collected in the USA (2004) and, more distantly, to an isolate from an olive ridley turtle in Mexico (2000). The geographical location of these isolates was consistent because most of those turtles inhabit the Pacific Ocean. Herbst et al. (2004) showed that virus isolates from Atlantic loggerheads were more related to ChHV5 from Pacific olive ridleys than to other Atlantic, Caribbean or Hawaiian variants.

In conclusion, the black and olive ridley turtle populations had good general health and were well fed according to the CI, Ht and TP values obtained. The CI was slightly lower in olive ridley turtles with poor condition. Only 4 olive ridley turtles with poor condition presented FP-related tumors, 3 of which were ChHV5-positive by 1-step PCR. Sequencing of the PCR product showed 96% identity with the ChHV5 DNA polymerase gene. This is the first record of FP and its associated herpesvirus (ChHV5) in olive ridley turtles in foraging grounds off northwest Sinaloa, Mexico.

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