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New Record of Black Turtle *Chelonia mydas agassizii* in High Latitudes of Eastern South Pacific Ocean

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Abstract: A juvenile specimen of the black turtle, *Chelonia mydas agassizii*, was located in the shores of Cabo Tamar Island in the western entrance of the Strait of Magellan, Chile. A king crab angler found the specimen, and donated it to the Río Seco Natural History Museum's vertebrate collection for further studies and exhibition. Morphological measurements and a genetic analysis derived from an mtDNA fragment amplified and sequenced, suggest that this specimen corresponds to the species *Chelonia mydas agassiizii* and that it is closely related to the Galapagos Islands black turtle population. Possible influences of the El Niño Southern Oscillation phenomenon on this tropical species in the Eastern South Pacific Ocean are briefly discussed.

Key words: Magellan Strait, mitochondrial DNA, sea turtle, Testudines.

1. Introduction

The green turtle is one of the five species of sea turtles known to occur in Chilean waters [1-3]. Genus *Chelonia*, includes two subspecies, the eastern Pacific green turtle (also called black turtle) *C. mydas agassizii* (Boucort, 1868), considered to be a melanistic form of the genus *Chelonia*, and the green turtle *C. mydas* (Linnaeus, 1758). The former is normally distributed from Baja California to Peru including Revillagigedo and Galapagos Islands; however, vagrant individuals have been recorded in British Columbia and in Alaskan waters in the Northern hemisphere, as well as in Patagonian waters in the Southern hemisphere, nesting mainly on the coasts of Michoacán and Revillagigedo Islands (Mexico), Costa Rica's Pacific coast, and in the Galapagos Islands, Ecuador [4]. The green turtle, on the other hand, is found throughout all of the World's oceans, excepting Polar Regions [5-13].

In Chile, green turtles have been present since prehistorical times and modern records exist at least for the past two centuries [14]. Their presence has regularly been reported from Arica (18°27′ S) to Coquimbo (29°58′ S), where they feed mainly on seagrass, and to a lesser extent until Chiloé Island (42°29′ S) [1, 2, 15-22]. To date, there is only one previously published record for the green turtle at the extreme southern Chile waters. A 12-kg green turtle, with a 47-cm long carapace, was found in the western sector of the Patagonian channels at Desolation Island (52°57′ S, 74°05′ W), at the entrance of the Magellan Strait, on March the 12th of 1973 [23] (Fig. 1). Here, we record a new finding of the eastern Pacific green turtle in the southern tip of South America, updating the record list of this species in the southern region of Chile and providing a possible natal origin of the studied specimen.

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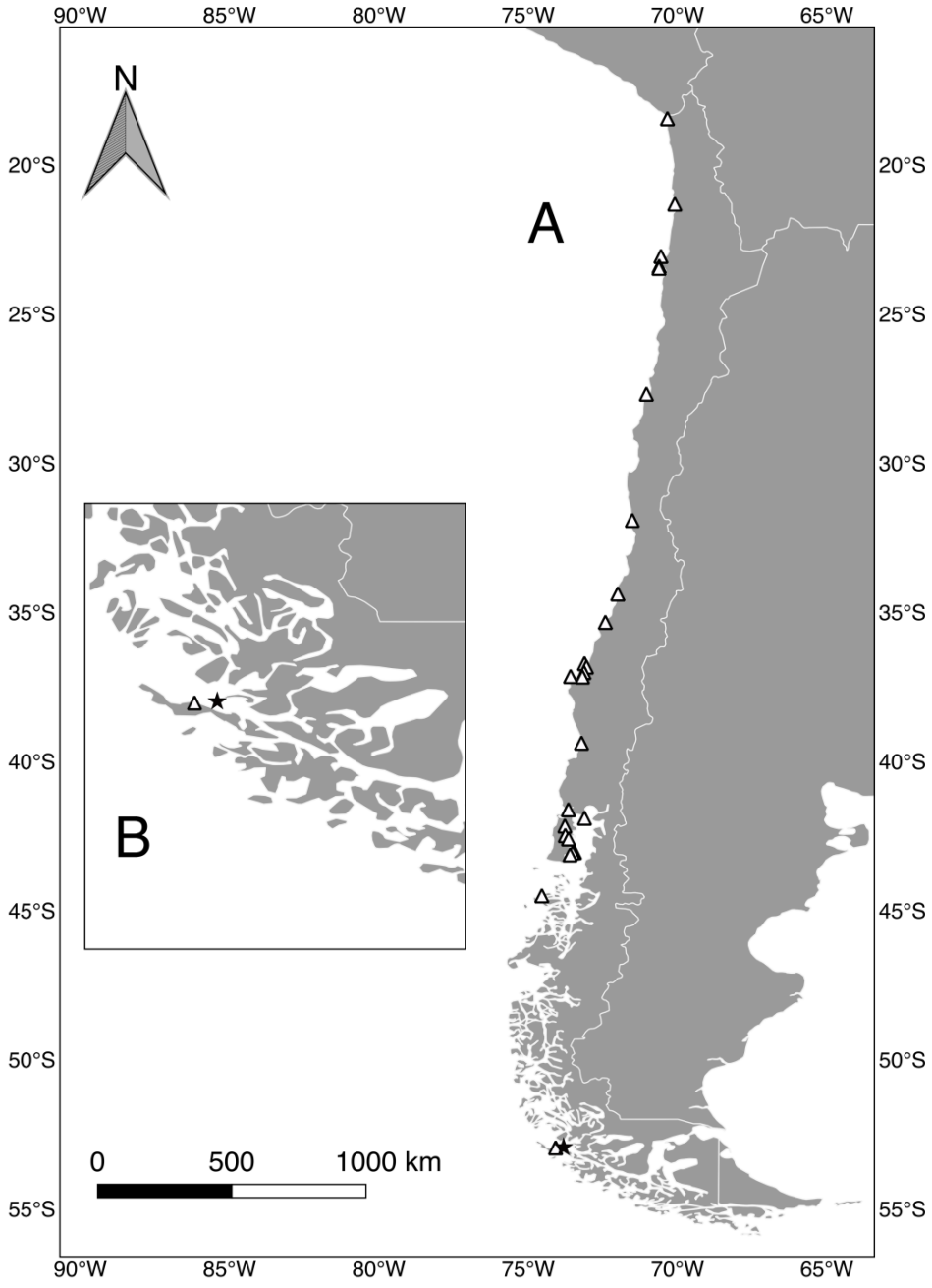

Fig. 1 Records of east Pacific green turtles (triangle) along the Chilean coast (A), and zoom of the region (B) where the new specimen was found at Tamar Island, Magellan Strait (star).

2. Materials and Methods

The specimen was found dead at Tamar Island (52°56′00″ S, 73°49′00″ W) in July 30, 2015, by the fisherman Erwin Soto onboard "Helvecia Cuarta". It showed an advanced stage of decomposition, and was being consumed by a small flock of kelp gulls (*Larus dominicanus*). The right hind limb, lower jaws, intermaxillary and hyoid apparatus were missing, as well as scales on the head and scutes of the carapace and plastron. The carcass was donated to the zoology collection of the Río Seco Natural History Museum (MHNRS) under the MFSI-tes-0001 code, in order to prepare the skeleton for further studies and exhibition.

Species identification was based on the skull shape, diagnostic patterns of bones of the palate, and shape of the carapace and entoplastron [24, 25]. Standard carapace length from the anterior-most point on the midline of the nuchal scute to the posterior-most tip of the last marginal (supracaudal or postcentral) scute, was measured in straightline (SCL) with calipers and curved (CCL) with a flexible tape measure, as well as the carapace width (SCW and CCW), respectively.

The presumptive *C. mydas* was also subjected to genetic analysis for taxonomic confirmation and identity of the species. DNA was extracted using a modification of the salting out method [26]. We amplified a ~380 bp fragment of the mtDNA control region with degenerate markers designed by Ref. [27]. The sequences of the primers were TCR5: 5´-TTGTACATCTACTTATTTACCAC-3 and TCR6: 5´-CAAGTAAAACTACCGTATGCC-3´. PCRs were performed in 25 μL reactions containing 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.8 uM of each primer and 1.25 U GoTaq DNA polymerase (Promega). The PCR protocol consisted of an initial denaturation step (95 ℃ for 3 min) following by 40 cycles of 45 s at 95 ℃ (denaturing), 45 s at 59 ℃ (annealing) and 90 s at 72 ℃ (extension) and a final extension step of 10 min at 72 ℃. Quality and size of the PCR product were evaluated using 1% agarose gel with Gel-Red™ and PCR Markers 50 bp Promega, and nucleotide sequences were determined for both strands of the PCR amplification products at the Macrogen (Korea) sequencing facility.

Available *C. mydas* sequences (eastern South Pacific = 17; western South Atlantic = 5) were downloaded from GenBank database (http://blast. ncbi. nlm. nih. gov/Blast .cgi) using the Blast procedure, to be included in the analysis for taxonomic confirmation and identity of the specimen (named as C_sp_CH). BIOEDIT Sequence Alignment Editor Program [28] and the ChromasPro program (version 1.7.7) were used to visualize, edit and align all sequences. Clustal W (in MacVector version 7.2; Accelrys, Madison, WI) was used for multiple alignments with the algorithm of Ref. [29]. We used a sequence of the loggerhead *Caretta caretta* (ID GenBank: DQ924967) as outgroup. All aligned sequences were uploaded at MacClade 4.0 program [30] to compare and evaluate the nucleotides composition, percentage of transitions and transversions, and number of polymorphic sites or their lengths. The most conserved and variable regions were explored in the sequenced regions. The relationship between the number of transitions and transversions was made comparing the sequences obtained from a pair, using the nucleotide substitution model of Ref. [31] with a bootstrap of 10,000, and with substitution rate per site under the gamma parameter (5 categories, parameter 0.0500). Finally, a phylogenetic tree of haplotypes was generated using the Maximum-likelihood method with bootstrap method (1,000 replications) as implemented in the program MEGA 7.0 [32]. The best model was selected on the AIC value in JmodelTest 2.1.7 [33] and MEGA.

3. Results

Based on the state of decomposition, the death of the specimen is estimated at around 60 days (Fig. 2). A macroscopic visual inspection of the carapacial and plastron were without apparent epibionts, and no piece of plastic was found.

3.1 Species Identification from Skeleton

The skull (splanchnocranium) was small (about

Fig. 2 Discovery of the turtle by the fisherman Erwin Soto at Tamar Island (a).

24.3% of SCL), rounded shape with a short snout and shallow parietal notches. The upper jaw has a smooth U-shaped outline. In the palate, a pair of ridges run parallel to the outer edge of the jaw between the margins of the upper jaw and the internal nares (Fig. 3).

The carapace is oval and elevated, with an outer margin slightly scalloped but not serrated. Its dorsal view is subcardiform and slightly emarginated over the neck and fore flippers; and the last third of the carapace has indentations between each marginal scute, typical of juvenile individuals. The carapace width attains 92% of its straight-line length (SCL) (Fig. 4 and Table 1).

The plastron has four pair of bones, epiplastron, hyoplastron, hipoplastron and xiphiplastron, and the distinctive elongated and narrow entoplastron with an arrow shape (Fig. 4).

3.2 Genetic Analysis

The mtDNA sequence of the studied turtle was nearly identical to the *Chelonia mydas* (*n* = 19) and *Chelonia mydas agassizii* (*n* = 2) sequences are available in GenBank. The phylogenetic tree grouped the haplotype of our specimen together with the Pacific clade (Fig. 5) and was compatible with the Galápagos haplotypes (Galap_KX499514 and AY540071).

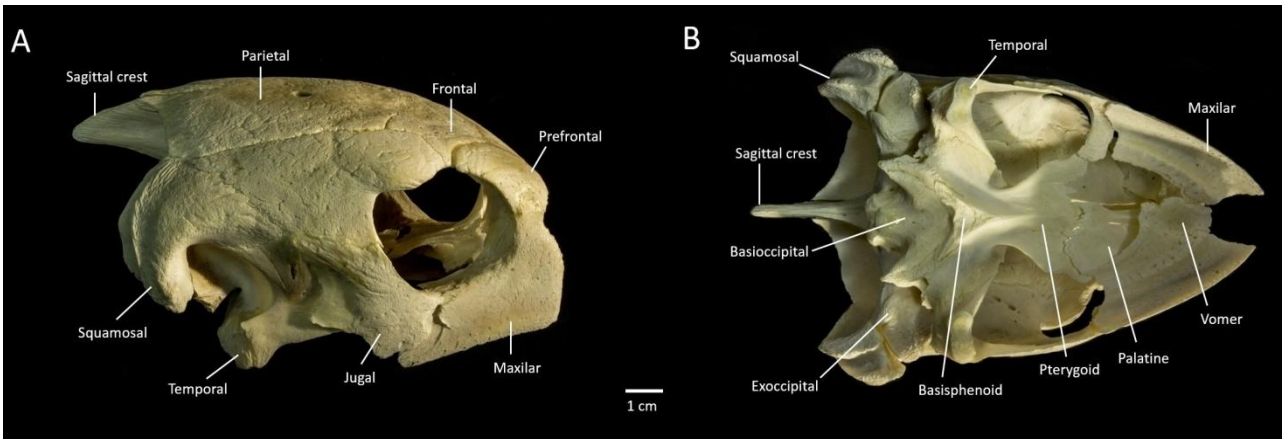

Fig. 3 Skull of the specimen at Tamar Island in lateral (A) and ventral (B) views.

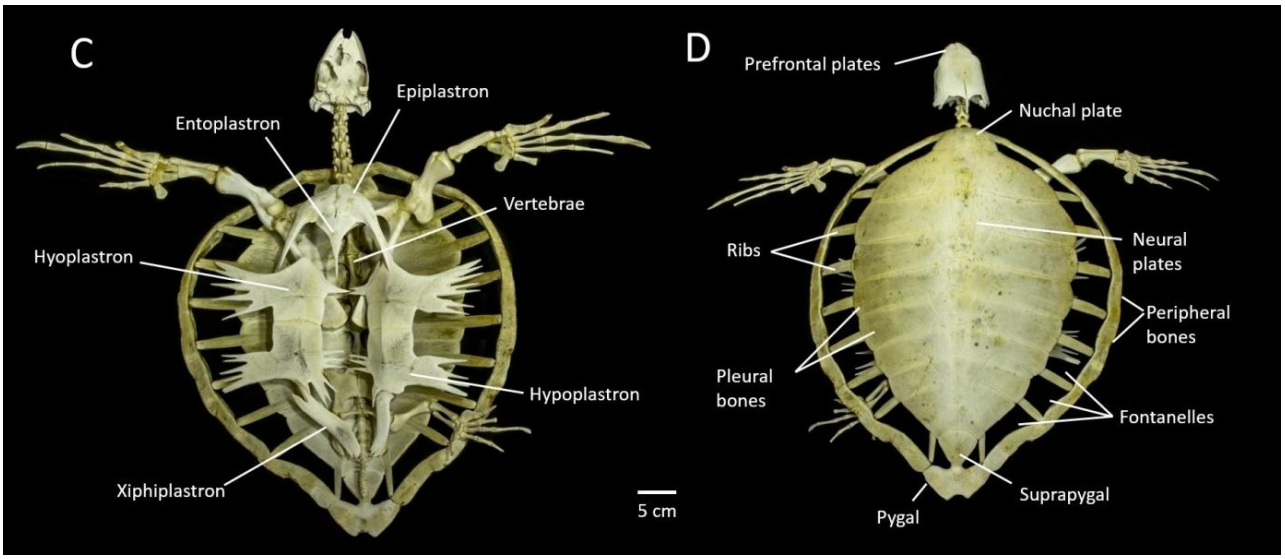

Fig. 4 Skeletal system of the specimen; (C) dorsal view and (D) ventral view.

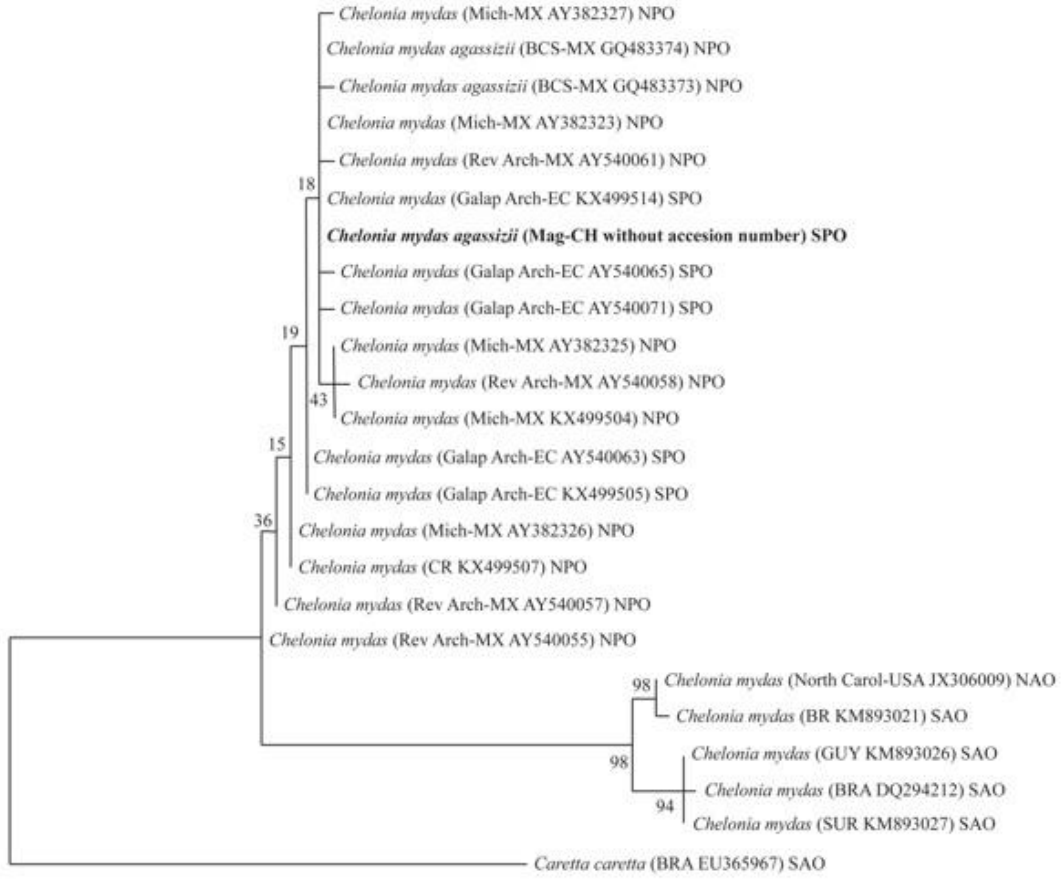

Fig. 5 Phylogenetic tree based on a 380 bp fragment of the mtDNA control region.
The phylogenetic tree is rooted on *Caretta caretta*. Nodal numbers are Bayesian posterior probability. Accession numbers of sequences derived from GenBank are shown in brackets. NAO: North Atlantic Ocean; SAO: South Atlantic Ocean; NPO: North Pacific Ocean; SPO: South Pacific Ocean; Mich: Michoacan; BCS: Baja California Sur; Rev Arch: Revillagigedo archipelago; Galap Arch: Galapagos archipelago; Mag: Magallanes; North Carol: North Caroline; MX: Mexico; EC: Ecuador; CH: Chile; CR: Costa Rica; USA: United States of America; BR: Brazil; GUY: Guyana; SUR: Suriname. The studied specimen is reported in bold.

Table 1 Summary of measurements of skull and carapace of the specimen found at Tamar Island in July 2015.

Measurements	Value (mm)
Standard length of skull	124
Standard width of skull	79
Standard carapace length (SCL)	500
Standard carapace width (SCW)	460
Minimum standard carapace length (SCL_{min})	480
Maximum standard carapace length (SCL_{max})	525
Curved carapace length (CCL)	520
Curved carapace width (CCW)	480
Minimum curve carapace length (CCL_{min})	520
Maximum curve carapace length (CCL_{max})	530

4. Discusion

The morphological characteristics of the skull of the specimen from Cabo Tamar match with those of the green turtle *C. mydas*, which is also reinforced by the results of the phylogenetic tree. Moreover, the nucleotide sequence of the studied turtle grouped in the haplotypes observed for the eastern Pacific green turtle, confirms that our specimen is *C. mydas agassizii*. In Chilean waters, eastern Pacific green turtle is present with most records clustered in northern regions and shows some evidence of movements of individuals south [1, 2, 14, 17, 19-22, 34, 35], however, records in the extreme southern Chile are limited, with only one previously published record [23]. Our specimen was found only 19 km NE of the previous record and represents the second

southernmost record of an eastern Pacific green turtle in the Southern hemisphere. According to current evidence, the occurrence of this species in the southern tip of South America should be considered as accidental, although efforts to assess sea turtle in that high latitude are markedly reduced.

It is also interesting to note that the measurement of the carapace (SCL: 50.2 cm) of our specimen was a juvenile [8, 36, 37], just like the specimen (SCL: 47 cm) reported at Desolation Island [23]. These measures would be in line with the life stage reported for feeding areas in Peru [38, 39] and with the available size of measured specimens in Chilean waters (SCL mean: 49 cm; [14]), indicating that most are juveniles or subadult individuals.

The green sea turtle is a highly migratory species in the eastern Pacific using different habitats during their life cycle [8, 36]. Origins of eastern Pacific green turtle individuals observed in Chilean waters have been assumed to come from the nesting population of Galápagos Islands [40], because multiple records of specimens tagged in Galápagos have been recaptured in Peruvian coastal waters [41]. Although no record exists of tagged eastern Pacific green turtles from Chilean coastal areas [14], this migratory pattern is supported by recent analyses of mtDNA from samples taken in the north of Chile (e.g., Ref. [22]). Our genetic analysis also grouped the Tamar Island specimen most closely with the Galápagos haplotypes (see Fig. 5), thus supporting this link between Galápagos and Chilean coasts, and extends the longest distance travelled by *C. mydas agassizii* (up to ~5,900 km minimum travelling distance). The longest previously movement was reported for a tagged specimen that moved between Michoacan, Mexico and Buena Ventura, Colombia (~3,500 km) [8].

Green turtles inhabit in tropical, subtropical, and warm temperate regions and rarely stray into cold waters [42]. In fact, most green turtles seek optimal sea water temperature near 18 ℃ [43, 44]. Even when the cause of death of the Tamar Island specimen is unknown, the sea surface temperature in June and July 2015 was below 10 ℃ and probably the specimen was cold-stressed and cannot survive to return to warmer waters. Under that sea surface temperature, cheloniid turtles are very close to their minimum thermal tolerance and cannot survive for extended periods [43-45]. Malnutrition and/or disease could have also been potential cause of death of the Tamar Island specimen; however, the advanced stage of decomposition does not allow us to provide details about the state of health, and as such, it is difficult to comment on the role of putative physiological problems or disease associated.

The presence of sea turtles in high latitudes has been associated to warm-water years [42]; however, the occurrence of *Chelonia* in, for example, Alaskan waters do not reflect a direct warm-water year connection [12]. Disorientation due to physiological anomalies and signs used in migration may be other possible cause; yet, as mentioned above, it is difficult to comment on the putative physiological problems or disease with our Tamar Island specimen. In Peru and north of Chile, it has been observed that the species expand their distribution and can be found in greater numbers during periods of oceanographic-atmospheric anomalies resulting from the El Niño Southern Oscillation [22, 46-48] and it is reasonable to expect that such events could affect their movements from foraging areas, but no conclusive evidence has been presented yet [22].

Among various physical and biological factors that might be involved, specimens found in both, 1973 (Desolacion Island) and 2015 (Tamar Island) at the Magellan Strait occurred during strong El Niño Southern Oscillation events (http://ggweather.com/enso/oni.htm), with maximum SST anomalies of +2.1 ℃ and +2.6 ℃ for 1972/1973 and 2015/2016 El Niño events, respectively (http://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php). Thus, from an

oceanographic point of view, the incursion of superficial warm waters from tropical latitudes moving southward along the Chilean coastline during strong El Niño episodes, could explain the approach of both sea green turtles to the entrance of the Magellan Strait during 1973 and 2015, reflecting a direct warm-water year connection.

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