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HISTOLOGÍA ÓSEA DE UNA TORTUGA GIGANTE DEL PLEISTOCENO (TESTUDINIDAE) DE ECUADOR CONTINENTAL, CON COMENTARIOS DEL ORIGEN DE LAS TORTUGAS DE GALÁPAGOS

Disertación previa a la obtención del título de Licenciado en Ciencias Biológicas

FERNANDO JOSÉ TORRES CHIRIBOGA

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Certifico que la Disertación de Licenciatura en Ciencias Biológicas del Sr. Fernando José Torres Chiriboga ha sido concluida de conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación correspondiente.

Ph.D., Omar Torres-Carvajal

Director de la Disertación

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LISTA DE ABREVIATURAS

Abreviatura	Significado
PO	Primary Osteon
PVC	Primary Vascular Canal
SO	Secondary Osteon
SF	Sharpey Fiber
LB	Lamellar Bone
F	Foramina

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1. RESUMEN

Los testudínidos (tortugas terrestres) llegaron a Sur América durante el Mioceno aproximadamente hace 23 millones de años. Durante este período las corrientes oceánicas y la geografía del continente Americano pudieron haber ayudado a que animales con una buena capacidad para sobrevivir a la deriva, como tortugas gigantes, puedan haber llegado desde África a Sur América. En este estudio se realizó la descripción y caracterización histológica, mediante láminas delgadas, así como una descripción general de fósiles pertenecientes a una tortuga gigante de la formación Tablazo (Pleistoceno), provenientes de la provincia de Santa Elena, Ecuador que se encuentran en el Museo de Historia Natural de la Escuela Politécnica Nacional. Se observó que los especímenes pertenecen a una tortuga de la familia Testudiniae del género *Chelonoidis* de especie indeterminada. Esto nos lleva a pensar que probablemente existieron asentamientos de tortugas gigantes, muy similares a las tortugas de Galápagos, en el continente, es decir que el gigantismo ya evolucionó antes de su llegada al archipiélago, y que probablemente esta fue una de las características que les permitió llegar a Sur América.

Palabras clave: Testudinidae, *Chelonoidis*, histología, láminas delgadas, gigantismo, deriva transoceánica, colonización, formación Tablazo.

2. ABSTRACT

The testudinids (tortoises) reached South America during the Miocene about 23 million years ago. During this period ocean currents and the geography of the American continent may have aided animals capable of drifting, like giant tortoises, to reach South America from Africa. In this study, we describe the histology of bone tissue using thin sections of fossils of giant turtles from the Tablazo formation (Pleistocene) in the province of Santa Elena, Ecuador. In addition we present a general description of the most complete specimens of giant tortoises belonging to the Museo de Historia Natural of the Escuela Politécnica Nacional collection. We concluded that the specimens belong to a turtle of the family Testudinidae, genus *Chelonoidis*. This proves that giant tortoises, very similar to the Galapagos tortoises, existed on the continent, prior to their arrival to the archipelago. Thus, gigantism evolved in the continent, and was probably one of the characteristics that allowed them to reach South America.

Keywords: Testudinidae, *Chelonoidis*, histology, gigantism, drifting, Tablazo formation.

3. MANUSCRITO PARA PUBLICACIÓN

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TÍTULO

Bone histology of Pleistocene giant tortoises (Testudinidae) from continental Ecuador, with comments on the origin of Galápagos tortoises

AUTORES

Fernando Torres, José Luis Román, Edwin Cadena, Omar Torres-Carvajal

Museo de Zoología QCAZ, Escuela de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador.

F. Torres: O. Torres-Carvajal (*)
Museo de Zoología QCAZ, Escuela de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales,
Pontificia Universidad Católica del Ecuador, Quito, Ecuador
e-mail: fernando_torres2025@hotmail.com
Tel: +593 2 2-991-700 ext.1222

J. Román
Laboratorio de Paleontología, Instituto de Ciencias Biológicas, Escuela Politécnica Nacional
e-mail: joseluis.roman@epn.edu.ec; smilotun@yahoo.com
Telefax: +593 2 2-236-690

E. Cadena
Escuela de Ciencias Geológicas e Ingeniería, Yachai Tech
e-mail: ecadena@yachaytech.edu.ec
Tel: +593 6 2-999-130 / Ext.: 2613

ABSTRACT

The testudinids (tortoises) reached South America during the Miocene about 23 million years ago. During this period ocean currents and the geography of the American continent may have aided animals capable of drifting, like giant tortoises, to reach South America from Africa. In this study, we describe the histology of bone tissue using thin sections of fossils of giant turtles from the Tablazo formation (Pleistocene) in the province of Santa Elena, Ecuador. In addition we present a general description of the most complete specimens of giant tortoises belonging to the Museo de Historia Natural of the Escuela Politécnica Nacional collection. We concluded that the specimens belong to a turtle of the family Testudinidae, genus *Chelonoidis*. This proves that giant tortoises, very similar to the Galapagos tortoises, existed on the continent, prior to their arrival to the archipelago. Thus, gigantism evolved in the continent, and was probably one of the characteristics that allowed them to reach South America

Keywords: Testudinidae, *Chelonoidis*, histology, gigantism, drifting, Tablazo formation.

INTRODUCTION

Testudinid turtles (tortoises) arrived in South America by oceanic dispersal during the late Oligocene and early Miocene about 35 years ago (Ma), when South America was isolated from Africa, Europe, and North America (Simpson 1943, Auffenberg 1971, Zacarías, Fuente et al. 2013). Similarly, the fossil record shows the presence of large tortoises in Central and South American Miocene formations (Simpson 1943, Cadena, Bourque et al. 2012, Zacarías, Fuente et al. 2013, De la Fuente, Sterli et al. 2014, Cadena and Jaramillo 2015). During this period, Atlantic Ocean currents moving westward through the northern part of South America could have helped drifting animals to reach islands in the

Panamanian region, as well as North America. However, the Panamanian region currents moving toward the Gulf of Mexico may have restricted oceanic migration from North to South America (Tarling 1982). Some of these currents may have aided turtle migration from Africa to South America. The most significant migration routes between North and South America occurred during the Miocene and Pliocene; during this time, the distance between the two continents decreased, leading to the formation of the Isthmus of Panama. The absence of clear migration routes during the estimated time of colonization, in addition to the direction of the oceanic mainstream, and the fact that Africa was closer to South America during the late Eocene and early Oligocene, suggests that the origin of the lineage that gave rise to the giant Galápagos tortoises lies in the African continent, as recent phylogenetic data shows (Tarling 1982, Crumly 1984, Meylan and Sterrer 2000, Le, Raxworthy et al. 2006, De la Fuente, Sterli et al. 2014), opposed to the North-Central American or Antilles origin proposed by several authors (Simpson 1942, Simpson 1943, Auffenberg 1971, Broin 1991, Gerlach 2001).

Tortoises have developed a waterproof integument and a great ability to float, characteristics that facilitate their transoceanic dispersal (Tarling 1982, Meylan and Sterrer 2000). However, the geographical source of tortoises in South America is still controversial.

During the Pleistocene giant tortoises were distributed in almost all continents except Australia and Antarctica, today they are restricted to two oceanic archipelagos (the Galápagos and Seychelles) (Caccone, Gibbs et al. 1999). Galápagos giant tortoises (measuring up to 1.5 meters long) are included in the genus *Chelonoidis* (*Geochelone*) (Gerlach 2001, Le, Raxworthy et al. 2006, De la Fuente, Sterli et al. 2014, Poulakakis, Edwards et al. 2015, Torres-Carvajal 2015), with 12 living species: *C. hoodensis, C. chathamensis, C. abingdoni, C. ephippium, C. darwini, C. vandenburghi, C. microphyes, C.*

guntheri, C. vicina, C. elephantopus, C. becki and the recently described *C.donfaustoi* (Poulakakis, Edwards et al. 2015). Despite this important number of genetically defined Galápagos species, morphologically speaking the Galápagos tortoises have been traditionally considered as belonging to a single species *C. nigra* (Fritz, Alcalde et al. 2012, De la Fuente, Sterli et al. 2014, Itescu, Karraker et al. 2014), with identical skull morphology and two distinct carapace morphs according to their habitat (domed, for tortoises living in hot and humid areas and saddleback, for those in low and arid areas). Based solely on the morphology of the shell it has been argued that the extinct *C. gringorum* from the northern part of the formations of Patagonia and Sarmiento (early Miocene), close to the Argentinian Chaco, represents the closest extinct relative to the giant Galápagos tortoises (Auffenberg 1971). In previous studies (Auffenberg 1971, Sánchez 2013) *C. gringorum* is considered an ancestral species of *C. chilensis* based on the presence of a depressed shell with circular outline as seen from above, a slightly bifurcated epiplastron and a proportionately long pygal (anal) plate. In addition, the geographic distribution of the extinct *C. gringorum* overlaps with that of the living species *C. chilensis*, which is the living species most closely related to giant Galápagos tortoises (Le, Raxworthy et al. 2006), so it can be argued that *C. gringorum* represents the lineage that gave rise to both the Galápagos tortoises and *C. chilensis*, or together with *C. chilensis* it forms the sister group of Galápagos tortoises.

Although *C. chilensis* is much smaller in size (it can reach a maximum straight-line carapace length of 36 cm approximately), it is unlikely that the direct ancestor of the Galápagos tortoises is a turtle with a smaller shell, as gigantism in tortoises seems to be an important pre-adaptation for successful colonization of remote oceanic islands, as opposed to a feature that evolves on islands due to adaptation to different environmental conditions (Jaffe, Slater et al. 2011). Colombian fossil records show that during the early Miocene (14

Ma) tropical South American turtles had a tendency to gigantism (Podocnemididae, Chelidae), suggesting that other groups of large tortoises other than Testudinidae may have reached South America by trans-oceanic drifting (Cadena and Jaramillo 2015). Moreover, the fossil record shows continental extinct members of *Chelonoidis* within a clade that also contains *C. chilensis* (Auffenberg 1971, Caccone, Gibbs et al. 1999).

It has been estimated that the separation between *C. chilensis* and the Galápagos tortoise lineage took place at approximately 3.2 Ma, while the oldest island in the Galápagos, at the present time, has an age less than 5 Ma (Caccone, Gibbs et al. 1999, Poulakakis, Russello et al. 2012). Although precursors of these islands may have been available for colonization for a longer period of time than was previously thought (Christie, Duncan et al. 1992, Werner, Hoernle et al. 1999, Hoernle, van den Bogaard et al. 2002, Torres‐Carvajal, Barnes et al. 2014), the presence of giant continental fossils, transoceanic drift as a probable mean of dispersion, and gigantism as a possible requirement for dispersion, are sufficient grounds to hypothesize that the separation between *C. chilensis* and the lineage of Galápagos tortoises happened on the continent (Caccone, Gibbs et al. 1999). Additionally, the first speciation event of Galápagos tortoises is estimated to have happened 2 Ma, which is consistent with the idea of a diversification within the archipelago (Caccone, Gibbs et al. 1999, Jaffe, Slater et al. 2011).

Giant tortoise fossils in continental Ecuador have been reported by Hoffstetter (1952) from Santa Elena peninsula and more recently from Punta Canoa by Cantalamessa (2001). Both studies suggest that these fossils differ morphologically (mainly in general thickness) from the Galápagos giant tortoises (Hoffstetter 1952, Cantalamessa, Di Celma et al. 2001). In addition, a giant tortoise Holocene fossil identified as *Geochelone elephantopus* (*Chelonoidis nigra*) was reported from Isla Floreana (Galápagos islands) (Steadman and Steadman 1986). These fossils provide an excellent opportunity to gain insights about the possible origins of Galápagos tortoises, supporting the ideas of (i) gigantism as a feature acquired before trans-oceanic colonization, and (ii) a continental colonization (i.e., South America) prior to the colonization of the Galápagos Islands.

The turtle shell provides a great source of histological and morphological information that can be used to infer aspects of the origin and natural history of these animals (Scheyer and Sánchez-Villagra 2007). Analysis of bone histology and dermal bone microstructures can be correlated to other variables such as habitat, body size, and age, although bone microstructures can also be constrained by phylogenic history. The structure of the dermal bone exhibits three well-differentiated layers: external cortex, cancellous bone and internal cortex. The external cortex is the most external layer of bone, which is in contact with the osteoderms and is often poorly vascularized. The cancellous bone is mostly composed by bone trabeculae and vascular spaces. The internal cortex is the most internal layer, and it is in contact with soft tissue structures like muscles (Scheyer and Sander 2007, Cadena, Ksepka et al. 2013). Recent studies (Cadena and Schweitzer 2012, Cleland, Schroeter et al. 2015, Cadena 2016) report great microstructure (osteocytes and blood vessels) preservation, and in cases that show exceptional preservation of soft tissue elements, even organic molecules like proteins may be preserved in fossils over extended time periods.

Geology of the Santa Elena peninsula

The Santa Elena Peninsula (Fig. 1) is geologically formed by marine terraces known as tablazos that emerged from the Pleistocene to the Holocene (Lindsey and Lopez 2015). The changes in sea level during the Quaternary, followed by a renewed rise in recent times, have left extensive horizontal deposits of these marine terraces exposed (marine transgression) (Tablazo Formation: Quaternary).

The Tablazo formation (Bosworth, Woods et al. 1922) is composed of fossiliferous sandstone and has been divided in three sections, from top to bottom: Tablazo I (Lower Pleistocene), Tablazo II (Middle Pleistocene), and Tablazo III (Upper Pleistocene) (Bosworth, Woods et al. 1922, Lindsey and Lopez 2015). This formation can exceed 40 meters in thickness, and is composed of calcareous sandstone, sand, fine sandy limestones and conglomerates with abundant gastropods, bivalves, vernacular echinoderms and other fossils. These deposits are cut by numerous dry river beads that contain water only during rainy periods and during strong episodes of the "El Niño" current (Spillmann 1941, Lindsey and Lopez 2015).

At La Carolina locality, layers of river sediment deposits corresponding to an estuary have replaced Tablazo III. The sandy layers are impregnated with thick rusty oil that probably has gone up by rupture cracks in Tertiary deposits of the subsoil (Sauer 1965).

In the peninsula of Santa Elena cycles of transgression and regression have a strong influence in the sediment layers, of which the moments of transgression formed the platforms (Tablazos) followed by a strong tectonic process that elevates the older platform (Tabazo I) to its current position at the top of the lithostratigrafic unit, and leaving the newest platform (Tablazo III) at the base of the unit, (Hoffstetter 1948, Hoffstetter 1952, Ficcarelli, Coltorti et al. 2003); as opposed to the proposal made by Marchant (1961), which states the existence of a single terrace.

The mammal fauna of sites "La Carolina" and "El Cautivo" was found within the lithostratigraphic unit that characterizes the terrace Tablazo III, which Hoffstetter (1952) attributed to the Upper Pleistocene (Ficcarelli, Coltorti et al. 2003). Franz Spillmann was the first who carried out excavations between 1931 and 1940 especially in camps of the "Carolina Oil Company" at that time. Later, Hoffstetter (1948, 1952), and Ficcarelli et al. (2003) were able to obtain and study a large number of vertebrate specimens, especially mammals, birds and reptiles of great size, that have been assigned to the end of the Pleistocene.

Here we describe new fossil fragments of a giant tortoise from Santa Elena peninsula (continental Ecuador), as well as previously collected fossils from the same province, examine for the first time their bone histology and microstructures, and discuss possible implications of these fossils on the origin of Galápagos giant tortoises.

MATERIALS AND METHODS

I examined previously collected samples from Salinas and La Libertad in Santa Elena province. Additionally, new samples were collected in Atahualpa locality in the same province (Fig. 1). All samples were deposited in the paleontology collection at Escuela Politecnica Nacional in Quito, Ecuador.

Previously collected samples had not been subject to treatment for preservation; therefore a process of restoration and cleaning was performed in some of the samples. For this purpose small tools (brushes, needles, spatulas) and an alcohol soluble consolidator (polyvinyl alcohol) were used; for more feeble samples, Paraloid B72 mixed with paint thinner was used.

To obtain new samples Quaternary sediments were located, specifically belonging to Upper Pleistocene, were the probability of finding mega-fauna fossils is high (Spillmann 1941, Lindsey and Lopez 2015). Such locations were found using geologic and topographic maps, as well as information from previous expeditions. Five potential localities were chosen, where excavations were performed using big and small tools. Once samples were obtained they were treated with polyvinyl alcohol and wrapped in tin foil for transportation.

Some of the samples in the collection did not provide significant taxonomic information. Therefore some of them were selected to make thin sections to explore the potential preservation of osteocytes (one of the most abundant cells in bone tissue).

Thin section preparation

For thin section preparation four samples of marginal carapace from the paleontology collection at Escuela Politecnica Nacional (La Carolina locality; V-1356, V-497, V-1363, V-1361), were used. Procedure follows Green (2001) and Cadena and Schweitzer (2012). Explained briefly, the sample is cut in cross-section with the bedding plane using an industrial saw, trimmed to fit in a standard glass slide (Green 2001). One side is prepared by grinding it at high speed on a horizontal diamond impregnated wheel until it is approximately 5 cm thick (Green 2001). Then the sample is mounted onto the glass slide using epoxy resin. After the resin has dried at room temperature or using a hot plate, the sample is brought down to the desired 60-150 µm thickness (Cadena, Ksepka et al. 2013) using caborundum powders by successively applying finer grade powder and washing the sample in every grade transition to avoid cross contamination of the plates with different grades of powder, thus reducing the possibility of scratching the sample (Green 2001).

Osteocyte preservation

Fragments of bone from carapace from five different specimens from the Atahualpa locality were treated in ethylene diamine tetraacetic acid (EDTA; 0.5M; pH8) adding EDTA solution every 2 days. After 5 and 10 days, 1ml of the solution was placed in a glass slide, covered with coverslips, and observed in an optical microscope (Cadena and Schweitzer 2014).

RESULTS

Bone histology

As the specimens stored in the paleontology laboratory from the Escuela Politecnica Nacional have not been characterized as one or various individuals, bone histology is described individually for each thin section prepared.

V-1356 (La Libertad) (Fig. 2) Peripheral plate fragment

External Cortex: It is highly vascularized, which can be observed in the high density of primary vascular canals and primary osteons that are embedded in a matrix of interwoven structural collagenous fiber bundles that are arranged parallel and oblique to the external surface of the bone.

Cancellous bone: This portion is composed mostly by secondary osteons; their size seems to decrease near the cortical areas. Primary vascular canals can be observed scattered through all the callous section, with its density increasing towards the cortical portions.

Internal cortex: Less vascularized than the external cortex, primary osteons and primary vascular canals are present but in less quantities. The fibers of the collagenous matrix have a parallel disposition. Some of the vascular canals reach the external surface of the bone as small foramina.

V-497 (La Libertad) (Fig. 3) Costal plate fragment

External cortex: Scattered primary vascular canals can be observed in low quantities, primary osteons are present as well, but in even fewer numbers. Fiber bundles have primarily a parallel disposition but some are oblique.

Cancellous bone: Secondary osteons surrounded by lamellar bone tissue are predominant in this section, although some primary osteons can also be observed.

Internal cortex: Higher vascularization than the external cortex due to higher density of primary vascular canals, primary osteons are present in similar quantities as observed in the external cortex. Fibers have a predominantly parallel arrangement.

V-1363 (La Libertad) (Fig. 4) Peripheral plate fragment

External cortex: It exhibits a moderate vascularization; primary osteons are present while primary vascular canals are almost absent. Fiber bundles have mostly a parallel arrangement but some can be perpendicular or oblique.

Cancellous bone: Conformed primarily by secondary osteons surrounded by lamellar bone tissue. Some primary vascular canals and secondary osteons are scattered through the cancellous bone in the trabeculae.

Internal cortex: Moderate vascularization, abundant primary vascular canals are present, primary osteons are also present but in low quantities. Fibers have a perpendicular and oblique disposition; Sharpey fibers are present. Vascular canals extend to the bone surface as foramina.

V-1361 (La Libertad) (Fig. 5) Costal plate fragment

External cortex: Primary vascular canals have a moderate distribution and very few primary osteons are observed. Most of the fiber bundles have a parallel arrangement but some are oblique as well.

Cancellous bone: Secondary osteons are predominant. Some primary vascular canals can be observed scattered through the lamellar bone, as well as a few secondary osteons.

Internal cortex: High levels of vascularization given by primary osteons, these are predominant and bigger than the ones observed in previous samples; some primary vascular canals are present. Fibers bundles have a parallel arrangement and Sharpey fibers can be observed indicating proximity to a costal bone or a muscle insertion. No foramina are present.

Osteocytes

In the thin sections osteocytes can be observed and two morphs are present: flattened osteocytes and stellate osteocytes (Fig. 6); the presence of these morphs vary depending on the bone layer that is being observed. Flattened osteocytes are found on the internal cortex, in the lamellar bone surrounding primary osteons, while in cancellous bone they are found surrounding secondary osteons. Stellate osteocytes are more common in the interstitial lamellae between osteons, primarily between primary osteons in the external cortex and between secondary osteons in cancellous bone. Isolated Haversian canals can also be found (Fig 8).

While most osteocytes are degraded (Fig. 7a, 7b), some are well preserved (Fig. 7c, 7d). Flattened osteocytes (Fig. 6a, 6b) are 50-100 µm long and 5-30 µm wide, they present filipodia that in some cases can have secondary ramifications, or have no filipodia at all (Fig. 7D). Usually they show a clear green color on the interior and some of the most well preserved ones exhibit blue colored dots (Fig. 6a, 6c, 7b, 7c and 7d), in some cases they may show yellow round structures that resemble nuclei (Fig. 7c). Stellate osteocytes (Fig. 6c) have a rounder shape, they are far more uncommon than flattened osteocytes, they are 25-40 µm long and 25-30 µm wide; filipodia are distributed more randomly than in flattened osteocytes, ramifications are bigger and tertiary ramifications can be seen (Fig. 6c). Colors are the same as those observed in flattened osteocytes.

Systematic Paleontology

Order TESTUDINES Batsch, 1788

Family TESTUDINIDAE Gray, 1825

Genus CHELONOIDIS Fitzinger, 1835

Sp. Indt.

Referred material— V-1124, V-1132, V-1144, V-1145, V-1146, V-1149, V-1154, V-1356, V-1362, V-1384, V-1386, V-4983, V-4987, and V-6291.

Occurrence—Most specimens were collected by Robert Hoffstetter from La Carolina locality in Santa Elena province in Ecuador. Specimens were found in the Tablazo formation, specifically in the Tablazo III lithostratigrafic unit that corresponds to late Pleistocene. Specimen V-6291 was collected by Fernando Torres on the Atahualpa locality in the same province, and on the same formation.

Diagnosis— The specimens described can be assigned to Testudinidae due to the presence of characteristics such as thick carapace bone plates, costal plates moderately arched indicating a domed shaped carapace, and pronounced horizontal crest in marginal plates (Auffenberg 1974, Pritchard and Trebbau 1984, De la Fuente 1988, Ernst and Barbour 1989, Cabrera 1998). Due to the fragmentation of the specimens the synapomorphies for the *Chelonoidis* genus could not be found, nevertheless because of the similarities with other *Chelonoidis* that occur in South America, such as size, terrestrial habitat, domed shape carapace, peripheral plates wider towards the medial edge, and cervical plate absent, we tentatively refer this specimen to *Chelonoidis* (Cabrera 1998).

Description—V-1146 (Fig. 9), complete nuchal plate, with hexagonal form, the dorsal surface presents a V-shaped sulcus in the anterior region that continues downwards dividing the plate in 3 regions corresponding, from top to bottom, to the vertebral, and the first right and left marginal scutes from the epidermal shield cover of the carapace. In the lateral margin of the segments corresponding to the marginal scutes the sutures with the first peripheral plates can be seen in the anterior region. The posterior region bears the suture with the first costal plates. The top suture that joins with the first nuchal plate presents little damage. The ventral surface presents a conspicuous crest where the latissimus dorsi muscle inserts. This crest corresponds also to the boundary that separates the vertebral and marginal shield regions in the dorsal surface. The specimen shows notorious dragging marks.

V-1149 (Fig. 10), left hypoplastron is slightly rectangular as preserved, with fragments from left anterolateral and posterolateral ends missing, spaces between fragments are filled with plaster. The ventral surface has a slight concavity medially. The central suture that joints left and right hypolastron is found towards the medial edge. In the anteriormost edge there is another suture for the joint with the left hyoplastron. In the posterior edge, medially, a fragment of the suture that joins the hypoplastron with xiphiplastron can be seen. In the posterior part of the ventral surface there is a sulcus that corresponds to the edge between the abdominal and femoral scutes, anterior and lateral to this sulcus another one can be observed, but with a curvature that is the continuation of the same sulcus. The dorsal surface is smoother than the ventral one, and it exhibits a slight convexity. No sulci can be observed on the dorsal surface but several dragging marks are present.

V-4983 (Fig. 11), fragment from a left hypoplastron missing a portion of its anteromedial region, only the inguinal part of the bone is preserved. On the ventral surface several dragging marks are present, the sulcus that represents the edge between the abdominal and femoral scutes can be observed. On the external surface a prominent invagination is present, and the sulcus from the inguinal scute can be observed lateral to this invagination. There is a curvature on the dorsal surface that is followed by the marginal plates from the carapace. In the anterior, posterior and interior edges, only the fragmentation of the bone is observable.

V-6291 (Fig. 12), fragmented peripheral plate, the specimen was restored and it has various parts with fragmentation. The sulcus corresponding to the edge between marginal scutes is visible on the dorsal and ventral surface. Horizontal crest on the ventral surface can be observed. The size and curvature of the specimen shows that it is a posterior peripheral plate.

V-1386, V-1356, V-1384, V-1124, V-1144 and V-1145 (Fig. 13), peripheral plates, samples V-1384, V-1356, V-1145 and V-1144 are fragmented and only one of the sutural edges is observable. Only in samples V-1124 and V-1386 both sutural edges can be observed. On the ventral and dorsal surfaces of all the samples, except in sample V1144, the sulcus corresponding to the edge between marginal scutes can be observed. On samples V-1124 and V1386, there is a crest on the ventral surface where the intercostal muscles insert.

V-1132, V-1154, V-4987 (Fig. 14), costal bones, most of the plates are well preserved. In sample V-1132 there is a sulcus along the dorsal surface that corresponds to the edge between pleural scutes. Specimens V-1154 and V-4987 correspond to a costal plate where pleural scutes sulci are not visible. On the ventral surface of samples V-1132 and V-4987 the fusion between the costal plate and the rib is evident in the form of a crest towards the lateral edge of the plate. On the three samples both sutural edges are present. Dragging marks are clear on the three costal plate fragments.

CONCLUSIONS AND DISCUSSION

Based on the size and thickness of the samples examined in this study, we conclude that they belong to a large tortoise. Among testudinoid (Emydidae, Geoemydidae and Testudinidae) taxa, there is little variation in shell bone microstructures, with only three histotypes recognized (Scheyer 2007). Thus, based on the patterns of vascularization and microstructures observed in our samples, we conclude that the species examined in this study belongs to the clade Testudinoidea. Many features of the samples described here match those of histotype II of Scheyer (2007), however some characteristics from hitotype III can be observed and we consider or samples to be between histotypes II and III. For instance an external cortex with somehow homogeneous arrangement of fiber bundles, moderate to low vascularization on the internal and external cortex, and the presence of primary vascular canals that may extend to the bone surface forming the foramina. Moreover, our samples match the description of Testudinidae by Scheyer (2007) in that (1) the cancellous bone is formed almost completely by large secondary osteons, (2) the internal cortex is similar to the external cortex, but with lower levels of vascularization that is given mostly by primary vascular canals and some scattered primary osteons. Strikingly, the size and macro characteristics of the samples from La Carolina and Atahualpa show that these fragments once belonged to a testudinid with similar size and shell bone characteristics as the Galápagos tortoises, as well as four extinct giant *Chelonoidis* from South America found in Argentina (*C. gringorum, C. gallardoi, C. lutzae* and *C. australis*). These similarities, in addition to similarities in bone microstructure, lead us to believe that the fossils from La Carolina and Atahualpa correspond to a species of *Chelonoidis*. Unfortunately, we were not able to fully diagnose the identity of our fossil fragments, in part due to the inability to perform statistical analyses because of the lack of availability of enough specimens to have a representative sample and the great levels of fragmentation of the samples. Despite this, the fact that the fossils belong to a giant testudinid tortoise, possibly a species of *Chelonoidis*, provides insights on the evolution of giant tortoises in the Galápagos Islands. For instance, the presence of a giant tortoise in continental Ecuador as well as in Argentina, Panama, and Colombia would confirm the idea that gigantism evolved in the continent, and it would also suggest that gigantism was probably an important characteristic that helped these tortoises reach South America, most likely from Africa as recent molecular systematic studies suggest (Le, Raxworthy et al. 2006). Some giant tortoises fossils found in the north of South America have not been identified to the species level due to fragmentation, nevertheless they have been referred as a Testudinidae that resembles the extant Galápagos tortoises; while tortoises found in Argentina are roughly the same age (Miocene), and all have been identified as a different extinct species, suggesting that the Galápagos tortoises colonized South America before it reached the archipelago where it diversified in the species we know today. The extinction of giant tortoises in the continent may be related to climate change during the early Holocene; this idea is supported by data showing that climate change in the late Pleistocene and early Holocene caused adverse ecological conditions for the mammal megafauna, which might have influenced its extinction (Ficcarelli, Coltorti et al. 2003). On the other side extinction in the archipelago has been well documented (Van Denburgh 1914, Hansen, Donlan et al. 2010) and most happened during the 1800's due to over expedition of the islands where giant tortoises were hunted as a food source because its ability to survive long periods of time without food or water. It is important to mention that even though bone histology studies for other turtle groups (Scheyer 2007, Scheyer and Sánchez-Villagra 2007, Scheyer and Sander 2007, Cadena and Schweitzer 2012, Cadena, Ksepka et al. 2013, Cadena and Schweitzer 2014, Cleland, Schroeter et al. 2015, Cadena 2016), especially in fossils, have been performed, there are no studies that explore the bone histology of the extant Galápagos tortoises or other giant tortoise fossils. Description of the histology of such fossils will help to elucidate the identity of the continental giant tortoises presented in this study. Presence of high quantities of iron in exceptionally well preserved fossil samples may indicate the presence of organic molecules, like proteins (Cadena 2016); thus, isolation of these molecules, together with comparative analyses may also provide valuable information concerning the origin of Galápagos giant tortoises.

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4. NORMAS PARA PUBLICACIÓN

5. ANEXOS

Figures

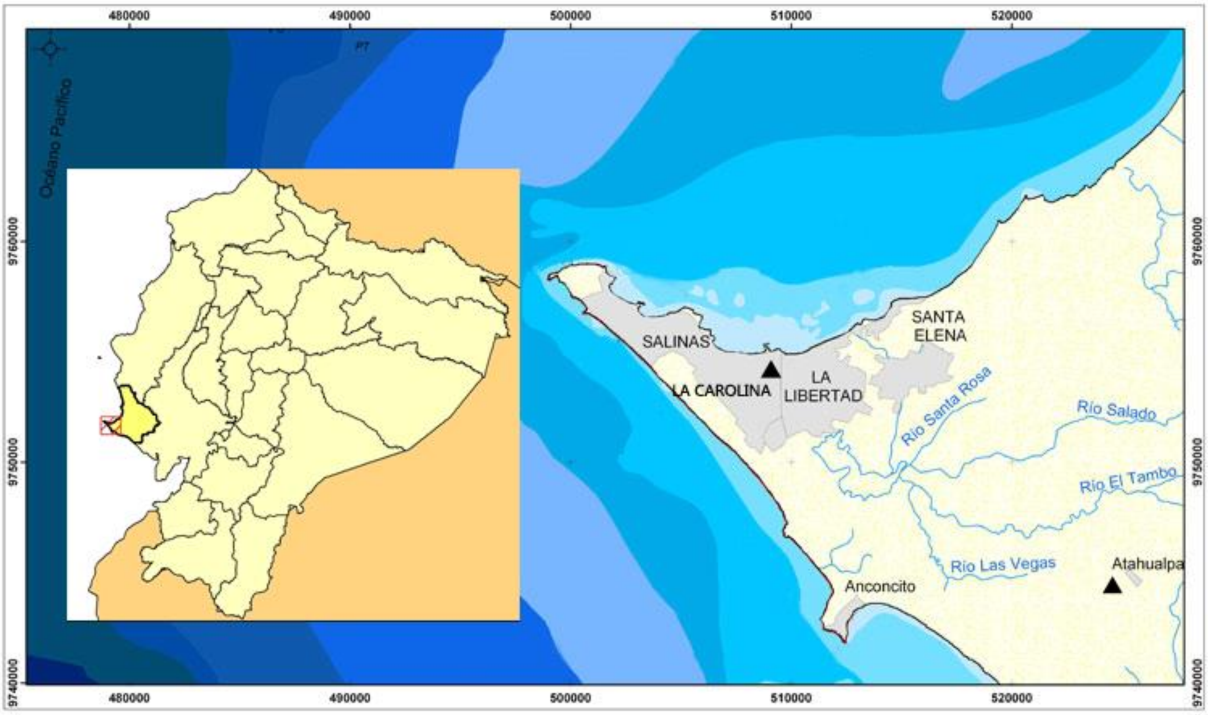

FIGURE 1. Map showing La Carolina and Atahualpa localities in Santa Elena province in Ecuador (tomado de http://www. http://institutonazca.org/es/santa-elena/)

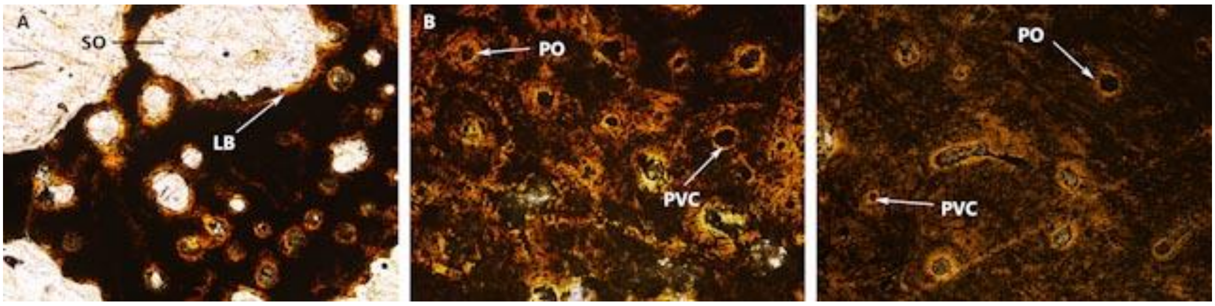

FIGURE 2. Thin section of sample V-1356 from peripheral plate fragment. **A**, cancellous bone (10x); **B**, internal cortex (10x); **C**, external cortex (10x). Abbreviations: **SO**, secondary osteons; **PO**, primary osteons; **PVC**, primary vascular canals.

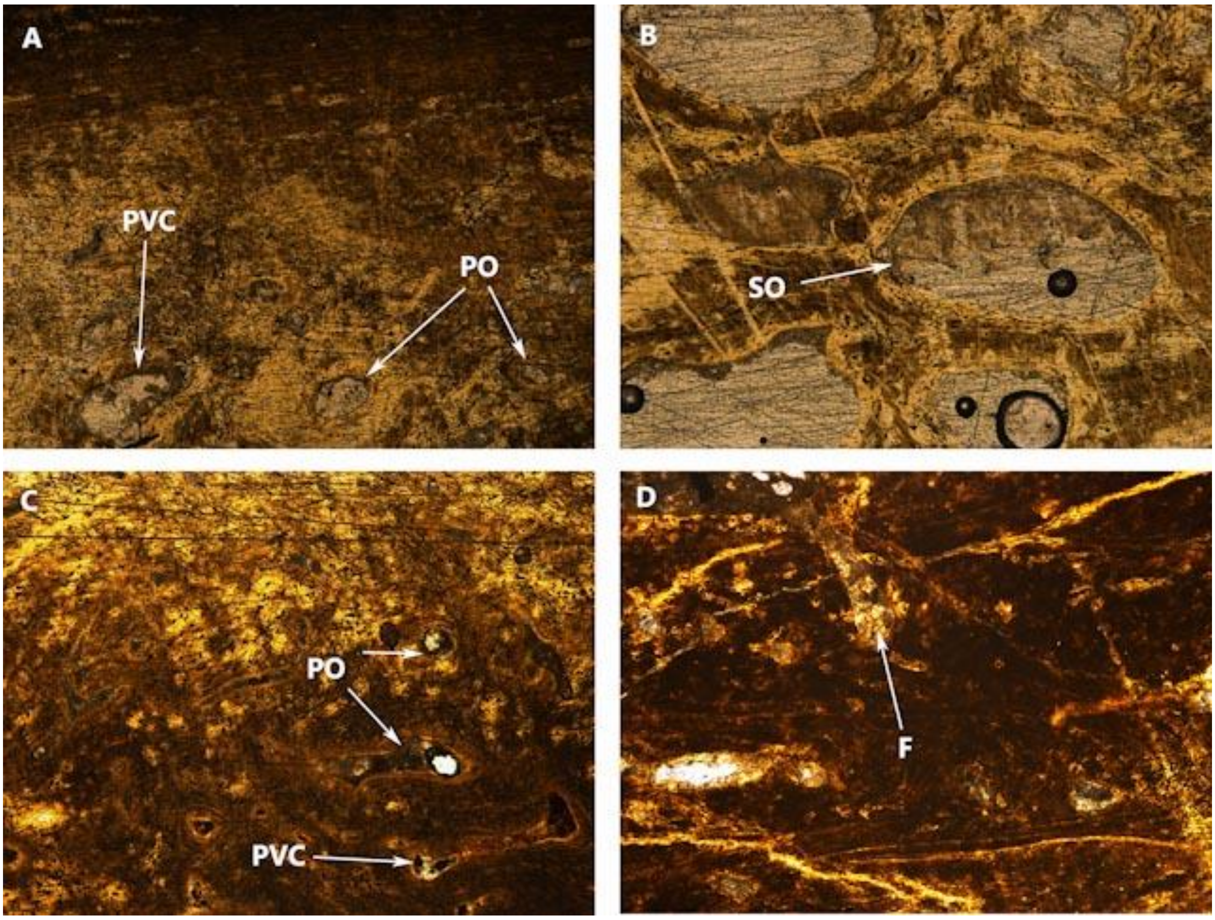

FIGURE 3. Thin section of sample V-497 from costal plate fragment. **A**, external cortex (10x); **B**, cancellous bone (10x); **C**, internal cortex (10x). Abbreviations: **SO**, secondary osteons; **PO**, primary osteons; **PVC**, primary vascular canals; **F**, foramina.

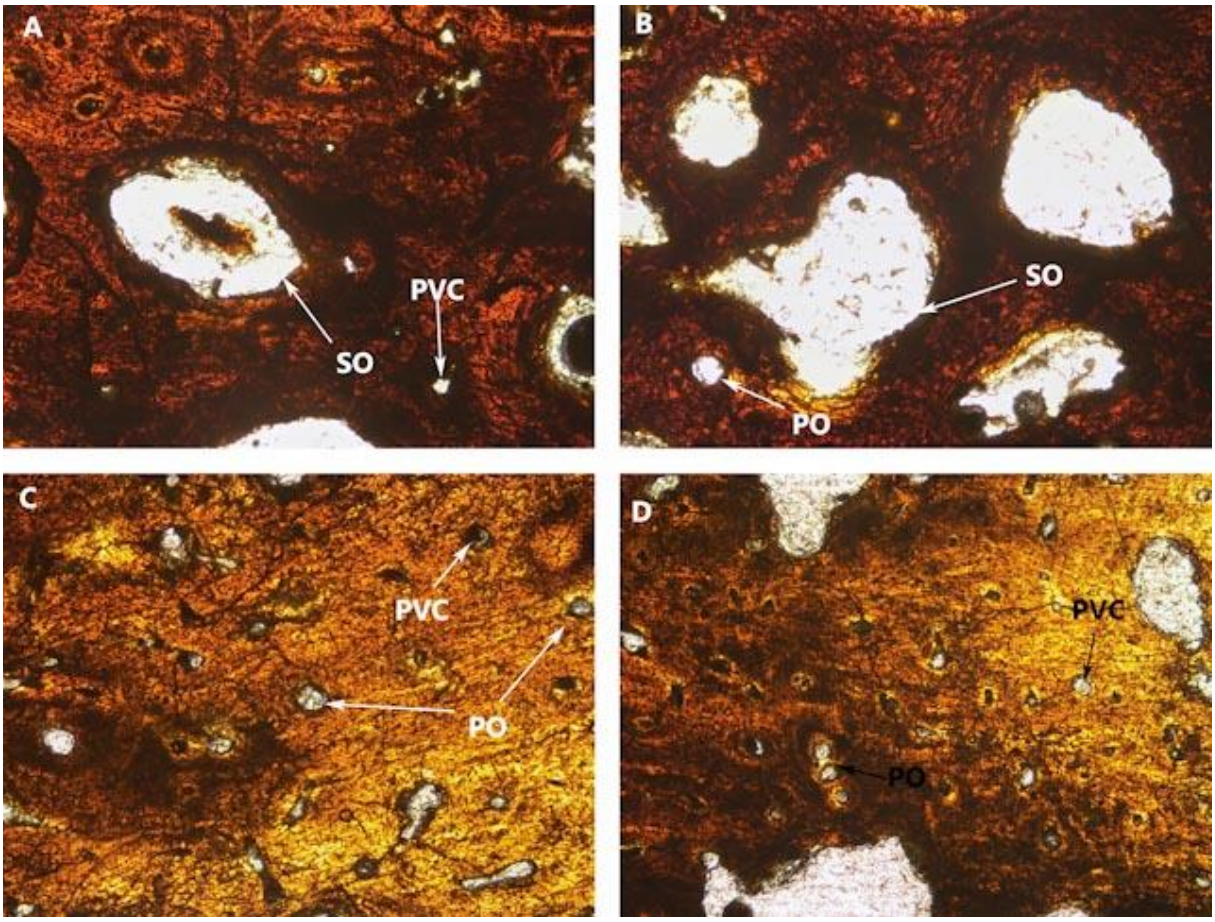

FIGURE 4. Thin section of sample V-1363 from peripheral plate fragment. **A and B**, cancellous bone (10x); **C**, external cortex (10x); **D**, internal cortex (10x). **Abbreviations: SO**, secondary osteons; **PO**, primary osteons; **PVC**, primary vascular canals.

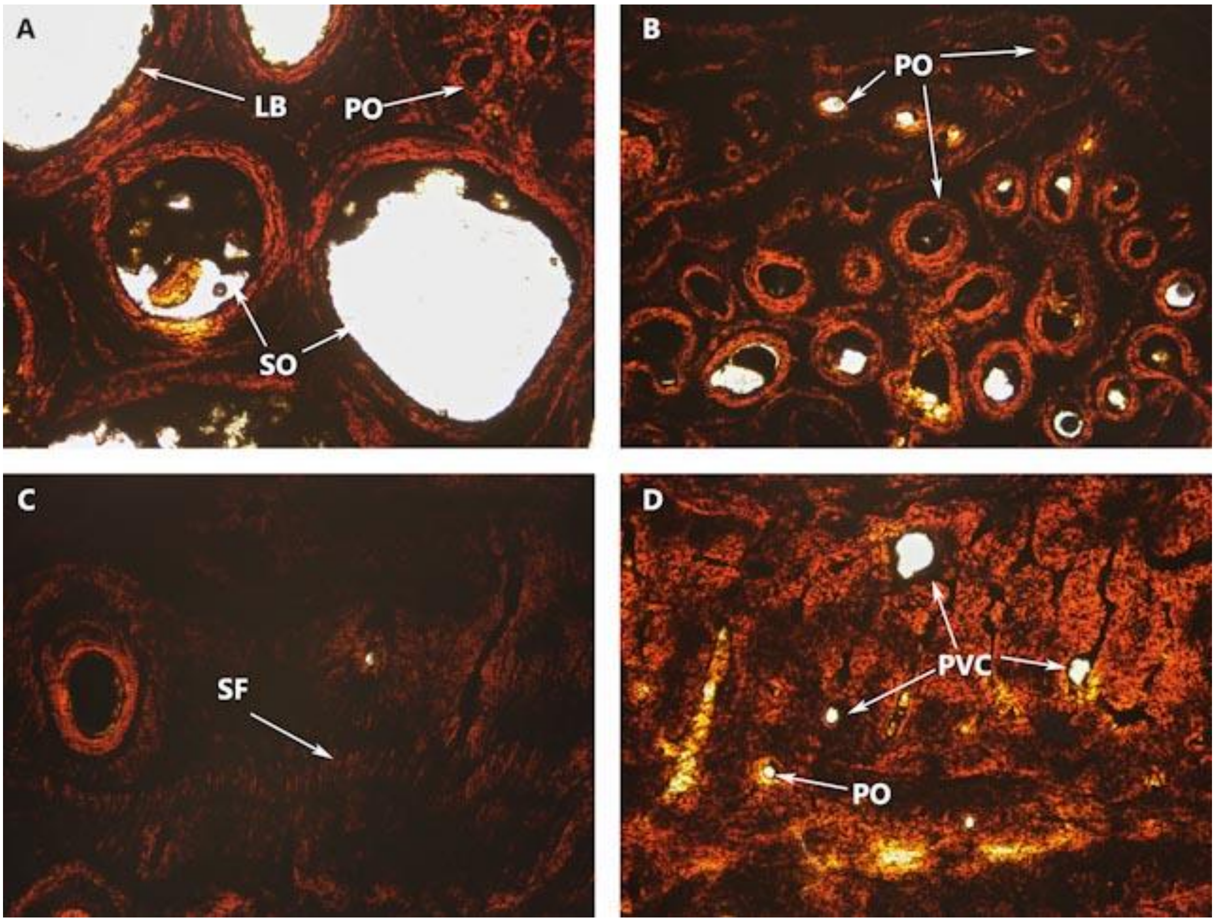

FIGURE 5. Thin section of sample V-1361 from costal plate fragment. **A**, cancellous bone (10x); **B and C**, internal cortex (10x); **D**, external cortex (10x). **Abbreviations: SO**, secondary osteons; **PO**, primary osteons; **PVC**, primary vascular canals; **SF**, sharpey fibers; **LB**, lamellar bone.

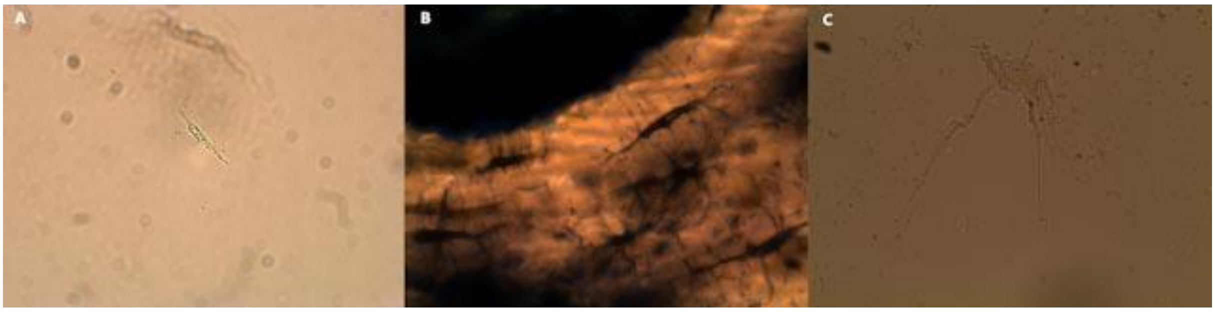

FIGURE 6. Osteocytes. **A** (40x)**, B** (100x)**,** flattened morph; **C** (100x)**,** stellate morph.

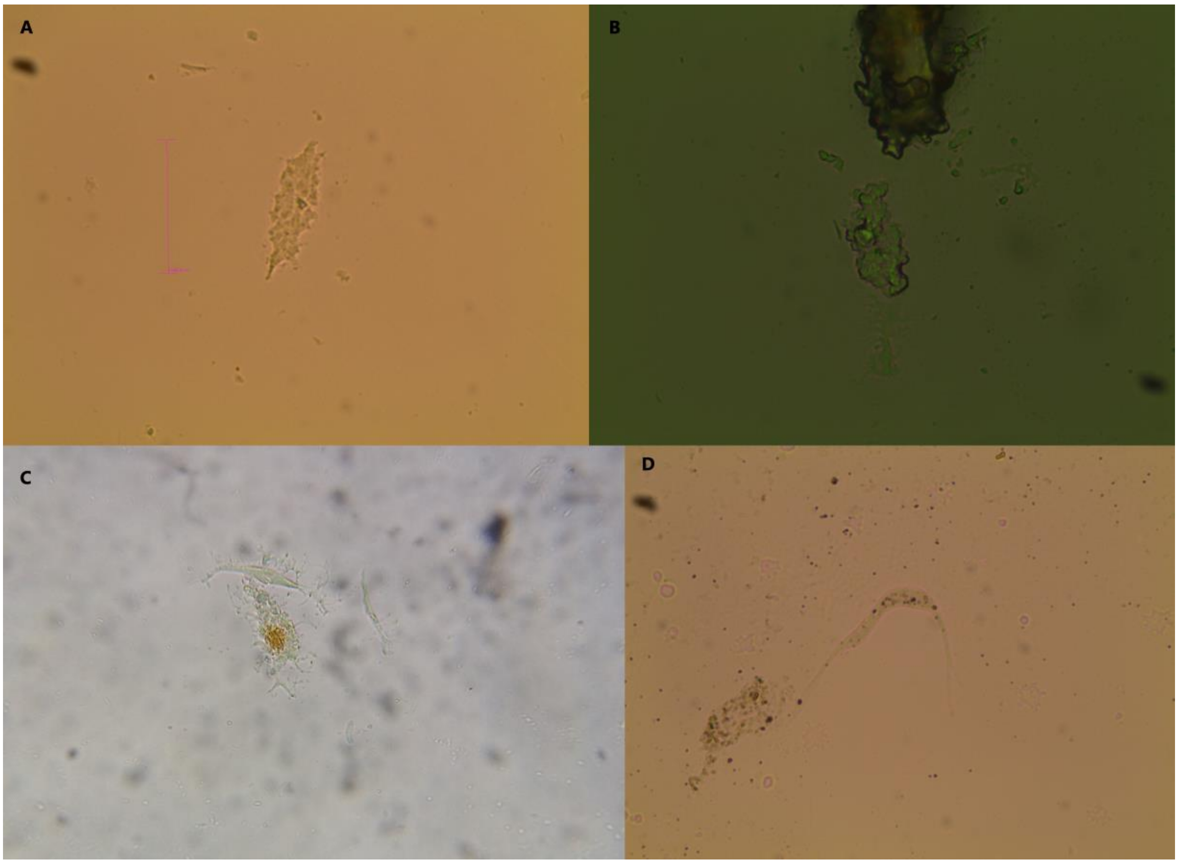

FIGURE 7. **A, B,** Isolated osteocytes that show low levels of preservation (100x). C, D, Well-preserved osteocytes (100x). **C**, filipodia present; **D**, filipodia absent.

FIGURE 8. Isolated Haversian canals (10x).

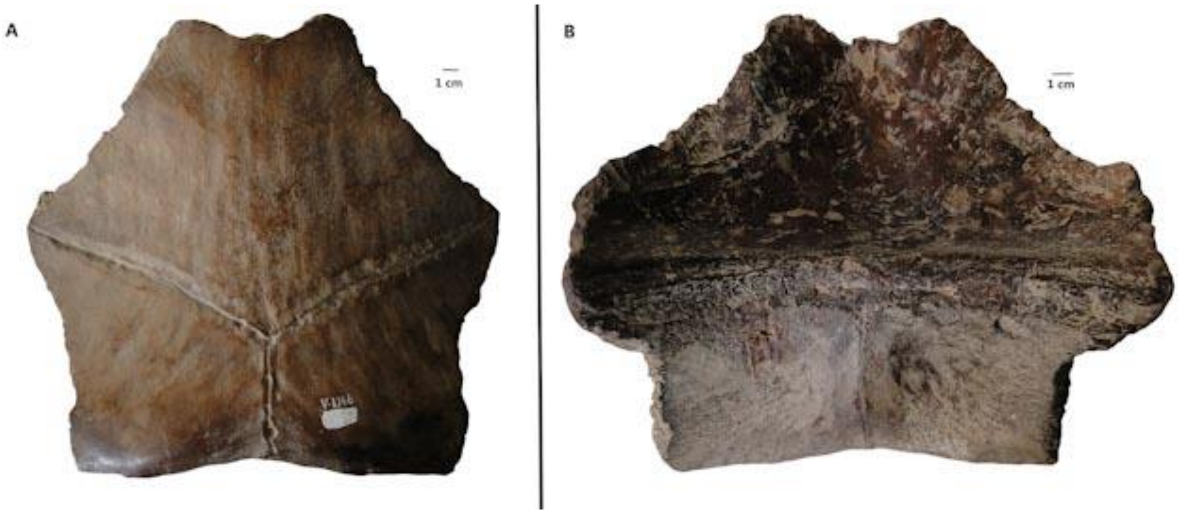

FIGURE 9. Specimen V-1146, complete nuchal plate. **A**, dorsal surface; **B**, ventral surface.

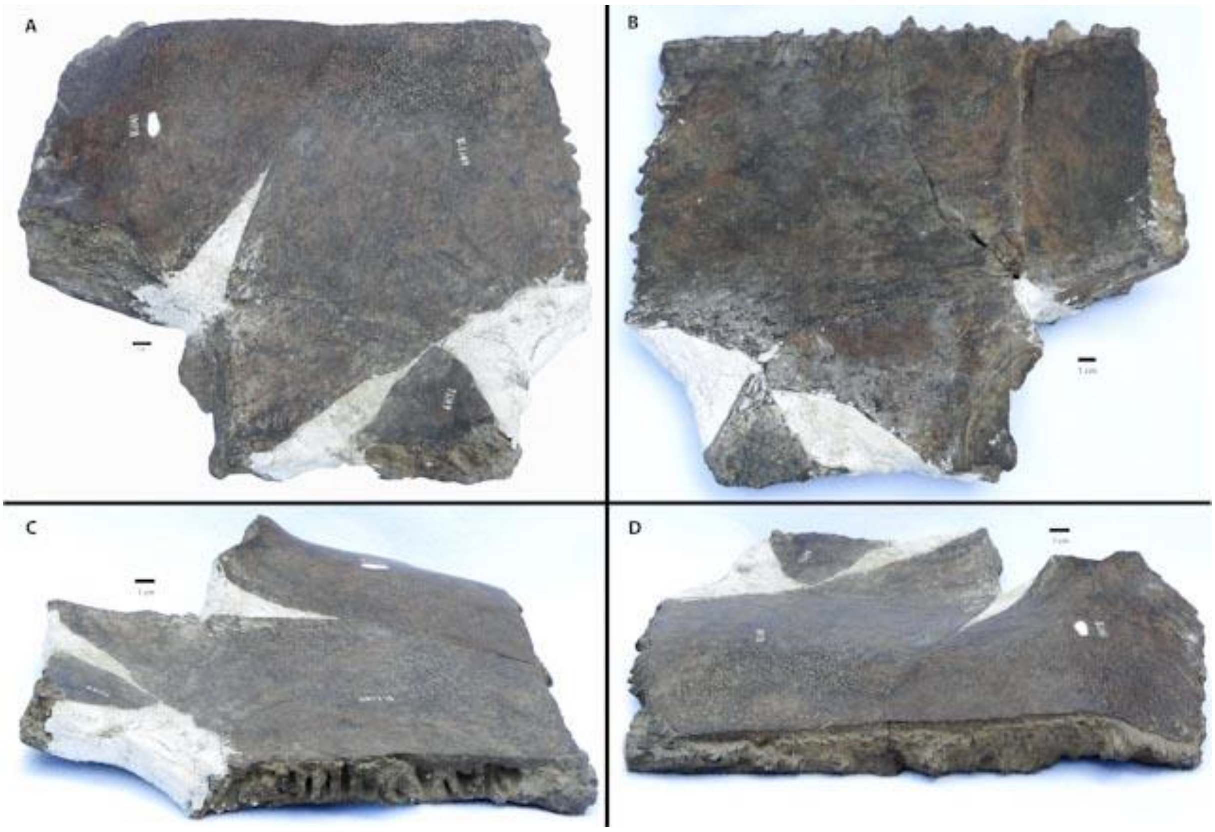

FIGURE 10. Specimen 1149, fragmented left hypoplastron. **A**, dorsal surface; **B**, ventral surface; **C**, external edge; **D**, internal edge.

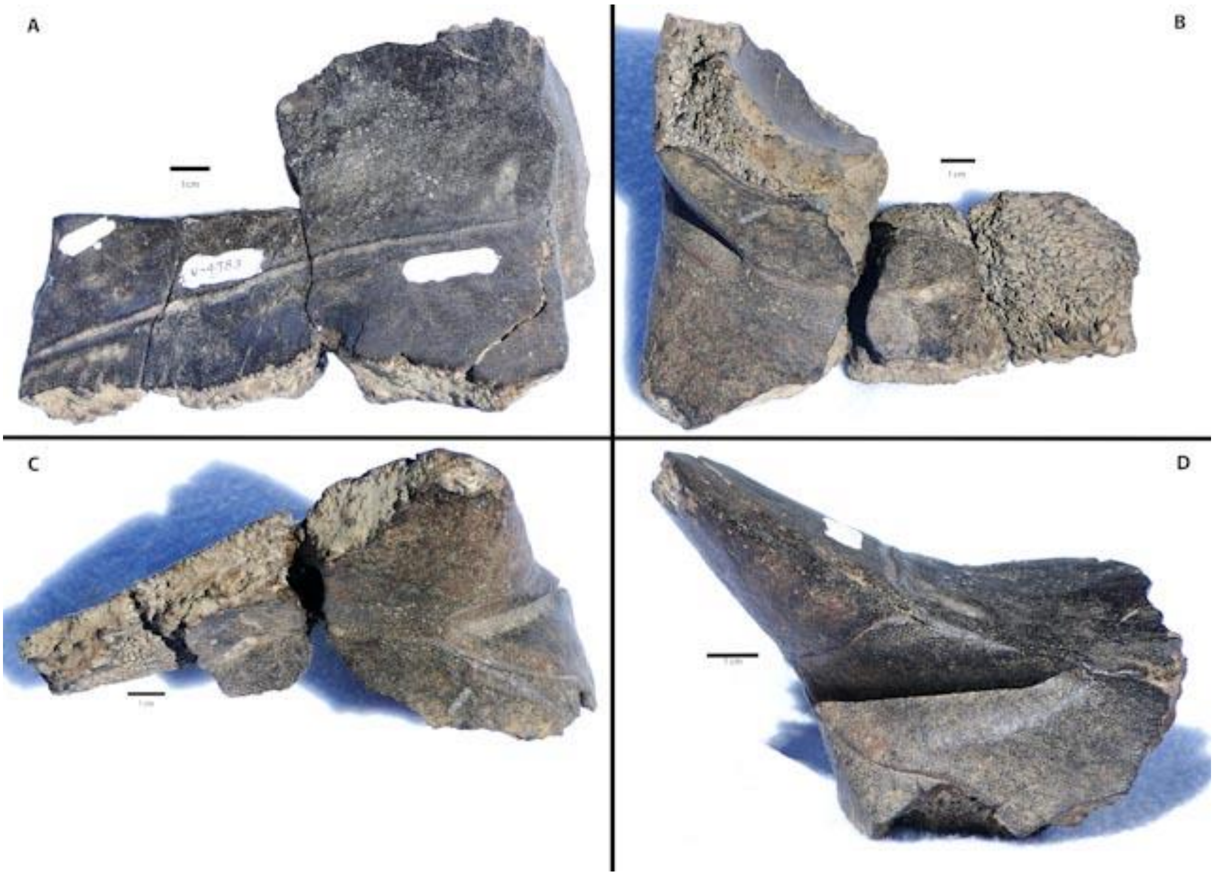

FIGURE 11. Specimen V-4983, fragment from left hypoplastron. **A**, dorsal surface; **B**, ventral surface; **C**, internal edge; **D**, external edge.

FIGURE 12. Specimen V-6291, fragmented peripheral plate. **A**, dorsal surface; **B**, ventral surface; **C**, internal edge; **D**, external edge.

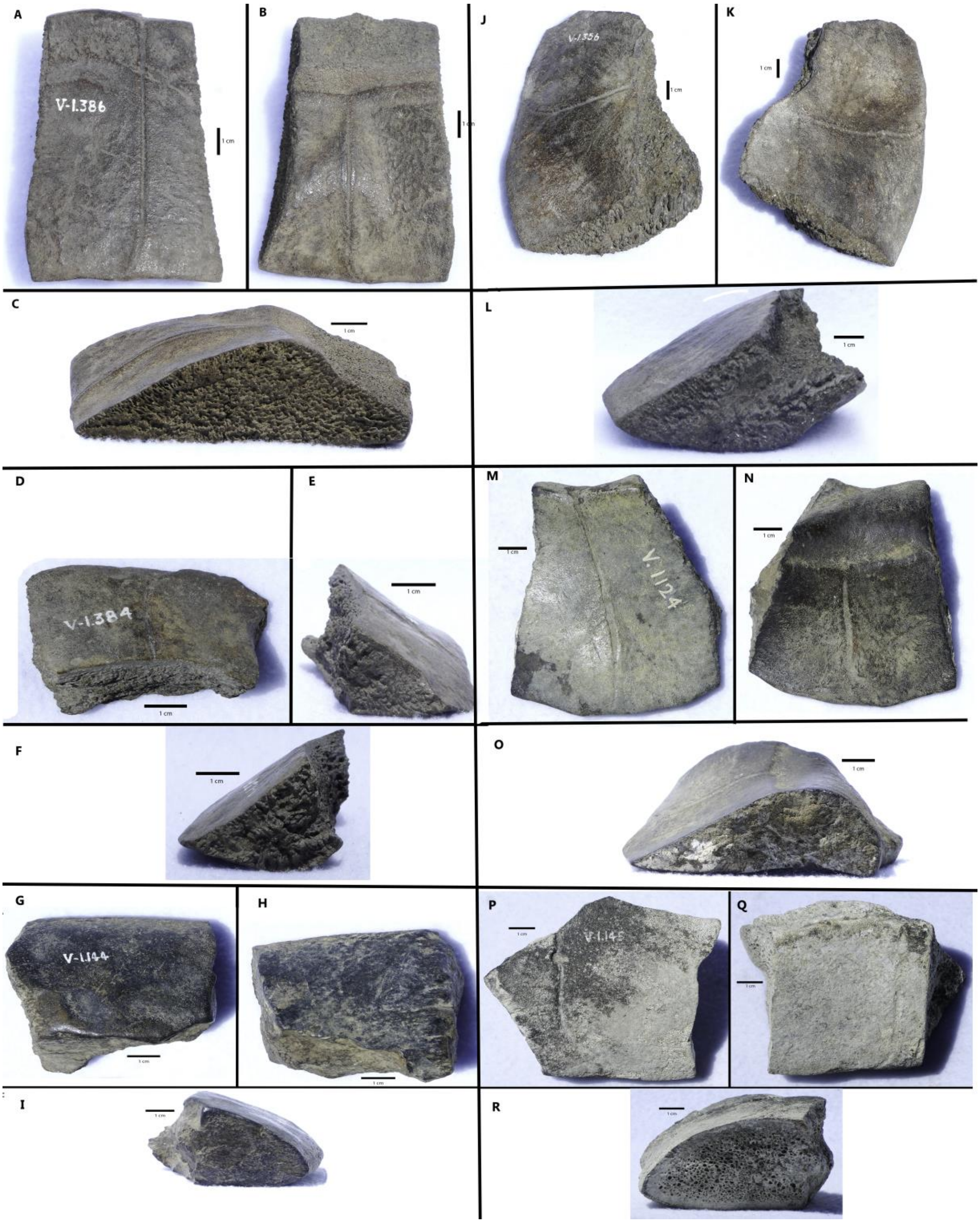

FIGURE 13. A, B, C: Specimen V-1386, peripheral plate. **A**, dorsal surface; **B**, ventral surface; **C**, sutural edge. D, E, F: Specimen V-1384, peripheral plate. **D**, dorsal surface; **E** sutural edge; **F**, sutural edge. G, H, I: Specimen V-1144, peripheral plate. **G**, dorsal surface; **H**, ventral surface; **I**, sutural edge. J, K, L: Specimen V-1356, peripheral plate. **J**, dorsal surface; **K**, ventral surface; **L**, sutural edge. M, N, O: Specimen V-1124, peripheral plate. **M**, dorsal surface; **N**, ventral surface; **O**, sutural edge. P, Q, R: Specimen V-1145, peripheral plate. **P**, dorsal surface; **Q**, ventral surface; **R**, sutural edge.

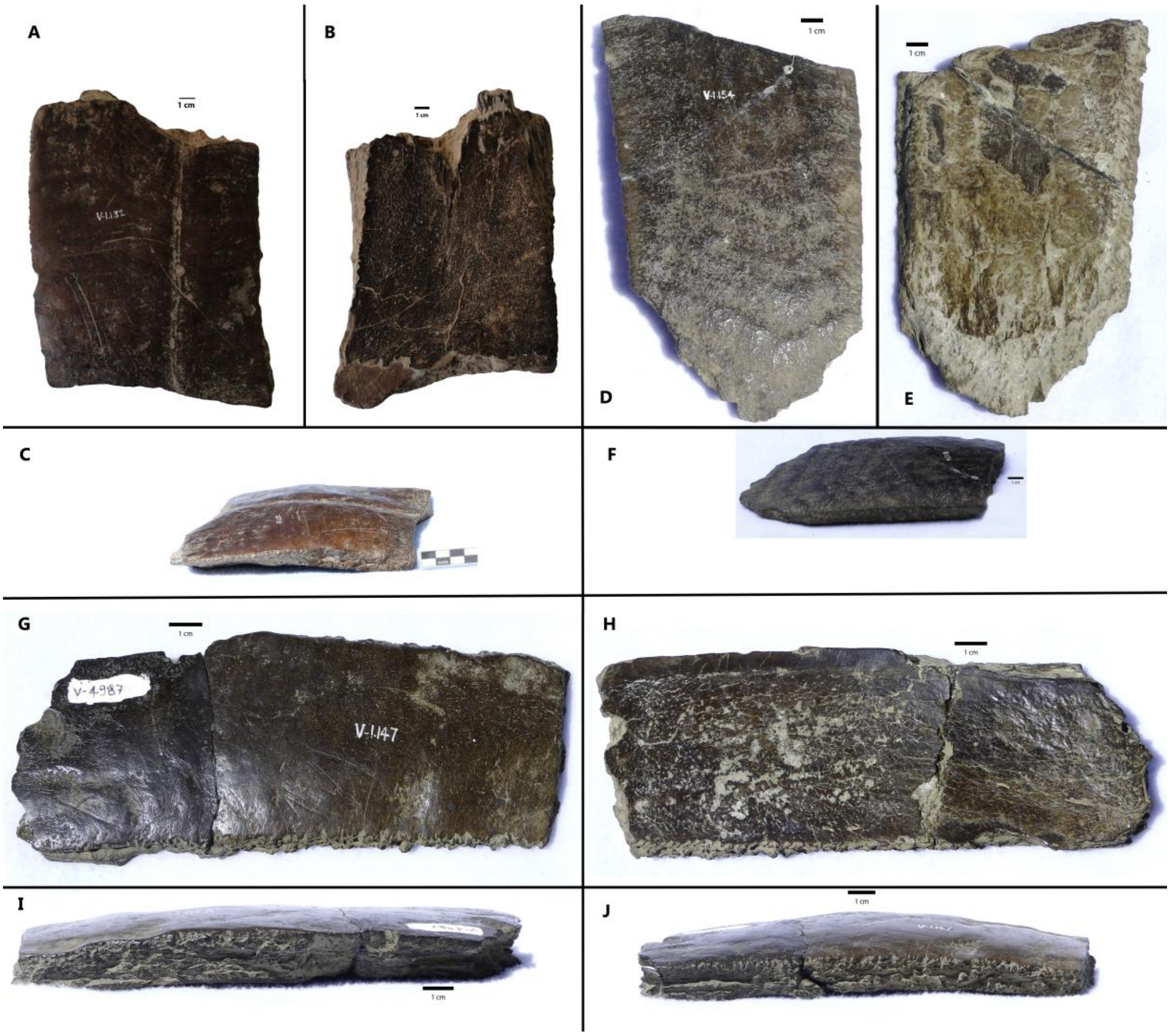

FIGURE 14. A, B, C: Specimen V-1132, costal plate. **A**, dorsal surface; **B**, ventral surface; **C**, sutural edge. D, E, F: Specimen V-1154, costal plate. **D**, dorsal surface; **E**, ventral surface; **F**, sutural edge. G, H, I, J: Specimen V-4987, costal plate. **G**, dorsal surface; **H**, ventral surface; **I**, sutural edge; **J**, sutural edge.

Tables

	Vascularization level	**Primary osteons**	**Secondary osteons**	**Primary vascular canals**	**Fiber bundles**
V1356					
E.cortex	High	Abundant	Absent	Abundant	Parallel and oblique
C. bone	High	Absent	Abundant	Scarce	Absent
I.cortex	Medium	Regular	Absent	Regular	Parallel
V-497					
E.cortex	Low	Scarce	Absent	Scarce	Parallel and oblique
C. bone	High	Scarce	Abundant	Absent	Absent
I.cortex	Medium	Regular	Absent	Regular	Parallel
V-1363					
E.cortex	Medium	Absent	Absent	Regular	Perpendicular and oblique
C. bone	High	Scarce	Abundant	Scarce	Absent
I.cortex	Medium	Scarce	Absent	Abundant	Perpendicular and oblique
V-1361					
E.cortex	Low	Scarce	Absent	Regular	Parallel and oblique
C. bone	High	Scarce	Abundant	Scarce	Absent
I.cortex	High	Abundant	Absent	Regular	Parallel

TABLE 1. Summary of the observation from the thin section of samples V1356, V-497, V-1363, V-1361.

PARA GRADOS ACADÉMICOS DE LICENCIADOS (TERCER NIVEL)

PONTIFICIA UNIVERSIDAD CATÓLICA DEL ECUADOR

DECLARACIÓN Y AUTORIZACIÓN

Yo, Fernando José Torres Chiriboga, C.I. 1713360962 autor del trabajo de graduación titulado: "HISTOLOGÍA ÓSEA DE UNA TORTUGA GIGANTE DEL PLEISTOCENO (TESTUDINIDAE) DE ECUADOR CONTINENTAL, CON COMENTARIOS DEL ORIGEN DE LAS TORTUGAS DE GALÁPAGOS", previa a la obtención del grado académico de **LICENCIADO EN CIENCIAS BIOLÓGICAS** en la Facultad de **Ciencias Exactas y Naturales**:

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