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Bone histology of Broad-snouted Caiman *Caiman latirostris* (Crocodylia: Alligatoridae) as tool for morphophysiological inferences in Crocodylia

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Abstract. Bone histology is an important tool for the interpretation of life patterns in animals of the past and extant fauna. The crocodylians have been studied as important inferential models for morphophysiological characteristics. We aimed to characterize the osteohistology of captive *Caiman latirostris*, identifying its microanatomy related to growth rates, ontogeny, and environmental conditions. We analyzed five pairs of humeri (proximal elements of the appendicular skeleton) and ribs (axial skeleton) of females' caiman. Ribs showed, in general, woven-fibered tissues, with low vascularization and parallel-fibered bone and many resorption and erosion cavities. It presented lines of arrested growth (LAGs) in three individuals, without skeletochronological compatibility. Humeri showed a gradient of woven-fibered to parallel-fibered and lamellar-zonal bone as the individuals aging. We observed compacted coarse cancellous bone (CCCB) and a higher number of LAGs in older specimens. Ribs remodel faster than humerus, showing an intra-individual histovariability. The humeri indicated an evident growth pattern with different ontogeny stages and growth rates in different ages. Fast-growing tissues are uncommon in crocodylians, but basal metabolism and optimal growth conditions can lead to this. Bone histology of *C. latirostris* shows patterns that can be used as inferential models for extant and extinct groups, but we encourage further studies for a better understanding, under different environmental conditions, such as temperature and food availability.

Keywords. Crocodylians, growth rate, ontogeny, osteohistology.

INTRODUCTION

The order Crocodylia is a monophyletic group of animals widely distributed in tropical regions, divided into three families: Alligatoridae, Crocodylidae, and Gavialidae (Zug, Vitt and Caldwell, 2001). They have great ecological importance within the ecosystems where they are inserted, as in the control of food chains (Fernández-Fernández, Arias and Khazan, 2015) and supply of nutrients from the aquatic environment (Fittkau, 1973), besides their economic relevance through meat and skin trade (Caldwell, 2017).

In South America, Broad-snouted Caiman *(Caiman latirostris* Daudin, 1801) is one of the most abundant species of Alligatoridae family, especially in Brazilian territory (Coutinho et al., 2013). It is considered as a medium-sized crocodylian with records of up to three and a half meters, although free-living specimens hard-

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ly exceed two and a half meters (Verdade, Larriera and Piña, 2010). It is commonly found in lentic freshwater ecosystems, such as dams and ponds (Filogonio et al., 2010), and is widely adapted to anthropized environments (Mascarenhas Júnior, Santos and Correia, 2018; Mascarenhas-Junior et al., 2020). It presents opportunistic and generalist feeding behavior, preying from invertebrates to fish, testudines, and small mammals (Diefenbach 1988; Melo, 2002). Its meat and skin have great commercial value, with its rearing being allowed for economic purposes, especially in the closed systems in Brazil (farming) and in ranching programs in Argentina (Coutinho et al., 2013).

As it is a well-known species, *C. latirostris* has an important potential for interpretive and inferential studies, especially in understand past species conditions. Knowledge at microscopic level of bone structures of crocodylians is an important tool for biological, behavioral, evolutionary, and ecological inferences through ontogenetic stages (Chinsamy, Codorniú and Chiappe, 2009). Due to physiological and morphological similarities between fossil and extant crocodylian taxa, the later are widely used as inferential histological models for past groups (Storrs, 1993; Andrade and Sayão, 2014; Woodward, Horner and Farlow, 2014; Sayão et al., 2016, Company and Pereda-Suberbiola, 2017). To understand the microstructural information of a fossilized bone, for example, a series of analyzes of comparative structures in existing organisms through osteohistology are necessary (Woodward, Horner and Farlow, 2014).

The histological methods of inference are complementary to the traditional models of morphological descriptions (Sayão et al., 2016), as the bone structure consists of biomineralized tissue, formed by deposition of hydroxyapatite and crystalline calcium phosphate, and its inner region consists of bone cells known as osteocytes and various blood and lymph canals (Andrade and Sayão, 2014). After death, all organic compounds are decomposed, the inorganic part is fossilized, and the shape of microstructures is preserved (Ricqlès, Horner and Padian, 1998), allowing their interpretation even in extinct organisms. However, although we can infer on the natural history of extinct groups with analyzes on extant crocodylians, individual skeletal variations may affect generalizations. Factors such as unfamiliarity of environmental influences, bone resorption and remodeling during growth can interfere in a determination of a pattern (Hutton, 1986).

Broad-Snouted Caiman is one of the extant species of Caimaninae subfamily, originally from the Cenozoic period (Brochu, 2010). Osteohistological information serving as inferential tool on the morphology, physiology, and environmental stresses in Caimaninae are still incipient, restricted to data of *C. yacare* (Andrade et al., 2018). Therefore, we aim to aggregate and refine information on microanatomical bone tendencies in Crocodylia by using bone elements from specimens of *C. latirostris* as a model for deducting structural responses to morphological, physiological, ontogenetic, and environmental questions.

MATERIAL AND METHODS

Sample study

Here we used skeletal elements of *C. latirostris*, kept in captivity and then slaughtered for commercial purposes. They were supplied by the Aruman Slaughterhouse Ltd, located in the municipality of Porto Feliz, São Paulo State, Brazil.

The slaughterhouse donated bone elements from ten females according to the availability, and we selected pairs from one to six years old (except for five years old specimens), totaling five pairs of different ages. All caimans were slaughtered in the same period and had their body mass weighted at slaughter, ranging from 6.99 kg to 21.35 kg. The individual classification of each specimen follows the control protocols used by the slaughterhouse (Table 1).

Like other crocodylians, *C. latirostris* exhibits temperature-dependent for sex determination. In captivity, eggs are generally incubated at temperatures ranging from 28 ºC to 34 ºC, to avoid embryonic death. For safety, the hatchery's incubation chamber in the caiman farm is adjusted to maintain constant temperature of 31 ºC, making 100% of females in its production (Parachú-Marcó et al., 2017, Simoncini et al., 2019).

Caiman farming

In the rearing pens, specimens were fed daily until reach two years old and three times a week at the beginning of the third year of life. Pens were formed by water tanks, dry areas, and rooftops, functioning as greenhouses for thermal retention and accelerating the growth of animals. Each pen has a total area of 7x5 m, of which 14 m^2 meters are dry and 21 m^2 meters are wet, with 80 cm deep pools. The walls measure 1.20 m from the surface of the tanks and the dry floor.

Abiotic data

We obtained the minimum and maximum daily temperatures of Porto Feliz city during the years that specimens lived in the rearing pens from data obtained from the National Meteorological Institute (INMET). We categorized temperatures considering each year of the animal's life as absolute minimum; average minimum; average; average maximum; and absolute average. We note that temperatures tend to be higher in the rearing pens for the use of greenhouses to retain heat and opti-

Table 1. Age, weight and morphometric data of the *Caiman latirostris* specimens from the Aruman Slaughterhouse Ltd. used in this study. IDnum = identification number; IDlabel = identification label; Humerus and Rib: L = length (mm); D = diameter (mm); S = Lenght of the sectioned sample.

IDnum	IDlabel	Age (year)	Weight (Kg)	Humerus			Rib		
				L	D	S	L	D	S
1	3VD4E259	6	15.08	84.07	9.20	6.40	111.65	5.65	6.71
2	3VD4E239	6	21.36	92.11	10.63	6.51	123.57	6.38	6.82
3	5VD2D348	4	19.17	87.54	8.84	6.80	122.84	5.38	7.15
4	5VD6D467	4	12.62	81.98	9.75	6.23	94.50	5.58	6.16
5	6VD3D568	3	10.00	72.56	8.66	4.23	105.62	5.39	6.39
6	6VD3D237	3	10.80	69.14	7.93	4.47	129.82	5.92	6.32
7	7VD5E248	2	8.49	70.55	8.02	4.51	105.43	6.66	5.52
8	7VD5D479	2	7.12	64.12	8.25	5.67	109.05	5.86	6.87
9	8VD7D9	1	6.99	67.49	7.27	3.83	118.90	5.24	5.11
10	8VD2E345	1	7.31	64.85	7.93	4.76	105.91	5.24	4.50

mize animal growth, especially in the hottest periods of the year (Sarkis-Gonçalves et al., 2001).

Inside greenhouses, the temperature is not constant, forming microclimates in different areas, such as on the dry floor, on the surface, and at the bottom of the water tanks. To determine the temperatures estimated in these three microhabitats, we used the study by Fincatti and Verdade (2002) as reference for presenting similar rearing pens to those applied by the caiman farm in Porto Feliz City. We performed temperature estimates for this study based on the difference between the averages measured by Fincatti and Verdade (2002) in the rearing pens and the approximate local temperature of the external environment, based on data from INMET (2018).

The difference between the three microclimates (dry area, tank surface, and tank bottom) and the external environment, considering the physical conditions of the rearing pens, ranges in approximately 8 °C, 10 °C, and 10 °C in the absolute minimum data; 8 °C, 10 °C, and 9 °C in the average minimum data; 9 °C, 4 °C, and 0.1 °C in the maximum averages; and 14 °C, 11 °C, and 0.4 °C in the absolute maximum temperatures (Fincatti and Verdade, 2002) (Available in supplementary material). Each same age pair of individuals were subjected to different temperature variations, considering that some of them spent more time in the farm for being older.

Bone elements

We selected 20 bones, one axial (rib) and one appendicular (humerus) element of each specimen, which we removed from the same anatomical position (Fig. 1). We measured each bone according to its length, diameter, and the length of the sample used. We defined rib length by the distance between proximal and distal portions.

The humeri presented maximum length and diameter ranging between 64.12 mm and 103.34 mm and 7.27 mm and 11.36 mm, respectively. The ribs indicated length ranging between 94.5 mm and 129.82 mm and diameter gradient ranging between 5.24 mm and 8.01 mm. The average length of the sectioned sample were 5.35 mm and 6.08 mm for humerus and rib, respectively (Table 1).

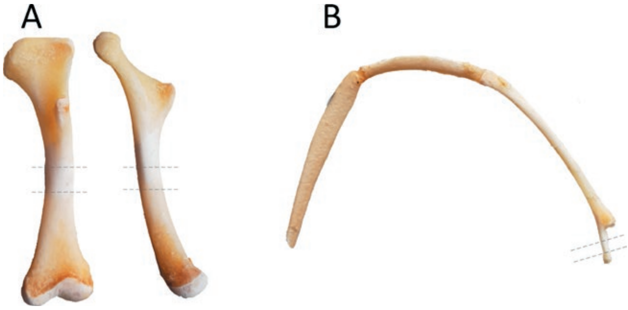

Fig. 1. Bone elements selected for osteohistological description. Section location highlighted between dashed lines. A: Humerus; B: Rib.

Sample preparation

We prepared all the material in the Laboratory of Paleobiology and Microstructures of the Academic Center of Vitória (CAV), advanced campus of Federal University of Pernambuco (UFPE).

For preparation of histological slides, we performed thin sections from the medial regions of the bone diaphysis (Fig. 1), using standard osteohistological techniques (Chinsamy and Raath, 1992; Lamm, 2013). Sampled portions were removed using a Dremel 400 rotary tool, embedded in epoxy clear resin RESAPOL T-208 and catalyzed with BUTANOX M50, using a ratio of 1 mL of catalyst for every 100 mL of resin. The samples were adhered to microscopy slides with glue and Epoxy Araldite hardener. The blocks were roughed and polished using a metallographic polishing machine (ARAPOL VV) with Arotec abrasive paper of increasing grit size (grain sizes 60/ P60, 120/P120, 320/P400, 1200/P2500) until a final thickness of 30 to 60 μm was reached.

Analysis of histological material

We analyzed the bone sections using an AxioImager M2 transmitted and polarized light microscope equipped with AxioCam digital sight camera. Inferences and descriptions followed the study by Francillon-Vieillot et al. (1990). We selected all the structures of interest observable in sections of the bone cortex. We evaluated humeri together in pairs of the same age for presenting similarities between the observed patterns. Ribs were not described in pairs for not presenting microanatomical similarities between same-aged individuals. Images of all histological sections are available in the supplementary material.

Growth curve

Older specimens presented lines of arrested growth (LAGs), enabling to estimate the initial growth curves of these animals based on the distances between lines and the medullary cavity. We also calculated the artithmetic means of distances between LAGs.

Data analysis

For the lack of information on animal size, relationships between specimens were tested according to age, weight, humerus lengths, and rib length. Notably, we tested the significance of the bivariate Pearson's correlation coefficient (r_P) between all the possible pairs. Analyzes were performed using the BioStat 5.9.8 software. The level of significance was set 0.05.

RESULTS

We observed a significant positive correlation between caimans' weight and humerus length (r_P = 0.962; t-test: t = 9.897; df = 8; P <0.001). On the other hand, we did not observe this relationship between weight and rib length (r_P = 0.396; t-test: t = 1.221; df = 8; P = 0.257) and between humerus and rib lengths (r_P = 0.192; t-test: t = 0.552; df = 8; P = 0.596).

Humeri

One-year-old specimens: we observed a highly vascularized cortex, mainly on endosteal portion, with presence of woven-fibered tissue. Anastomosed vascular canals have a reticular pattern, with the presence of simple longitudinal canals and primary osteons distributed throughout the cortex, forming a fibrolamellar bone (Fig. 2A).

Two-year-old specimens: the bone cortex of specimen 8 indicated the presence of fibrolamellar complex. At the endosteal level it shows a woven-fibered bone followed by a lower vascularized transition zone of parallel-fibered tissue towards the subperiosteal region (Fig. 2 B). We could observe a similar pattern of decreased vascularization in the medullary-cortex direction in specimen 7, with evidence of secondary lamellar tissue in perimedullary portion (Fig. 3 A), woven-fibered and parallel-fibered bone at inner the cortex. Simple and anastomosed canals are found inside the cortex, with the presence of primary osteons throughout the cortex and opening towards the periosteum (Fig. 2 B).

Three-year-old specimens: we observed moderate vascularization in the matrix, with mostly simple and few anastomosed canals, presenting longitudinal and reticular patterns and primary osteons (Figs. 2 C, 3 B). We noticed an anisotropic tissue in perimedullary portion, indicating secondary remodeling bone with the presence of a compacted coarse cancellous bone (CCCB). We also observed parallel-fibered and woven-fibered in medial (Fig. 3 B) and parallel-fibered in endosteal portions of the cortex (Fig. 2 C).

Four-year-old specimens: perimedullary portion of both individuals presented remodeling bone, with CCCB in specimen 4 (Fig. 3 C) and lamellar remodeling tissue in specimen 3 (Fig. 3 D), both with mineral erosion cavities, opening to medullary cavity in specimen 4. Also, in medullary-periosteum direction, both bones presented a decrease of primary osteons and vascularization, woven-fibered tissue in medial portion of matrix and parallel-fibered tissue in periosteal region (Fig. 2 D), with an erosion cavity in specimen 3 (Figs.2 E,3 D). We observed simple and anastomosed vascular canals pattern in both samples and plexiform canals in specimen 3 (Fig. 2 F). The most external portion of both bones presented LAGs (three in specimen 4, Fig. 2 D, and two in specimen 3).

Six-year-old specimens: both specimens 1 and 2 presented remodeling bone in perimedullary portion (lamellar in specimen 2 and CCCB in specimen 1, Fig. 3 E). We observed woven-fibered and parallel-fibered tissue (Figure 2 G), besides primary osteons throughout the cortex (Fig. 2 H), with simple vascular canals and reticular anastomoses within the matrix and low vascularization in periosteal portion. In addition, we observed four LAGs in specimen 2 (Fig. 2 G) and three in specimen 1.

Ribs

Specimen 10 (one-year-old): cortex is composed of woven-fibered tissue low vascularized, with few simple

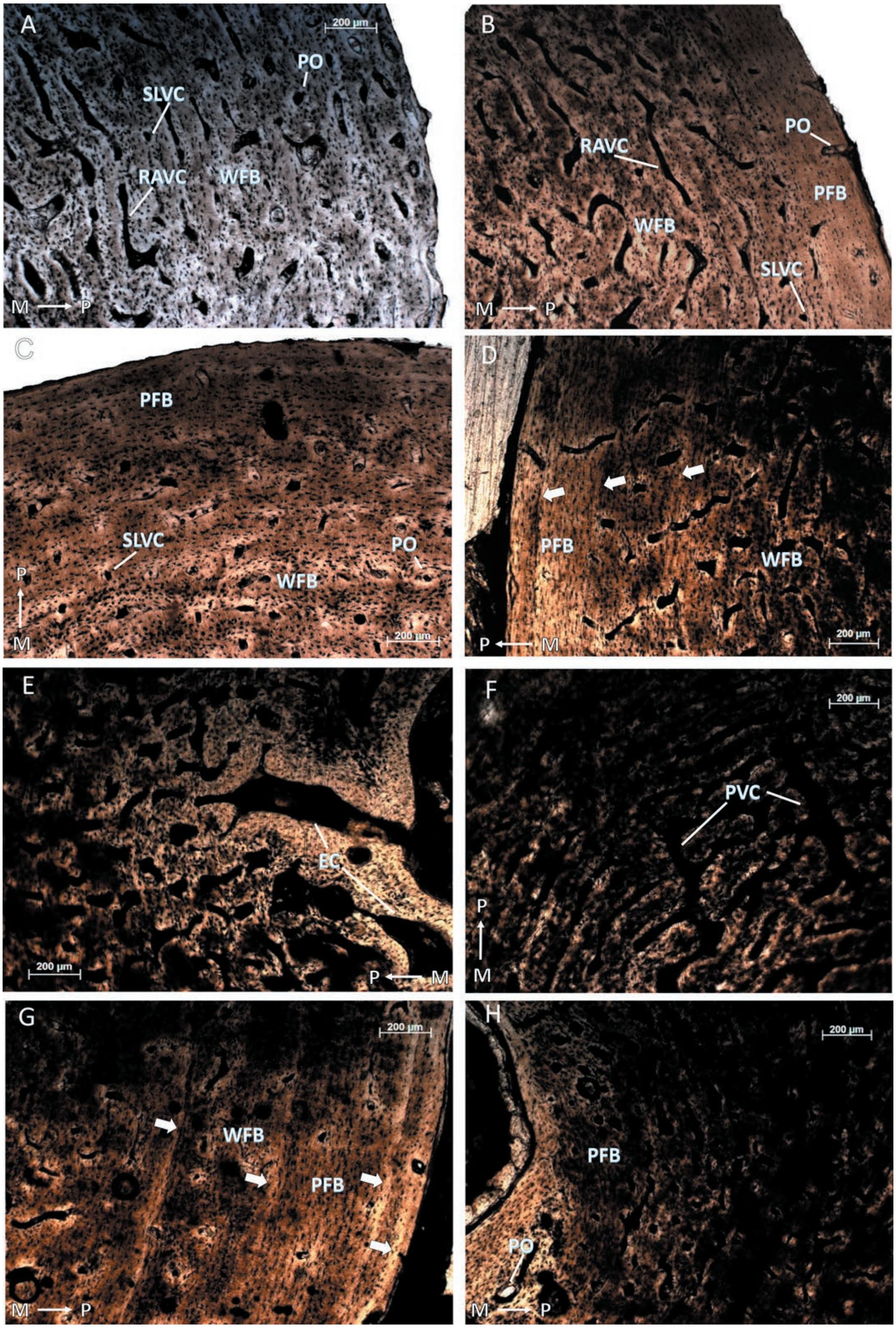

Fig. 2. Histology under transmitted light of humeri of *Caiman latirostris* specimens in captivity slaughtered in a rearing pen in the municipality of Porto Feliz, São Paulo. The specimens were one year old (A), two years old (B), three years old (C), four years old (D, E, and F), and six years old (G and H) at the time of slaughter. EC: erosion cavity; M: medullary portion; P: periosteal portion; PFB: parallel-fibered bone; PO: primary osteon; PVC: plexiform vascular canal; RAVC: reticular anastomosing vascular canal; SLVC: simple longitudinal vascular canal; WFB: woven-fibered bone. White arrows show lines of arrested growth (LAGs).

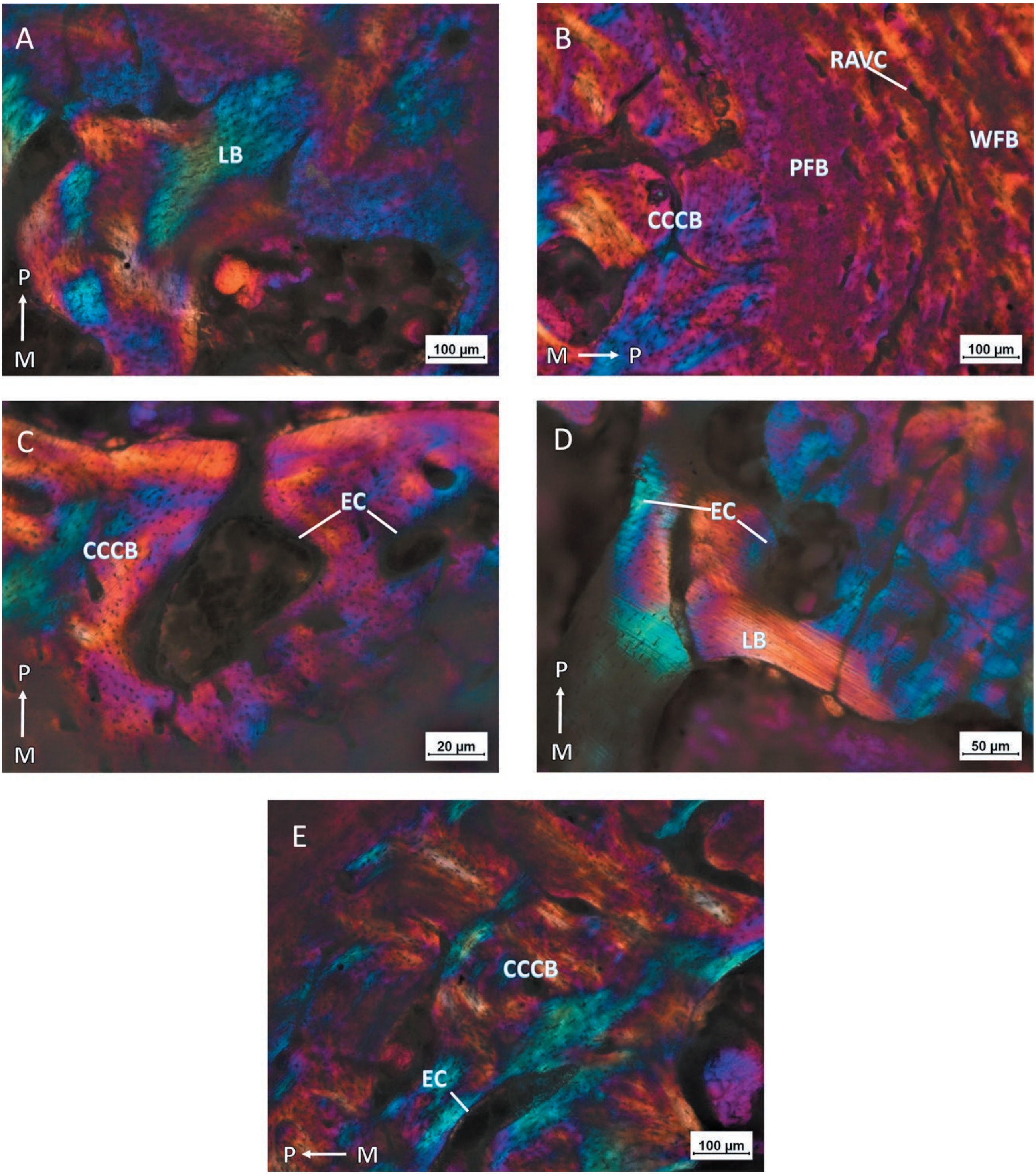

Fig. 3. Histology under polarized light combined with gypsum filter of humeri of *Caiman latirostris* specimens in captivity slaughtered in a rearing pen in the municipality of Porto Feliz, São Paulo. The specimens were two years old (A), three years old (B), four years old (C and D) and six years old (E). CCCB: compacted coarse cancellous bone; EC: erosion cavity; LB: lamellar bone; M: medullary portion; P: periosteal portion; PFB: parallel-fibered bone; RAVC: reticular anastomosing vascular canal; WFB: woven-fibered bone.

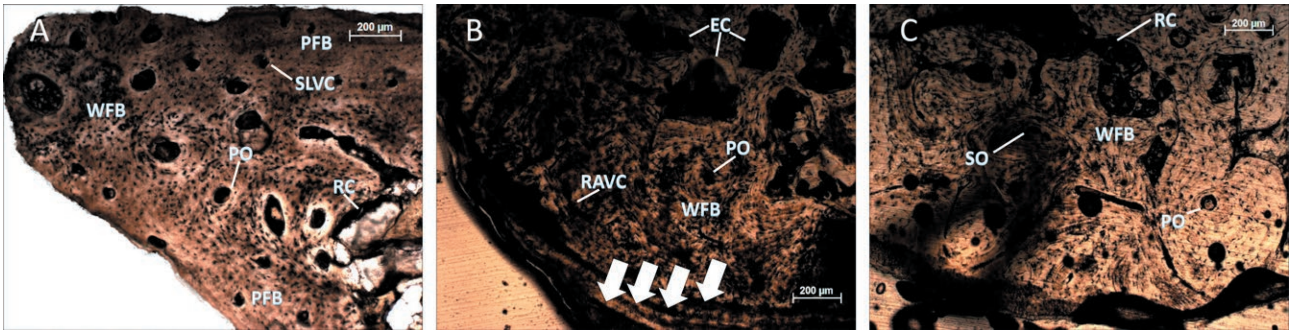

Fig. 4. Rib histology under transmitted light of three specimens of *Caiman latirostris* in captivity slaughtered in a rearing pen in the municipality of Porto Feliz, São Paulo. The individuals were two (A), three (B) and four (B) years old at the time of slaughter. EC: erosion cavity; PFB: parallel-fibered bone; PO: primary osteon; RAVC: reticular anastomosing vascular canal; RC: resorption cavity; SLVC: simple longitudinal vascular canal; SO: secondary osteon; WFB: woven-fibered bone. White arrows indicate lines of arrested growth (LAGs).

and anastomosed vascular canals, and few primary osteons. Large resorption cavities appear in the endosteal bone.

Specimen 9 (one-year-old): the tissue is predominantly woven-fibered, with a matrix vascularized mainly through simple canals and presenting primary osteons. Within the cortex, we found secondary osteons. Resorption cavities are also visible.

Specimen 8 (two-years-old): woven-fibered bone is observed at endosteal level and parallel-fibered bone evidenced in the periosteal layer, with simple longitudinal vascular canals presenting primary osteons. Resorption cavities present mainly within the cortex (Fig. 4A).

Specimen 7 (two-years-old): we found little vascularization in the cortex, presenting simple longitudinal and reticular anastomosing canals with primary osteons. The tissue exhibits woven-fibered pattern and two possible LAGs in the cortex.

Specimen 6 (three-years-old): we noticed a large amount of erosion cavities, found in the bone structure until close to the periosteal region. We evidenced woven-fibered tissue in the regions between erosion cavities, comprising most of the tissue and parallel-fibered tissue in the periosteal region. We found little vascularization, with simple longitudinal and reticular anastomosing canals and primary osteon.

Specimen 5 (three-years-old): we found tissue of woven-fibered type with moderate vascularization, presenting simple longitudinal and reticular anastomosing vascular canals, as well as primary osteons. In the region of the inner cortex, we identified several bone resorption cavities. In one of the periosteal portions, we identified three LAGs.

Specimen 4 (four-years-old): we found woven-fibered tissue in this bone with moderate vascularization, presenting simple longitudinal and reticular anastomosing vascular canals. We observed primary osteons and erosion cavities. In addition, it presents four LAGs in the periosteal region (Fig. 4B).

Specimen 3 (three-years-old): cortex is composed of woven-fibered tissue full of resorption cavities, comprising most of the bone. Vascularization is low, composed by primary osteons and simple longitudinal vascular canals.

Specimen 2 (six-years-old): presence of several bone resorption cavities with woven-fibered bone. The cortex is poorly vascularized, with small amounts of simple longitudinal and reticular anastomosing vascular canals and primary osteons.

Specimen 1 (six-years-old): primary and secondary osteons compose the cortex. Vascularization is low with simple longitudinal and reticular anastomosing canals. Bone resorption cavities concentrated endosteal region and woven-fibered tissue is present within the cortex (Fig. 4C).

Distance between lines of arrested growth

LAGs are present only in older specimens. We calculated distances between intervals among lines, as well as the total thickness of the cortex. The distances between the medullary cavity and periosteum ranged between 8,040 µm in specimen 3 and 9,830 µm in specimen 2. The minimum and maximum distances observed between LAGs ranged from 105 µm to 475 µm. The average spacing between LAGs was 282.2µm, ranging between 35 µm from the first to the second LAGs in specimen 1 and 475 µm from the second to the third LAGs in the same specimen (Fig. 5).

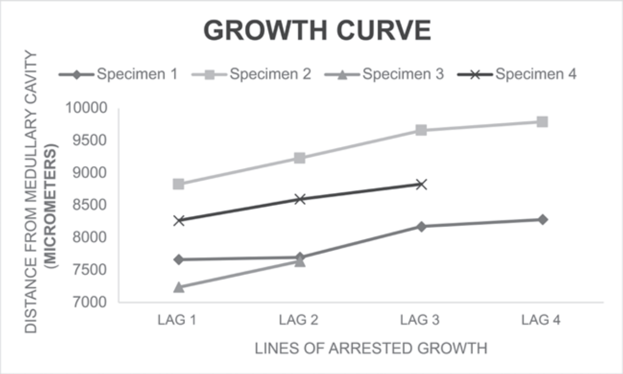

Fig. 5 Distance between highlighted lines of arrested growth (LAGs) in humeri of specimens 1, 2, 3, and 4.

DISCUSSION

In general, we observed a fast-growth pattern in ribs than in humeri. Younger specimens presented mostly woven-fibered (fast-growing) tissues in the humeri, and during their ontogenetic development, began to form parallel-fibered tissue, indicating growth stabilization. The ribs demonstrated a prevalence of woven-fibered tissue in all ages, with reduced vascularization. This information may be related to a lack of relationship in the growth trend between these bone elements, leading us to believe that the ribs may have a faster growth and remodeling than humeri (Enlow and Brown, 1958), demonstrating histovariability in axial and appendicular bone elements the same specimen (Sayão et al., 2016; Sena et al., 2018). This also corroborates what has already been described in Alligatoridae crocodylians: *Alligator* and *Caiman* (Lee, 2004; Woodward, Horner and Farlow, 2014; Andrade et al., 2018).

The ribs showed major remodeling in the medullary region, which expanded over a large part of the bone cortex, as already observed in other Crocodylomorpha (Andrade et al., 2015; Sayão et al., 2016; Sena et al., 2018). These remodeling processes can directly influence determinations by skeletochronology. Specimens 7, 5, and 4 (two, three and four years old respectively) were the only with LAGs, showing the same number of lines as its age, as found by Waskow and Mateus (2017). On the other hand, the other ribs showed no evidence of LAGs, indicating that the bone had possibly been remodeled and the lines lost during the processes of bone absorption and redeposition throughout its development. Deficiency to determine ages from axial elements has also been previously reported in the literature in both living (Hutton, 1986) and fossil specimens (Sayão et al., 2016; Sena et al., 2018). For the inconsistency in microanatomical patterns, ribs are not the most suitable elements for skeletochronology (Padian, 2011), although having already been used in skeletochronological studies in other groups of archosaurs, especially in fossils, such as Dinosauria (Gallina, 2012; Waskow and Sander, 2014; Waskow and Mateus, 2017; Woodruff, Fowler and Horner, 2017; Brum et al., 2021). Also, Waskow and Sander (2014) stated that the posteromedial side of the proximal shaft of the ribs record best growth information in osteohistological observations. It was suggested that in studies without promising results, samples were collected from distal portions of the ribs, not showing lines or other growth marks (Waskow and Sander, 2014). However, in this study all samples were collected from the proximal portions of ribs (see material and methods), and we identified LAGs in three of our samples, contradicting this idea, at least for the group under study. We did not identify significant relationship between rib length increase and animals aging, although we found association between the size of the axial element and the presence of LAGs. The three specimens that presented LAGs (specimens 4, 5, and 7) had the smallest ribs, being related to bone tissue remodeling, which had not been enough remodeled for the destruction of the lines.

The humeri showed tendencies in microanatomy and osteohistology related to animal age. The youngest caimans (10 and 9) presented a homogeneous pattern in tissue composition, being of woven-fibered type. This tissue is quite common in young specimens, with high metabolic rates and rapid growth, presenting great vascularization and primary osteons (Huttenlocker, Woodward and Hall, 2013). The presence of woven-fibered tissue with primary osteons, as already reported in crocodylians, indicate physiological strategies of rapid growth, forming a fibrolamellar complex (Ricqlès, 1983; Reid, 1984, 1990, 1997; Ricqlès, Padian and Horner, 2001; Chinsamy and Hillenius, 2004; Tumarkin-Deratizan, Vann and Dodson 2007; Woodward, Horner and Farlow, 2014; Andrade et al., 2018). This bone composition supports the hypothesis that crocodylians retain an ancestral characteristic of Archosauria (Cubo et al., 2012), evidenced in young animals.

We did not expect the presence of fibrolamellar bones in crocodylians, although it has already been identified in young specimens with optimal growth conditions in farms, constant feeding, and ideal temperature of approximately 30 °C to 33 °C (Lang, 1987; Woodward, Horner and Farlow, 2014). During the day, caimans tend to move within the pens, being exposed to the temperature ranges close to the ideal for growth (Verdade, 1995). Generally, during winter and days of lower temperatures, caimans remain in the dry areas in sun periods and in humid areas at night for the different rates of heat accumulation between air and water, as the air is a better heat disperser than water. During summer or in periods with high temperatures, the animals will follow the opposite, preferring wet microenvironments during sun periods and the dry ones at night (Verdade et al., 1994). Despite lower temperatures, the pens have greenhouses that tend to retain heat, leaving the environment with a higher temperature in relation to the outside (Fincatti and Verdade, 2002). It is possible that, through thermal stimuli and constant feeding, individuals tend to maintain higher rates of basal metabolism, consequently accelerating their growth with longer lasting strategies of rapid growth. This fact is understandable, since crocodylians spend less energy producing heat, living with a low metabolic rate. Therefore, we assume that the physiological demand for energy, especially glucose, for the growth and maintenance of ectothermic animals is low if compared to that of endothermic carnivores, since there is no need to produce heat from this sugar to maintain temperature body. Consequently, a large part of its energy supply is invested in growth and reproduction. This result is obtained rather through predominantly behavioral mechanisms than physiological mechanisms by seeking the ideal temperature range for their metabolic activities, which in general are eight to ten-fold lower than that of endothermic animals, somehow explaining their dependence on room temperature (Smith, Robertson and Davies, 1978; Staton, 1988; Silva, 2000).

Specimens 8 and 7, despite being the same age (two years old), presented differences in metabolic growth strategies, observed in the bone. During animal aging, the tendency is the reduction of growth rate, with a transition between tissues that demonstrate fast-growing metabolism strategies (woven-fibered bone) and slow-growing tissues (Ricqlès, Padian and Horner, 2003; Huttenlocker, Woodward and Hall, 2013). With maturation, we observed parallel-fibered tissue, especially in the periosteal region, and secondary remodeling bone, such as CCCB and lamellar bone in endosteal portion. There is a gradient associated with age which reflects the start of slow-growth strategy, although without finalization of definitive bone growth, evidenced by the absence of an External Fundamental System and the opening of primary osteons in the cortex towards the periosteum (Ricqlès et al., 2003; Woodward et al., 2011; Andrade et al., 2018). The decreasing presence of fibrolamellar complexes implies the reduce of fast-growth rates, a baseline characteristic of tetrapods in general (Woodward, Horner and Farlow, 2014).

In some specimens CCCB in response of secondarily remodeling processes can be observed, mainly in older individuals or breeding females (Hutton, 1986; Schweitzer et al., 2007). In female crocodylians, bone remodeling can occur faster than in males due to calcium mobilization for the formation of eggshells (Hutton, 1986). The beginning of the reproductive stage in the life of crocodylians is related to their growth, also influenced by temperature, and feeding. Females of *C. latirostris* reaches sexual maturity with approximately 68 centimeters of snout-vent length (Leiva et al., 2019) or between four and five years of age in captivity (Verdade et al., 2003). In commercial farms, caimans are maintained in greenhouses under high temperatures to reduce their time for slaughter and sexual maturation. We observed a high remodeling bone in females over 10 kg (three years old), which can be an important indicator of reproductive stage beginning. Klein, Scheyer and Tütken (2009) suggest that captive females have great absorption in long bones due to mineral mobilization to form eggshells or due to nutritional deficiencies, and, associated with secondary remodeling bone, the cyclic growth marks may be destroyed. For free-living animals, Schweitzer et al. (2007) stated that long bones in wild female *Alligator mississipiensis* do not show major remodeling activities, which may indicate histovariability between wild and captive animals, even in similar ontogenetic stages.

As in other reptiles, LAGs provide clues to both skeletochronology and the growth rates of specimens on an annual basis (Castanet et al., 1993; Guarino et al., 2020). These thin lines that occur parallel to the periosteum margin (Wilson and Chin, 2014) are observed in greater quantities with increasing age. According to Castanet et al. (1993), the deposition of LAGs occurs annually in crocodylians, regardless of food, temperature, or photoperiod. Tucker (1997) suggested that the appearance of LAGs is associated with winter periods (food shortages and decreased metabolism). In this study, specimens that were in the first, second, and third years of life did not present evident lines and specimens that were in the fourth and sixth years of life presented underestimated numbers of growth marks regarding age.

Considering the deposition of one LAG per year, we could identify the growth speed of animals that showed these marks. Four-year-old specimens had a relatively similar spacing between LAGs, which may indicate a similar growth rate between the first and second years. The third line of Specimen 4 is close to the periosteum, indicating that the animal died before formation of the fourth LAG. On the other hand, six-year-olds had different intervals between lines. Specimen 2 presented a large spacing between the first and second LAG and between the second and third. Between the third and fourth, the distance reduced, leading us to believe that the caiman grew at high rates between the first three years and had a decline in the growth curve in the fourth year. Specimen 1 had little spacing between the first and second LAG, with a larger distance between the second and third one. It also had a reduction between the third and fourth lines, probably indicating a low growth rate until reaching the third year of life, growing quickly until reaching the fourth year, in which apposition decreased again. Both specimens 1 and 2 may have been slaughtered before the formation of the fifth line. These differences in growth patterns can be related both to the intrinsic physiological conditions that influence growth and external factors, such as dominance in the competition for food and the selection of thermal ranges in the pens (Hutton, 1986; Verdade et al., 1994).

Scarcity of osteohistological studies conducted with a significant sample of specimens and taxa hinders the confirmation that LAGs are necessarily formed annually in crocodylians. Based on our observations, it is likely that this concept is not consistent with the patterns observed in captive specimens of living taxa or even with those of the genus *Caiman* or of the species *C. latirostris*. In general, studies that performed osteohistological interpretations in extant crocodylians are restricted to less than ten specimens sampled (Schweitzer et al., 2007; Woodward, Horner and Farlow, 2014; Andrade et al., 2018), the only exception was a study conducted with 30 different individuals of *A. mississipiensis* (Woodward, Horner and Farlow, 2011). Researches with larger samples are still scarce (Hutton, 1986; Tumarkin-Deratzian, Vann and Dodson, 2007) and must be incentivized. So far, any other research has been performed with crocodylians from the subfamily Caimaninae, which elucidates the incipience of concrete data on the pattern of growth marks in crocodylians.

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