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Physicochemical analysis of *Catonyx cuvieri* (Mammalia, Xenarthra) remains from the Pleistocene of Paraguay

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ARTICLE INFO

Vibrational response

Keywords:

Paleometry

Diagenesis

Pleistocene

Hydroxyapatite

Catonyx cuvieri

San Lázaro district

ABSTRACT

The fossil-diagenetic characteristics, such as mineralogical aspects present in the ribs of *Catonyx cuvieri* (Lund, 1839) (Mammalia, Xenarthra), were analyzed in this work. These analyzed remains come from the Risso Cave, San Lazaro's District, Concepción Department, Paraguay. The vibrational response was studied using Raman and Fourier transform infrared (FTIR) and the mineralogy and elemental compositions were examined using multiple characterizations techniques. The main functional groups belong to the mineral part of the bone, and specific functional groups were associated with organic components. The characterization indicated that material was composed of apatites and the original mineral structure was affected by partial ionic substitutions, probably due to the interaction of the fossil with the environment. A Gaussian deconvolution model (GM) was used to identify the individual overlapping bands in the regions of interest in the spectra. A diagenesis pathway for the fossil involved the inclusion of calcium carbonate on the surface of the bone due to the accumulation of moisture, which allowed the recrystallization of hydroxyapatite and the precipitation of calcite in the pores and silicates of the bone. It's worth mentioning that this work is the first in the ground sloths *C. cuvieri* in South America. In addition, the material here constitutes the most complete fossil record of a member of the Pleistocene megafaunal of Paraguay.

Introduction

The bone structures of vertebrates have as their main inorganic constituent a mineral called hydroxyapatite (HA) [1]. HA is a calcium phosphate that has a chemical composition of $Ca_5(PO_4)_3(OH)$, which is of great importance in the fields of biomaterial and paleontology [2–4].

Apatites are particularly susceptible to ionic substitutions at both Ca and phosphate sites. These substitutions introduce disorder at the atomic level and thus destabilize the mineral. Because of this, crystals in bone materials readily undergo diagenetic changes after burial [5].

During the fossilization process, changes are generated in the bone

mineral structure, such as recrystallization and ion absorption, which lead to changes in its chemical composition and crystalline structure [6]. Diagenesis is a complex process involving physical and chemical alterations in the material during fossilization, a condition directly influenced by the sedimentation system [7].

The study of fossils in recent years has advanced by the development and use of characterization techniques for understanding of geobiology and paleoenvironments topic. Also, new discoveries and conceptual advances in paleobiology have made it possible to extract more information from the fossil record through paleometry. This line of research has greatly contributed to a better understanding of the environmental

https://doi.org/10.1016/j.rechem.2024.101745

Received 6 March 2024; Accepted 21 August 2024

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conditions under which a given diagenesis process occurred and provides us with tools to analyze the available data in more detail [8].

Vibrational spectroscopy techniques are currently an important tool for the study of fossils, mainly through the rapid identification of phosphate groups associated with apatite materials. There are several studies carried out in South America in which techniques such as Raman spectroscopy, X-ray fluorescence by dispersive energy (EDXRF), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray Energy Dispersive Spectroscopy (EDS) were used to perform physical–chemical characterizations to elucidate the fossilization conditions of a given material through the diagenesis process [4,9–11].

Studies of Pleistocene mammal fossils from Paraguay are very scarce compared to those from Argentina, Brazil and Bolivia. In turn, it should be noted that the paleofauna record is poorly known and that the material found so far is very fragmentary, isolated and, in most cases, lacking an adequate stratigraphic context [12–14].

The ground sloth *C. cuvieri* (Lund, 1839) (Xenarthra, Folivora) is recorded during the Late Pleistocene mainly in the intertropical region

of Brazil, more precisely in the State of Minas Gerais (Lagoa Santa), in Rio Grande do Sul ("Concheiro" and Chuí stream, [15]), Iporanga and Sergipe [16] and in São Paulo [17]. Corona et al. [18] recorded *C. cuvieri* from the Dolores Formation (Late Pleistocene) Department of Canelones Uruguay. In turn, Miño-Boilini et al. [19] referred new materials from the Late Pleistocene of Paraguay (San Lázaro district, Concepción Department) to *C. cuvieri* [20].

This work focuses on describing the characteristics of the vibrational response of *Catonyx cuvieri* ribs from the Risso Cave, San Lazaro's District, Concepción Department, Paraguay. The diagenetic alterations of the fossil were analyzed along with the potential processes involved, based on the data obtained.

Geological setting

The Risso cave, developed in the karstic system of Cerro Morado, is located to the NW of the Concepción Department, specifically in the San Lazaro's District (Fig. 1). This cave is related to underground karstic geoforms "endokarst" of the Itapucumí Group and has a depth of 30 m,



Fig. 1. A) Location map of Risso Cavern, B) Process of rescuing the specimen and C) Rib fragment, D) 1) mf1 lacks the vestibular lobe, E) humerus lacks the entepicondylar foramen, F) the tibia-fibula of *Catonyx cuvieri* (FACEN-PVert-0002).

measured from the location where the fossil was found to the top of Cerro Morado, where, in the middle of 2012, a partial fossil skeleton of a single individual assigned to *C. Cuvieri* was discovered in this cave with approximately 70 % of the bone remains preserved [19,21]. There are also a significant number of bones remains of reptiles, medium and small mammals from the late Pleistocene, all these corresponding to the first records at the country of fossil mammals in karstic systems and the first record in Paraguay of fossil micromammals [22].

The Neoproterozoic Itapucumí Group [23] is made up of oolitic calcarenites, marls, shales, and sandstones, intensely deformed in the Vallemí Folded Belt [24]. It has an age of 550 Ma, determined through 86Sr/87Sr dating [25,26]. Initially, it was described as a unit of carbonate rock. Bertoni (1921) identified ancient crystalline limestone rocks resting on granites and assumed for the latter as corresponding to the Archean [27]. Subsequently, Harrington (1950) considered this unit as from the Ordovician and described it as "Itapucumí Series" [28]. Eckel (1959) described this unit as composed of light to dark gravish oolitic carbonates at the base and composed of marls with shales at the top, in erosive contact with basement rocks [29]. Putzer (1962) describes this unit as tectonically disturbed and correlates it with the Tamengo Formation of the Corumbá Group of Brazil [30]. Wiens (1986) divided the Itapucumí Group into two, Vallemí Formation and Camba Jhopo Formation [23], later confirmed by Orué (1996) [31]. Finally, Warren (2011) reclassifies the Itapucumí Group as composed of; the Vallemí Formation, Camba Jhopo Formation, and Cerro Curuzú Formation associated with the Vallemí Folded Belt, while the Vallemí Formation and the Tagatiya Guazú Formation are associated with cratonic covers. Generally made up of laminar pelitic rocks, massive rocks, siliciclastic rocks, sandstones with cross-stratification, with planeparallel lamination, massive sandstones, massive conglomerates, conglomerates with cross-stratification, oolitic sedimentites, calcareous breccias, rhythmites, and marls [25].

Materials and methods

It was evaluated the mineralogy features of rib fragment (Fig. 1 C) of Catonyx cuvieri with FACEN-PVert-0002 collection number corresponding to an individual that is part of the collection of the Paleontology Laboratory of the Faculty of Exact and Natural Sciences of the National University of Asunción. The specimen is represented by the following elements: right Mf1, right and left Mf2, right and left Mf3, right and left Mf5, left mf4, right ulna, left humerus, right astragalus, right patella, fragment of right scapula, various rib fragments, eleven caudal vertebrae, left femur, right mf1, left tibia and fibula; and, right tibia distal end. Fig. 1 A) and B) shows the record of the sloth was made in 2011 in the Risso Cave (Lat: 22°17'31.83'S; Long: 57°52'34.87'W), located in the District of San Lázaro, Concepción Department, Paraguay [21]. The specimen assignment was performed by Miño-Boilini et al. [19] and Miño-Boilini and Quiñones [32]. The specimen is assigned to the Catonyx cuvieri based on the following morphological characteristics: Fig. 1 D) mf1 lacks the vestibular lobe, Fig. 1 E) humerus lacks the entepicondylar foramen, Fig. 1 F) the tibia-fibula are not normally fused [16,18]. Anatomical abbreviations: Mf/mf, upper and lower molariform, respectively. The recovered fossil remains were highly fragmented and exhibited few diagnostic features in the rescued elements.

X-ray diffraction (XRD) measurements were performed in a Panalytical XPert PRO diffratometer using Cu K α radiation source with a wavelength of $\lambda = 1.5418$ Å. The range of angles covered was from 20 to 80 degrees, advancing in increments of 0.025 degrees and accumulation time of 1 s for each angular position. The identification of the crystalline phases present in the analyzed powder material was carried out by comparing the experimental results with the crystalline phase patterns contained in the COD database Crystallography Open Database.

The infrared absorption analysis of the material was conducted using an FTIR system, specifically the VERTEX 70v model manufactured by Bruker. Acquisition was carried out over the range of 350–4000 cm⁻¹

with a resolution of 4 $\rm cm^{-1}$, utilizing 128 accumulation scans in ATR mode with a diamond crystal.

The Raman responses were obtained using a B&W Tek Model 465-785S Raman Spectrometer, equipped with a 785 nm laser and offering a resolution of 4 cm⁻¹. The selection of analysis regions involved identifying flat areas measuring 1 mm² to maximize signal intensity. Additionally, spectra were positioned beneath the measuring probe of the equipment. Measurements were conducted by adjusting the laser power and acquisition time to optimize signal quality as needed.

The spectral analysis of the vibrational signals was conducted based on the second derivative criterion, as described by Pleshko, Boskey, and Mendelsohn [38]. This approach entails identifying the minima in the second derivative spectrum of the raw data, corresponding to the positions of the band centers for both Raman and FTIR signals in the original spectrum.

Then, armed with the approximate centroid positions, the spectra were deconvoluted using a Gaussian model (GM) of coupled oscillators with coupled vibrational responses. The deconvolution process adhered to the methodology outlined by Davies and Vaughan [39], a mathematical procedure designed to pinpoint the overlapping peaks constituting a complex spectrum.

The fitting of the FTIR and Raman vibrational signal bands was conducted using the open-source software Fityk, employing Gaussian curves within the various regions of interest (ROI) and a linear baseline. For data processing and manipulation of spectral data obtained from Raman and FTIR spectroscopy, including baseline correction, smoothing, and calculation of the second derivative, the Spectragryph software was utilized.

The chemistry was characterized by X-ray photoelectron spectroscopy (XPS) in ultra-high vacuum using a PHOIBOS 100/150 analyzer from SPECS. The analysis was carried out using Al anode X-ray tube with 1486 eV (K α Al). Survey measurements were performed with an energy step resolution of 0.5 eV in the energy range of 1200 to 100 eV. In the high-resolution measurements the energy step was 0.02 eV. The measurement intervals were optimized using the survey data of the Ca 2p, O1s and P 2p elements. Data processing and analysis were carried out with Casa-XPS software.

The elemental composition was measured using an Energy Dispersive X-ray Fluorescence Spectroscopy (EDXRF) system manufactured by Bruker model S2 Ranger with Pd anode and SSD type detector. The fundamental parameter was used for the semi-quantitative calculation of the percentage by weight (wt. %) of the solid samples and identification of the elements present in the analyzed samples.

Scanning electron microscopy (SEM) analysis was carried out to visualize the microstructure in the sample, before SEM analysis, the sample was coated with carbon to reduce the effects of electron accumulation on the irregular surface of the sample. The coating was carried out under a voltage of 4 V and a carbon deposition time of 8 s. The images in secondary electron (SE) mode were taken using a Coxem CX-200 Plus scanning electron microscope operated at 20 kV. Chemical composition was measured in specific areas of the sample using an Energy Dispersion X-ray Spectrometer (EDS) with EDS OXFORD Xplore30 Detector.

Results

The XRD result shown in Fig. 2a reveals the crystalline phases commonly present in bone and fossil structures, confirming the apatitic nature of the material and reveals the presence of silicates. The presence of the functional groups $(PO_4)^{3-}$ and $(OH)^-$, characteristic of materials with apatitic structures, is detected through FTIR spectroscopy, as shown in Fig. 2b. In addition, organic groups and carbonates were observed. The specific assignments of the absorption bands are presented in Fig. 2b. In Table 1 depicts details that represents the conventional approach and analysis of FTIR spectra of fossil samples [33–37]. The detection of vibrational responses associated with calcium



Fig. 2. a) XRD plot, b) IR absorption response: ROI 4000 to 400 cm⁻¹, c) deconvolution in the ROI: 775 to 425 cm⁻¹ and d) deconvolution in the ROI: 1300 to 850 cm⁻¹.

Table 1

Assignation	in t	he	FTIR	bands	of the	FACE	N-PVEF	TO002	in	the	ROI	4000	to
400 cm^{-1} .													

Wavenumber (cm ⁻¹)	Assignment	Reference
470	Double degenerated bending mode	[38,39]
	(ν_2) of the O—P—O bonds of the PO ₄	
567	Triply degenerated bending mode	[36,38,39,40-42,46]
	(ν_{4}) of the O—P—O bonds of the PO ₄	[,,,
	group	
605	Triply degenerated bending mode	[36,37,38,40-42]
	(ν_4) of the O—P—O bonds of the PO ₄	
	group	
874	Stretching mode (ν_2) of the CO ₃	[34,35,38,40,41]
	group Non-apatitic	
1037	Triply degenerated asymmetric	[36,37,41,43,44,46,48]
	stretching mode (ν_3) of the P—O	
	bond of the PO ₄ group	
1102	Due to the presence of HPO_4 or CO_3 groups	[4,37,39,49]
1414	Stretching mode (ν_3) of the CO ₃	[34,36,38,39,42,43,46]
	group	
1453	Amide II	[4,47]
1647	Amide I	[40,41,43,45,47]
3430	Stretching mode (ν_s) of the OH ⁻	[4,33,48]
	group	

phosphates, whether stoichiometric or with imperfections, is complicated by the overlap of bands in the regions from 725 to 475 cm⁻¹ and from 1300 to 850 cm⁻¹. These regions are especially relevant since the phosphate groups manifest multiple absorption bands. This complexity is compounded by the proximity of the responses and the spectral resolution of the spectroscopy system, making accurate identification by mere visual inspection difficult. A curve fitting was generated using a response model with Gaussian curves, and the results of the fits are presented in Fig. 2(c–d), respectively.

The parameters and assignments corresponding to the adjustments

Table 2
Parameters and band assignments of the GM deconvolution of the ROI: 775 cm^{-1}
to 425 cm ⁻¹ FTIR spectra.

Band	Center	Area	FWHM	Assignment	Reference
Α	627.20	10.37	42.21	Vibrational mode (ν_L) of the OH	[38,39,45]
В	602.83	14.45	22.81	Triply degenerated bending mode (ν_4) of the O—P—O bonds of the PO ₄ group	[36-43]
С	580.83	4.37	15.39	Triply degenerated bending mode (ν_4) of the O—P—O bonds of the PO ₄ group	[37,39,40,41,42,44]
D	564.31	21.21	27.18	Triply degenerated bending mode (ν_4) of the O—P—O bonds of the PO ₄ group	[36-42]
E	535.68	9.82	38.59	Bending mode of the O—P—O bonds of the PO ₄ group	[39,42,44]

made in Fig. 2(c–d) are depicted in Tables 2 and 3. In the region from 725 to 475 cm⁻¹, triply degenerate bending modes (ν_4) of the O–P–O bonds of the PO₄ group are identified at frequencies of 564.31, 580.83 and 602.83 cm⁻¹ [36–44]. In addition, a vibrational absorption mode (ν_L) of the OH group is recorded in the vicinity of 627.20 cm⁻¹ [38,39,45]. The presence of a bending mode for the O–P–O bonds of the PO₄ group at a frequency of 535.68 cm⁻¹ is also highlighted [39,44]. In the region 1300 to 850 cm⁻¹, bands typically reported in stoichiometric calcium phosphates in the literature can be observed as the symmetric stretching mode (ν_1) of the P–O bond of the PO₄ group at 1032.67 and 1078.72 cm⁻¹ [36–46].

Through curve fitting and deconvolution, it is possible to identify typical calcium phosphates and those associated with structural defects, which manifest themselves at 992.31 cm⁻¹ [37,44,45]. Additionally, the frequency of 1125 cm⁻¹ is associated with the presence of HPO₄ groups [37,44,45]. Also, other responses are not associated with calcium phosphates could be related to the type of analysis. An example the identification of asymmetric bending mode corresponding to C—O—C bonds at 1179.25 cm⁻¹ [47].

The Raman spectra of the samples in Fig. 3a show the presence of organic components Amida I at 1707 cm⁻¹, and Amida III at 1286 cm⁻¹ and 1225 cm⁻¹ [4,38,50–57]. As expected, we also identify the presence of (PO₄)^{3–} phosphate groups recorded at 960 cm⁻¹ corresponding to the fully symmetric stretching mode (ν_1) of the P—O bond of the (PO⁴)^{3–} group and the stretching mode (ν_3) of the P—O bond of the PO₄ group at 1060 cm⁻¹ [38,39,50–58].

Using the GM model with coupled vibrational responses, the analysis of the organic components present in the sample was performed in the region of 1200 to 1800 cm^{-1} of the Raman spectrum, considering the organic structures and types of bonds they contain, as shown in Table 4 and Table 5.

Fig. 3b and Table 5 shows organic compounds identified by curve fitting, revealing characteristic bands of Amide I groups at 1727.78

Table 3

Parameters and band assignments of the GM deconvolution of the ROI 1300 $\rm cm^{-1}$ to 850 $\rm cm^{-1}$ FTIR spectra.

Band	Center	Area	FWHM	Assignment	Reference
Α	1179.25	14.04	80.03	Asymmetric bending mode of the C—O—C bonds	[47]
В	1125.00	18.53	70.76	Due to the presence of HPO ₄ groups	[37,44,45]
С	1078.72	34.12	78.89	Triply degenerated asymmetric stretching mode (v_3) of the P—O bond of the PO ₄ group	[39,42,44,45,46]
D	1032.67	22.58	58.72	Triply degenerated asymmetric stretching mode (ν_3) of the P—O bond of the PO ₄ group	[36–39,41,42,43,46]
E	992.31	21.86	55.58	Stretching mode of the P—O bond of the PO₄ group	[37,44,45]
F	952.44	7.51	34.78	Symmetric stretching mode (ν_1) of the P—O bond of the PO ₄ group	[37,38,39,43,45]
G	921.16	5.11	36.97	BG	



Fig. 3. Raman spectrum of the fossil sample a) ROI: 300 to 3000 $\rm cm^{-1}$ and b) deconvolution at ROI: 1100 to 1800 $\rm cm^{-1}.$

Table 4	
Raman active bands of the FACEN-PVERT0002	2.

Raman shift (cm ⁻¹)	Assignment	Reference
960	Totally symmetric stretching mode (ν_1) of the P—O bond of the PO ₄ group	[38,39,50,51,53,55,56,57,58,54]
1060	Stretching mode (ν_3) of the P—O bond of the PO ₄ group	[39,50,52,57,58]
1225	Amide III	[38,50,51,54,57,58,59]
1286	Amide III	[4,38,50,54,56,57,58]
1707	Amide I	[4,38,50,54,55,56,57,58]

cm⁻¹, 1673.63 cm⁻¹ and 1637.53 cm⁻¹ [38,50,54–59], Amide II at 1540.36 cm⁻¹ [4,50,51,59] and Amide III at 1223.39 cm⁻¹ and 1271 cm⁻¹ [38,50,53–59]. This fit also allows the identification of stretching and bending vibrational modes for various bonds present in the structure of organic molecules. These bonds include: C—C—H, C—O—C, C—O, C—C, =CH, N—H, C—N, C=C and C=O [4,38,50–53,58,59]. Additionally, vibrational modes associated with CH₂ and CH₃ radicals were detected [38,51,54,55–59], as well as vibrations specific to the carbonate group (CO₃) [48,50,58].

The surface analysis of the pulverized samples by XPS shows the presence of Ca, P, O and C, an expected response in apatitic structures frequent in fossil materials. Once the elements were identified (Fig. 4a) by the survey, the regions were identified for high-resolution analysis of Ca 2p (Fig. 4b), O1 s in Fig. 4c and P 2p Fig. 4d for analysis of the binding energies of the elements of interest. The fitted center of Ca $2p^{3/2}$ at 347.39 eV is assigned to Ca-O, Ca-OH bonds and Ca-Ca structures and the Ca $2p^{3/2}$ at 346.39 eV is associated with the presence of Ca-O and Ca

Table 5

Parameters and band assignments of the GM deconvolution of the ROI 1100 to 1800 cm⁻¹ Raman spectrum.

Band	Center	Area	FWHM	Assignment	Reference
Α	1194.89	35509.6	31.69	Bending mode (δ) of the C—C—H and C—O—C bonds	[58,59]
В	1223.39	110,583	33.42	Stretching mode (ν) of the C—O bond (Amide III)	[38,50,53,54,57,58,59]
С	1271.00	208,891	44.34	Stretching mode (ν) of the C—C and C—O bonds (Amide III)	[53,54,56,57,58,59]
D	1308.00	200,910	55.53	Bending mode (δ) of the =CH bonds	[50,58,59]
E	1373.69	112,657	62.41	Stretching mode (ν_3) of the CO ₃ or vibrations associated to macromolecules	[48,50,58]
F	1425.00	45141.3	51.60	Bending mode (δ) of the CH_2	[38,51,54,55–59]
G	1500.26	6345.97	23.24	Asymmetric bending mode (δ) of the $\rm CH_2$ and $\rm CH_3$	[51,54,57,58,59]
Н	1540.36	31838.9	56.45	Bending mode (δ) of the N—H and C—N bonds (Amide II)	[4,50,51,59]
I	1590.23	19397.3	39.22	Stretching mode (ν) of C=C bonds	[50,59]
J	1637.53	30973.5	52.68	Stretching mode (ν) of C=C bonds (Amide I)	[38,50,54,57,58]
К	1673.63	41023.5	55.57	Stretching mode (ν) of C=O bonds (Amide I)	[38,50,54,55–58]
L	1727.78	96585.9	79.32	Stretching mode (ν) of C=O bonds (Amide I)	[38,50,54,57,59]



Fig. 4. XPS spectrum: a) survey 1200 – 100 eV and high-resolution spectra of: b) Ca 2p, c) O 1S and d) P 2p of the analyzed sample.

(II) in apatitic structures [4,60–65].

The fit performed to the O 1 s reveals the presence of O—C, P—O and OH bonds at 531.21 eV associated with the phosphate and (CO₃) groups, and the P $2p^{3/2}$ shows the presence of frequent PO-(PO₄) type bonds in the phosphate groups [4,64,65]. The fitting parameters of the spectra are presented in Table 6.

The presence of Ca and P is confirmed by EDXRF, in addition to Mg, Si, Ti, Mn, Fe, Ni, Zn and Sr, frequent elements in fossil samples (See Fig. 5). The semi-quantitative analysis by EDXRF indicates that Ca with 69.57 % and P 16.13 % are the main elements detected together Al 4.42 %, Si 7.49 %, Mn 0.31 %, Fe 1.54 %, Zn 0.17 %, and Sr 0.37 %.

Fig. 6 shows a SE image of the fragment of the rib fossil fragment, this

Table 6
Fitting parameters of high-resolution Ca 2p, O 1s and P 2p spectra.

Element	Position (eV)	FWHM (eV)	Area
Ca 2p3/2	347.39	1.986	7579.2
Ca 2p1/2	350.91	2.025	3783.7
Ca 2p3/2	346.39	1.986	1138.3
Ca 2p1/2	349.39	1.986	568.4
O 1s	531.21	2.403	18121.9
P 2p3/2	133.02	2.085	1329.6
P 2p1/2	133.86	2.078	664.9



Fig. 5. EDXRF spectrum of the sample.

image contains the identification of 4 enlarged zones (named 1, 2, 3, and 4) that have been identified and are highlighted in yellow along with local chemical composition results obtained by EDS. In Fig. 6b, Zone 1, which is the flat part seen in Fig. 6a, has features that appear to be a silicate due to its morphology and chemical composition of Al and Si, as well as calcite, justifying the presence of Ca and no P. The central part of the fossil, named as Zone 2 and shown in Fig. 6c, displays dimple structures and some voids on the surface. Also, nodular particles of different sizes were noticed in this region. Zone 2 could be a mixture of smectite and amorphous phosphate due to the biodegradation of the material. Zone 3, shown in Fig. 6d, appears to be covered by a region very close to Zone 4. Zone 3 resembles a channel made up of thin material. The chemical composition of this region is Ca, P, K, Si, Al, and Fe, suggesting a mixture of bone and silicates. Zone 4, shown in Fig. 6e, corresponds to the fossil bone structure that was preserved and is chemically composed of Ca and P.



Fig. 6. Fossil secondary electrons images: a) low magnification and identification of four zones, b) zone 1, c) zone 2, d) zone 3, and e) zone 4 besides each EDS spectrum corresponding to each zone.

Discussion

Exposure of fossil materials to different characterization techniques such as chemical or elemental identification allows to obtain information on environmental and dietary characteristics, as well as and suggest a possible diagenetic pathway [53].

Materials characterization techniques such as FTIR and Raman spectroscopy provide structural and chemical information on calcium phosphates present in fossils and get information on stoichiometric apathetic phases with frequent defects in fossil materials. A more indepth analysis that considers the apatitic nature of the material and incorporates both theoretical and experimental answers for structures based on calcium phosphates can provide more information about stoichiometric and structure imperfections calcium phosphates. This refinement is achieved by curve fitting using deconvolution methods with GM responses [40,44,49].

The GM deconvolution model is not frequently reported in the literature and is a necessary method for analysis of vibrational spectra allowing the identification of overlapping bands that otherwise would be missed in the Raman and FTIR responses of calcium phosphates.

The use of GM in the analysis of FTIR spectra allows not only the identification of the typical responses found in the literature for materials based on calcium phosphates, but also the identification of bands associated with defects in the structure. The methods allowed to identify variations in the apatitic structure, not only by identifying the absorption bands, but also by parameters such as FWHM and intensity, which can be associated with the crystalline structures of the material analyzed. The vibrational response differs from the sedimentary material shown in Sup1 a) because the sedimentary material is primarily composed of silicates, whereas the fossil remains contain calcium phosphates.

Analysis of the FTIR spectra allowed the identification of other vibrational modes corresponding to phosphate group bonds [36–44,46,48], carbonate [4,34–41,49], hydroxyl [4,33,48] and amide [4,40,41,43,45,47]. Two regions of interest in each spectrum were selected for deconvolution: from 775 cm⁻¹ to 425 cm⁻¹ and from 1300 cm⁻¹ to 850 cm⁻¹.

Through GM model it was possible to identify binding and bending modes corresponding to the phosphate group. In the first region of interest (ROI: 775–425 cm⁻¹), several bands corresponding to the ν_4 bending mode of the O—P—O bonds of the phosphate group, and a band corresponding to the ν_L vibrational mode of the hydroxyl were identified [36–45]. In the ROI setting: 1300–850 cm⁻¹, we identified ν_1 and ν_3 bending vibrational modes of the phosphate group, a band associated with the presence of HPO₄ groups [36–46], in addition to a bending vibrational mode corresponding to C—O—C bonds [47].

The FWHM parameter in the GM model bands has a high value, these mentioned differences can be attributed to the inclusion of external elements [66] and to a decrease in crystallinity, since smaller FWHM values are associated to higher crystallinity [9].

Raman spectrum presents apatitic structure, with the 1060 cm⁻¹ band corresponding to the v_3 vibrational mode of the PO₄ group ([39,50,52,57,58]), slightly shifted a few wavenumbers higher compared to the spectra of pure hydroxyapatite reported in the literature [39], a parameter that may indicate diagenetic alterations in the initial structure of the bone [59]. The deviation is related to identification of carbonate ions (CO₃) and lower crystallinity of bone minerals [67]. Raman responses were not obtained from the sediment samples.

The organic compounds were identified through deconvolution due to enrichments produced by the interaction between the fossil and the supergenic medium during the diagenetic process. This information helps understand the mechanisms and diagenetic pathways of the material. The presence of organic compounds is recorded in the sedimentary material shown in Sup 2.

The morphological structure identification by SEM ascribed to possible present of smectite minerals within the sample (refer to the image in zone 2 of Fig. 6) may be ascribed to supergene processes after material burial. These minerals likely constituted an integral component of the sedimentary stratum within the confines of the Risso cave.

On the other hand, the presence of Sr was also identified in the analyzed sample, and this condition can be attributed to its subsequent inclusion from the sedimentary environment (As observed in Sup1 b). Previous reports have documented the proportional relationship between the amount of strontium in animal bones and their diet, with herbivores, in comparison to carnivores, carrying higher quantities of Sr [68]. However, later studies [69,70] confirm that biogenic strontium is not preserved in significant amounts in bone after diagenesis, but mostly corresponds to diagenetic inclusions from the medium, associated with the characteristics of the surrounding soils and groundwater.

The Ca presents sensitivity to conditions that propitiate structural variations in apatite due to environmental conditions [67]. Therefore, it could be an indicator of new crystalline or amorphous structures in the material. In addition, the presence of Sr in the sedimentary medium allowed to consider the substitution of Ca by that element, since Sr ions are considered strong substitutes for Ca in apatitic materials [70].

The functional groups identified in this work suggest a diagenesis route. The stretching and bending vibrational modes of the phosphate group are sensitive to ionic impurities, so a change in the position of the bands and FWHM can translate into alterations in the mineral structure [71]. It could suggest that microorganisms present in the environment produce organic acids during the decomposition process, causing the dissolution of hydroxyapatite and altering its biogenic structure [72].

According to Sousa et al. [9], an estimated route of diagenesis in carbonate environments involves the constant drip of water rich in calcium carbonate (CaCO₃), which allows the precipitation of calcite in the fossil, such as in limestone and dolomite caves. This condition favors the availability of CaCO₃ in the medium due to chemical weathering processes.

Although carbonate minerals were not identified by XRD (see Sup 3), specific bands in the FTIR spectra did reveal the presence of CO_3 groups. This suggests the existence of compounds related to carbonate structures, possibly derived from the dissolution of the cavern walls. This hypothesis is further supported by the detection of calcium, as observed in the analyses carried out with EDXRF and SEM-EDS (see Sup 1b and Sup 4).

The suggested diagenesis process is featured by precipitation of calcite minerals within the bone pores, in addition to changes in the molecular structure of hydroxyapatite, followed by recrystallization of hydroxyapatite [9,73].

For ideal recrystallization of hydroxyapatite, the availability of calcium and phosphorus is necessary, where crystals are generated by dissolution and recrystallization due to the existence of calcium and phosphorus sources under neutral or slightly alkaline pH conditions [74]. During diagenesis there is a degradation of the organic fraction of the bone and the dissolution of a fraction of the minerals present. It is presumed that the decomposition of the organic matrix is a source of the H₂PO^{4–} anion, while chemical weathering in carbonate composition rocks under subsurface conditions could be a source of the Ca²⁺ cation. The loss of collagen and water leakage might favor the demand for phosphorus and calcium for recrystallization, assuming a structure like unaltered bone [9]. Therefore, it is speculated that the crystallographic structure remains like bone without significant alterations.

This condition agrees with what has been observed, since the spectra show an apatitic structure with slight variations due to the inclusion of elements such as Sr in our case. In addition, vibrational modes corresponding to the carbonate group are identified according to our diagenesis pathway. It can be translated as the precipitation of carbonate minerals inside the fossil, a consequence of the accumulation of moisture on the bone surface. This conditions the formation of a calcium carbonate layer and constitutes physical protection against weathering agents [9].

This study complements, from a different perspective, our

knowledge of the Pleistocene palaeofauna that inhabited the current territory of Paraguay. As mentioned above, studies on Pleistocene fossil mammals from the Paraguay are very scarce compared to those carried out in other areas of South America (e.g., Argentina, Brazil and Bolivia [75]). The materials found so far are very fragmentary and isolated. According to Hoffstetter (1978), Báez-Presser et al. (2004) and Ríos-Díaz et al. (2014) the faunal association of Xenarthra recorded for Paraguay corresponds to Glyptodontidae, Dasypodidae, Megatheriidae and Mylodontidae [12–14].

More recently, and according to Miño-Boilini et al. (2015), xenarthrans recorded include Cingulata Glytodontidae (*Glyptodon* sp., *Neosclerocalyptus* sp., and *Panochthus* sp.) and Pampatheriidae (*Holmesina paulacoutoi*). Ground sloths include Mylodontidae (*C. cuvieri*) and Megatheriidae (cf. *Megatherium*) [19].

Moreover, the ground sloth *C. cuvieri* was probably linked to lowlands of eastern South America [32] in open forest environments of the intertropical region of Brazil [76].

Conclusion

In this work was performed a detailed materials characterization study of the most complete fossil record of a member of the Pleistocene megafaunal fauna of Paraguay, the ribs of *Catonyx cuvieri* (Lund, 1839) (Mammalia, Xenarthra). From the results obtained in this study, it can be concluded that:

The presence of vibrational modes corresponding to phosphate, carbonate, hydroxyl, and amide group bonds was identified in the samples by analysis of FTIR and Raman spectra. By doing the GM deconvolution of Raman spectra, the individual bands containing information about the bonds present in amides and other organic compounds were distinguished.

Absorption modes assigned to the phosphate group and the Gaussian model deconvolution procedure allowed the identification of the different superimposed vibrational modes corresponding to **P**—**O** bonds and characteristic bands of carbonates and hydroxyl ions.

The fossil remains showed that the diagenesis pathway of the bone remains was associated with a $CaCO_3$ rich environment inside to the cave. The CO_3 content in the system where the fossil remains were found originated from the dissolution of limestone rocks, a process that began through fractures and was transported vertically and laterally by waters that entered the system from the surface, supplied to the environment by precipitation. This condition provided the environment within the karst system with the ideal carbonate compounds for the recrystallization of hydroxyapatite, in addition to favoring the precipitation of calcite in the bone pores.

CRediT authorship contribution statement

Bella Sanabria: Writing – original draft, Formal analysis. Christian F. Colman: Writing – original draft, Formal analysis. A. Celeste Aquino: . Ángel R. Miño-Boilini: Writing – original draft, Methodology. Alfredo E. Zurita: Writing – original draft, Methodology. Ricardo Souberlich: Writing – original draft, Methodology, Funding acquisition. Sergio D. Rios: Writing – original draft, Methodology, Investigation. Fernando Mendez: Writing – original draft, Supervision, Project administration, Funding acquisition. Alex Matos da Silva Costa: Writing – original draft, Methodology, Formal analysis, Data curation. Edher Z. Herrera: .

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT) of Paraguay – Finance Code PINV15-120 and PINV18-17.

This research is a part of project code RLA1019: Strengthening Capabilities for the Utilization of Nuclear and Radiation Technology to Characterize, Conserve, and Preserve the Cultural Heritage, financed by International Atomic Energy Agency (IAEA).

Thanks to the scientific initiation program (PIC) of FACEN for the resources and infrastructure provided.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2024.101745.

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B. Sanabria et al.

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B. Sanabria et al.

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