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Journal of Historical Archaeology and Anthropological Sciences

Case Report
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Early Holocene human occupation in the lowlands of South America- Gruta Azul de Cocalinho, Brazil

Volume 9 Issue 2 - 2024

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Received: May 13, 2024 | **Published:** June 04, 2024

Abstract

Although there are signs of human occupation in the Americas older than 20.000 years, most of the evidence dated to be older than 10.000 years are based on signs of human presence, while human remains from the Early Holocene are relatively rare. In Gruta Azul, in the Cocalinho karst (central Brazil), where human remains were discovered, U-series disequilibrium methods were used for estimating local environmental changes and bone dating, along with optically stimulated luminescence (OSL) of quartz for estimation of ceramic age, adding important piece of information about early hunters-gatherers occupation in central South America. The results indicate that human presence in the site happened in two different periods, being the first older than 10.000 BP, in an area of the cave estimated to be submerged for at least the past 5.000 years, while the second started approximately at 2.000 years B.P., placing this site amongst the oldest sites where human remains are found in the lowlands of South America.

Keywords: Holocene, photogrammetry, central Brazil, underwater speleology, excavation, underwater archeology

Introduction

In the past 13.000 years the Americas experienced different climate changes, directly affecting human occupation. The end of the last deglaciation was marked by an event known as Younger Dryas, which happened between 12.900 and 11.700 years BP.[1] In most of South America this event was marked by a substantial increase in the pluviometric levels in comparison with to the dryer conditions that prevail from approximately 11.000 to 5.000 years BP, especially in the central part of the continent.[1,2] At approximately the same time there was a pioneer phase of colonization by hunters-gatherers, with dispersal inland following the major river systems and developing to a point when most of the territory had been occupied, or at least visited, by humans in the 8th millennium BP.[3]

Although there are signs of human occupation in Central and South America as early as 20.000 BP,[4] only indirect evidences of human presence are found in most of the sites older than 15.000 BP, like charcoal, seeds, shells and lithic artifacts, or evidences of activity, for instance, the ochre mines found in the underwater caves of the Yucatan peninsula.[5] Very few sites in the Americas have human remains directly dated to be older than 10.000 BP.[4]

The earliest reliable evidence of human presence in Brazil is dated to the 13th millennia BP. They are rock shelters found in the Serra da Capivara (northeastern Brazil) and the Peruaçu river valley (Central Brazil), both regions are within the São Francisco river basin, the main river valley that connects central and northeastern Brazil.[6] It provides a perennial water source and an exceptional place to obtain different kinds of resources, for both technology (lithic raw material and wood) and subsistence (fish, mammals, and edible plants). The locations of these sites could be related to the selection of this valley as one of the main entry and dispersion routes into the continental interior.[3]

The western limit of the early evidence of human occupation in the lowlands of South America during the early Holocene seems to be the Tocantins river basin, where there are several sites dated to the 11th millennium BP.[3] The main contemporary exception would be the Serra das Araras area, in the state of Mato Grosso, where the Santa Elina archeological site is found. The site is characterized by lithic artifacts and paintings dating as old as 11.000 BP.[7] In the same area is located another relevant site called Cidade de Pedra, where ceramic, charcoal, lithic artifacts and paintings were found.[8]

Although human presence is well documented, human remains from the 10th millennia or older are rare in the vast area to the east of the Rio São Francisco basin. The most famous exception is probably the Luzia woman, found in 1974 in the Lapa Vermelha archaeological site, considered one of the oldest humans remains found in the Americas. Later human remains and evidence of structured burial practices dated from 10.000 to 8.000 BP are found in other areas of central Brasil.[9] One of the most structured primary and secondary burial practices found so far in the lowlands of South America are in the well documented Lapa do Santo archaeological site,[10] where a ritualized decapitation, amongst other practices, were described.[11] Additional evidence of funerary rituals dated approximately from the same period, however less structured, are found in the Serra da Capivara archeological site, where lithic objects and charcoal were found with human remains dating back to 10.000 BP.[12]

While human presence can be traced back to the late Pleistocene, the earliest ceramic production in the Brazil was probably observed in Taperinha archaeological site, where grit- and shell-tempered pottery from the Mina phase were found, which, according to thermoluminescence dating, fall from 8.000 to 7.000 years BP.[13] The mid-Holocene is marked by a significant reduction in evidence of human occupation in lowlands South America, potentially caused by climate change, or simply by occupation dynamics (as predicted by general density-dependence models),[14] in what became known as the occupation hiatus,[15] therefore ceramic samples from this period are much less abundant.[16] Conversely, ceramic from the past two millennia, built using different technologies and for different purposes, is found in many sites in central South America.

Serra do Calcário, Cocalinho Karst

Located between the Araguaia and Rio das Mortes rivers flood plan, approximately halfway between the Tocantins river basin and the Santa Elina archeological site, this limestone formation extends for a distance of 18 km and a maximum width of 5 km, following an E-NE orientation. Many caves are found in its limestone walls, with one being particularly interesting, Gruta Azul (as known as Gruta dos Fósseis, name given to the archeological site), due to its size and to the fact that most of its area is permanently flooded. The cave is characterized by a large entrance that provides access to a lake with a diameter of approximately 40 meters that connects to the flooded cave galleries (Figure 1).

Figure 1 Gruta Azul. photo of the cave entrance. Flooded conduits develop to both sides of the lake, reaching a maximum depth of 47 meters. Taken at x:123.908, y: 100.133, z:1.5. Credit: Rodrigo Oliveira.

In 2004 a cave diver found an apparently complete human skeleton in the aphotic zone of the cave, in a conduit located at the western part of the lake, at a depth of approximately 5 meters.[17] In 2020, a second group of cave divers started to explore and map the underwater passages of Gruta Azul, having surveyed so far 1080 meters of submerged cave passages, reaching a maximum depth of 47 meters.

Speleothems are found above the lake and in the flooded galleries, down to a maximum depth of eight meters, which can be an indication that the area below this depth has been flooded most of the time since the formation of the cave.

For the purposes of the research, the archaeological site was arbitrarily divided in three different zones, according to its location in the cave, with the material observed in the area, and the initial dates obtained. The zones are: (i) in the eastern area of the lake, where there are pieces of ceramic, including a full vessel at a depth of 27 meters ("Zone 1") (ii) in a dry area at the far western shore of the lake were found human bones and ceramic ("Zone 2"); (iii) in the western area of the cave, starting in the transition to the aphotic zone and spreading to a maximum penetration of approximately 70 meters into the cave, at a depth range between 5 to 7 meters, the remaining of at least 6 individuals, including adults and children ("Zone 3"), can be found, along with animal bones, shells and at least one lithic artifact (please refer to Results, Photogrammetry). Amongst the animal bones, the following species were identified: tapirs (*Tapirus terrestris*), deers (*Ozotoceros bezoarticus*) and macaws (*Anodorhynchus hyacintinus*), while the shells are apparently aruá-do-mato (*Megalobulimus oblongus*). A detailed map of the cave be observed in Figure 2.

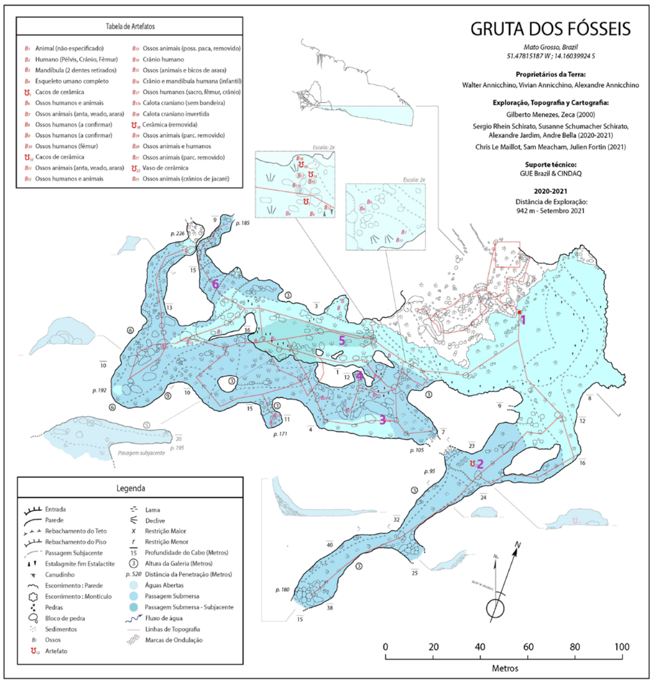

Figure 2 Gruta Azul. Map of all passages explored until September 2021 with the location of all submerged archeological artifacts found. The archeological site was registered under the name Gruta dos Fósseis (used in the map), due to the existence of many other sites named "Gruta Azul" in Brazil.

Water springs are found in the deeper section, in the easter area of the cave, where a small flow of clearer water can be seen flowing from small cracks on the limestone walls, although no noticeable flow can be observed neither in the lake nor in the western section. Despite no obvious indications of siphoning flow anywhere in the cave, there is debris accumulation observed on the deeper western walls, which might be an indication of water flowing in an E to W direction.

At 150 meters into the cave a shaft that abruptly changes the depth from 7 to 15 meters may have functioned as a trap in the past, when the water level was low enough for animals to penetrate the cave. On the bottom and at the wall of this shaft different fauna bones can be observed, from small rodents to a complete deer and what seems to be the skull of a large *Cuniculus paca* of Holocene, similar to those described in the Cuvieri cave,[18] at Lagoa Santa.[19]

Materials and methods

The current research has been conducted under the approval of Instituto Nacional do Patrimônio Histórico e Arquitetônico Nacional ("IPHAN"), under the license number 01425.000148/2021-59. The research on the site is on-going and its initial phase has been divided into two parts, both herein reported. The first part included the collection of speleothems, bones, and ceramic samples for initial dating, so that a timeline of the human occupation of the cave and an estimation of the water dynamics over time could be made. Water levels variation between the dry and the wet season were also recorded.

Citation: Schirato SR, Jardim A, Le Maillot C, et al. Early Holocene human occupation in the lowlands of South America- Gruta Azul de Cocalinho, Brazil. *J His Arch & Anthropol Sci.* 2024;9(2):99‒108. DOI: 10.15406/jhaas.2024.09.00304

Due to the difficulties of locating and recording the archaeological material in the submerged area of this zone of the cave, the second part was the photogrammetric documentation of the site, which allowed non-diving researchers to fully study the site, its artifacts, and their relative position in the cave in three dimensions, before the collection of any materials. After the initial survey and the identification of archaeological material on the surface of the site, both in dry and flooded areas, it was decided to begin the work of probing and excavation in Zone 2, due to the higher concentration of archaeological material on the surface as well as the presence of apparently anthropized sediment.

Speleothem dating

Gruta da Lagoa Azul is not a very decorated cave, with few areas where speleothems can be found. After a detailed analysis of the underwater images, divers were instructed to collect specific samples of speleothems from different areas and depths. Additionally, speleothems found in dry areas of the cave, including a chamber that is fully flooded during the wet season, when the water level is higher, were collected in order to provide further understanding of the water level variation in the cave over the years.

Chronological studies on speleothems were based on U–Th geochronology performed at the University of Minnesota (USA) and at Earth and Environmental Sciences and at the Isotope Laboratory of the Institute of Global Environmental Change, Xi'an Jiaotong University, China. Subsamples of 100 mg were obtained in clear layers, close to the growth axis of stalagmites trying to keep a maximum thickness of 1.5 mm, 10mm wide, and no more than 3mm depth. In both laboratories the Th and U isotopes were counted using inductively coupled plasma mass spectrometry (MC-ICP-MS Thermo-Finnigan NEPTUNE PLUS), and the results were calculated in a standard spreadsheet based on according to.[20] The ages are reported in BP (Before Present) and age uncertainties are of 2α statistical significance.

Bones dating

Archaeological samples (bones and teeth of humans and fauna) were collected from Zones 2 and 3, and were sent to Beta Analytic (Miami, Florida, USA) for dating. The first technique used was ^{14}C isotope dating of collagen extraction. Several bone samples were analyzed, however only one sample, a tooth root found in the shore of the lake, yielded enough collagen for ^{14}C isotope dating. Four samples (three human remains and one rib of an unidentified megafauna species) were dated using U/Th decay.[21] Sample surfaces were cleaned by means of abrasion and the most pristine area of the sample was chosen for sub-sampling. Sub-samples were sonicated and rinsed with Milli-Q water (18mΩ) and dried at 40°C. Between 20 to 100mg of homogenized sample was dissolved in 0.25 M HNO_3 to minimize leaching of uranium and thorium from detrital material. Uranium and Thorium were isolated from the samples by extraction chromatography and analyzed by MC-ICP-MS (Thermo Fisher Neptune Plus, Waltham, MA USA).

Age calculation and propagation of the uncertainties were performed by using Monte Carlo simulation[22] and by considering the measured isotopic ratios of U and Th derived along with other variables, such as the spike mass and concentration, sample mass, the decay constants of U and Th (as described in in the Speleothem dating section), along with their uncertainties.

Ceramic dating

Optically stimulated luminescence (OSL) of quartz was used for dating the ceramic samples collected from Zones 1 and 2.[22] Ceramic samples were crushed to release sand grains. Then, they were wet sieved to isolate the 63–180 µm grain size fraction for samples L1568, L1669 and L1570, and 180–250 µm grain size fraction for sample L1567. Afterwards, these specific grain size fractions were submitted to treatments with H_2O_2 and 10% hydrochloric (HCl) acid to remove organic matter and carbonate minerals, respectively. Quartz separation involved the use of lithium metatungstate solutions (LMT) to separate light minerals from heavy minerals (LMT = 2.75 g/cm^3) and to separate quartz from feldspar grains (LMT = 2.62 g/cm^3). After this step, quartz concentrates were submitted to a treatment with 38% hydrofluoric (HF) acid for 40 min to remove the external layer of quartz grains affected by alpha particles and eventual remnant feldspar grains. After the chemical treatments, which reduce quartz grain size, samples were sieved again to separate grains in the 63–180 µm and 180–250 µm size intervals. Aliquots of quartz grains were mounted on stainless steel discs for luminescence measurements on a Lexsyg Smart reader at the Luminescence and Gamma Spectrometry Laboratory (LEGaL) of the Institute of Geosciences, University of São Paulo, Brazil. The reader is equipped with blue and infrared LEDs, U-340 Hoya filter for light detection in the ultraviolet band, photomultiplier (Hamamatsu) and beta radiation source ($^{90}Sr/^{90}Y$) with a dose rate of 0.12 Gy/s. Equivalent doses were estimated using the single aliquot regenerative (SAR) dose protocol.[23] Only aliquots with recycling ratio within 0.9-1.1 range, recuperation less than 5% and negligible infrared stimulated luminescence signal (feldspar contamination) were used for equivalent dose calculation using the central age model.[24] Dose recovery tests were carried out to set up best preheat temperatures and to appraise the reliability of the SAR protocol for estimation of a known radiation dose using quartz retrieved from the studied samples of ceramics. Samples L1568 (six aliquots) and L1469 (four aliquots) were chosen for a dose recovery test using a given dose of 0.9 Gy, a preheat temperature of 200oC (10s) and cutheat temperature of 160oC (0s). Calculated-to-given dose ratios were 1.04 ±0,02 (sample L1568) and 1.03 ± 0.01 (sample L1569). Dose rate was calculated through radionuclides concentrations (U, Th e K) measured by high-resolution gamma spectrometry using a HPGe detector in an ultralow background shield (relative efficiency of 55% and energy resolution of 1.8 keV in 1332 keV, Canberra Industries). Samples were dried and packed in sealed plastic containers for radon requilibration for at least 28 days. Water saturation was calculated as the water weight to dry sample weight. Radionuclides concentrations were converted into dose rates by using conversion factor by.[25] The cosmic dose rate was negligible (<0.001 Gy/ka) due to shielding by the thick bedrock roof as the ceramic samples were collected inside the cave. The dose rate was calculated assuming an exclusive contribution of radionuclides within the ceramic. Thus, dose rate could be overestimated or underestimated if the surrounding sediments have respectively lower or higher radionuclides concentrations than the ceramics. This age uncertainty would be restricted to the gamma dose rate contributing with 50 to 65% of the total dose rate measured for the studied samples.

Water level and temperature monitoring

A depth and temperature data logger (Hobo, model U20-001-04, Bourne, Massachusetts) was left in the western end of Zone 3 in the begging of the wet season (summer) and retrieved at the end of the dry season (winter) in order to estimate the peak variation in the water level and the temperature in the lake during the different seasons of the year of 2021.[26]

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Photogrammetry

A complete photogrammetric model was done for Zone 3, as well as models for specific objects, such as ceramic and fauna remains, found in other parts of the cave. A three-dimensional virtual mesh was created for the precise location of all artifacts found in Zones 2 and 3. For this proposition, total Station Survey equipment (Total Station Sanding STS752R8, Sanding Optic-Eletrics, Guangzhou, China) was used to determine the precise 3-dimensional location of each artifact on the archaeological site and the angles or distances between them. This so-called "free stationing technique" was used to make maps to support the excavation process. The grid produced by this method, associated with a map of the "dry area" located at the western shore of the lake, allowed the connection of all surveyed area, whether in or out of the water, using the coordinates (X, Y and Z, values in meters) to every location of the cave. Coordinates X and Y are based on an arbitrary (0,0) point, located in the southwest end of the grid, while coordinate Z in relation to the water surface (which was measured in the dry season, when the lowest water level is encountered). All artifacts described in this article will be referenced by their coordinates.

The models were created based on a total of approximately twelve thousand photos taken at a resolution of 20 megapixels each. Camera settings used were adjusted to optimize image definition based on the ambient conditions and are detailed in Table 1. During image capturing divers used closed circuit rebreathers to reduce percolation, i.e., sediment dislodgement from the cave ceiling, which creates intense backscatter that negatively affects the quality of the images.[27]

Table 1 Camera settings used for image capturing

	From	To
ISO	800	1600
Speed (sec)	1/100	1/125
Shutter	5.6	9
Focal distance (mm)	10	15

Two computers were simultaneously used in parallel to compute the models: (i) first configured with a Threadriper 3990X 64-core processor (AMD, Santa Clara, California), 256 GB RAM memory and a GeoForce RTX 3090 video board, (NVIDIA, Santa Clara, California) and (ii) second assembled with a Rayzen 9, 12-core processor (AMD, Santa Clara, California), 128 GB RAM memory and a GeoForce RTX 3080 video board, (NVIDIA, Santa Clara, California). The software used for the photogrammetry was Metashape (Agisoft, Russia). Total processing time was approximately 500 hours.

Results

Speleothem dating

The dates obtained from each of the samples collected are reported in **Table 2**. It can be observed that development of the speleothems collected stopped approximately 7.500 years BP, while the mean minimum age of the samples is approximately 11.600 years BP. The exceptions are the samples collected in an area that remains partially dry during part of the year, highlighted in Figure 3 (MTCO-02, MTCO 05 and MTCO-21). The development of the speleothems over time, based on the U/Th dates from each piece is detailed in **Table 2**.

The piece collected deeper in the water column, LF-1, removed from a depth of 3 meters, dated older than the ones obtained from shallower areas. MTCO-02 was collected in the dry section of the cave.

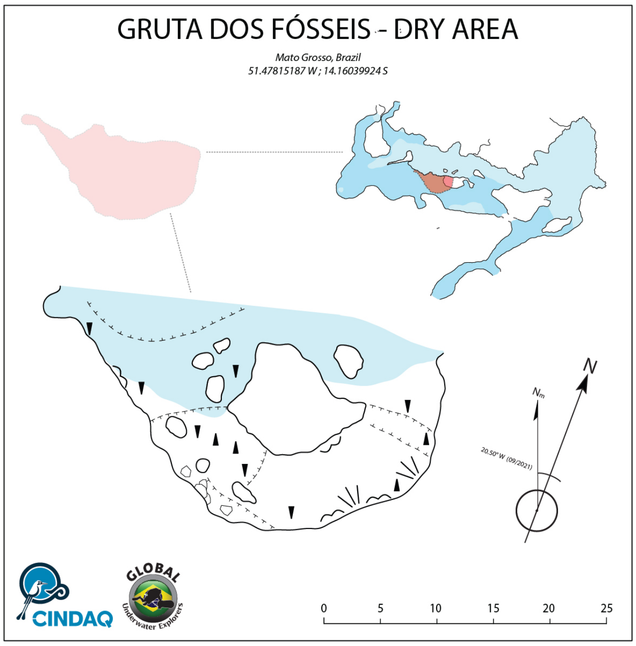

Figure 3 detailed view of the chamber that is partially dry during the dry season and the locations from where samples MTCO-02, MTCO 05 and MTCO-21were retrieved.

Ceramic dating

The results from the OLS dating are displayed in Table 3. As it can be observed, the oldest piece is a jar neck (Figure 4), estimated to have been made 770 years BP, while the mean age of the samples is 575 years BP.

Figure 4 Ceramic jar (L1567) found in Zone 2.

Bones dating

The only sample the yielded enough collagen for radiocarbon dating was a human tooth root coded GF-19 (Beta – 622112), which dated 1040 ± 30 years BP, collected in Zone 2. The results for the four samples dated using the U/Th decay method (U-series disequilibrium) are detailed in Table 4. It is important to note that sample IS-0934 is a megafauna bone found in Zone 3, while sample IS-0931 was collected in Zone 2, at the water shore, and the remaining two samples were collected from Zone 3.

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Table 3 Ceramic samples dating

Sample	Sample number	Recycling ratio		Dose (Gy) (CAM)		OD %	Age (years BP) (CAM)	
Jar neck	L1567	1.03	±0.01	0.811	±0.010	5.2	770	±50
Fragment	L1568	1.01	±0.01	0.658	±0.039	25.5	305	±32
Fragment	L1569	1	±0.01	0.703	±0.004	0	638	±54
Fragment	L1570	1.03	±0.01	0.975	±0.024	11.6	587	±51

Table 4 Bones samples dating - U/Th decay technique

Material	Origin	ID	Uncorrected age	±95% C.I.	Corrected age	±95% C.I.
Tooth Root	human	IS-0931	1722	29	1693	30
Bone	human	IS-0932	8630	526	8621	524
Bone	human	IS-0933	10981	187	10951	186
Fossilized bone	fauna	IS-0934	26453	590	26281	587

Water level and temperature monitoring

The results recorded by the logger device demonstrated that the water level varied 1.8 meters, which is in line with the measurement of the water level marks in the limestone walls of the lake, while the temperature stayed stable within a 1oC degree variation.

Photogrammetry

A scaled, high-resolution model was created for Zone 3. From this complete model, areas of specific interest, such as burial sites and areas with high concentration of fauna or ceramic, were extracted, allowing for 3-dimensional analysis of the material. Additionally, a model of a complete ceramic jar found in the deeper section of the lake in Zone 1 was also created. The full model with the main areas of interest highlighted can be accessed at https://construkted.com/asset/au4akjz4p8z/.

A 3-dimensional study of each piece of interest, including performing bone measurements, before any artifact was touched, was completed and the subsequent collection of material was documented with the creation of additional 3-dimensional models of the area. A map of the cave, with the different zones highlighted and the illustration of the human and fauna remains herein described (and their respective locations in the map) can be found in Figure 5.

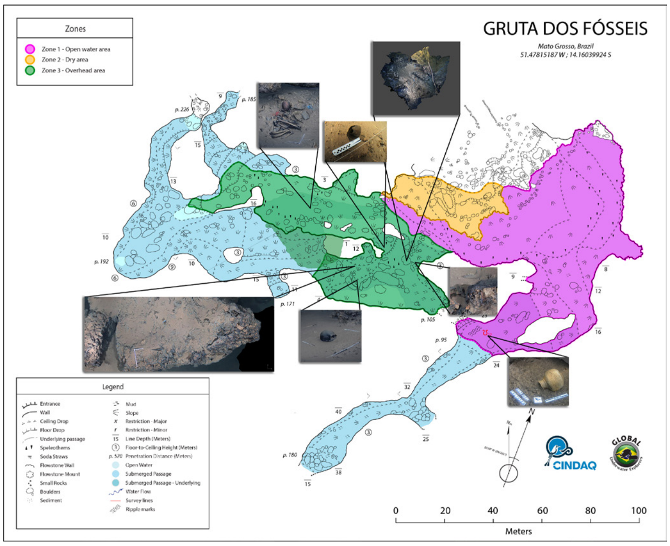

Figure 5 Cave map modified to highlight the archeological zones, pointing the locations where the artifacts herein reported were found.

An orthomosaic of the submerged part of Zone 3, extracted from the photogrammetric model, overlaid with the grid used for coordinates definition, can be observed in Figure 6. For the purposes of this paper, the human and fauna remains herein described will be numbered according to the original labeling of the stations, which followed the order of their findings.

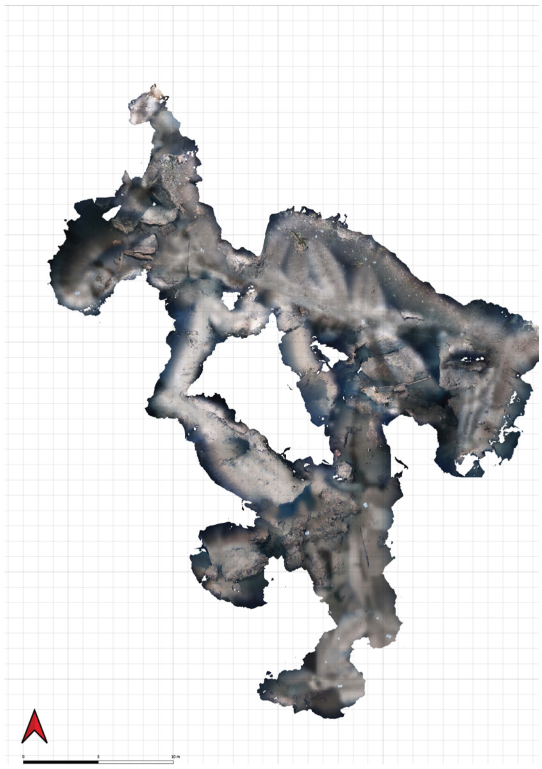

Figure 6 Zone 3 orthomosaic overlaid with grid for, amplifying the area highlighted in green in Figure 5.

Human remains, Station 4 (location: x:105.90, y:99.71, z:-4.85)

The most complete human skeleton found in the cave. An image of the site is displayed in Figure 7. An initial observation would presume, based on the apparent relative position of the bones that the body was left in that place in a flexed position. However, the detailed revision of the photogrammetric model revealed that limbs are inverted, as demonstrated in amplified image. The right Humerus is positioned parallel to the Una, in position where the Fibula and the Tibia would be expected, while the right Femur is positioned in a 90o degree angle with the left Humerus and left Femur, in a location where the left Tibia should be found. Additionally, the right Tibia is placed where the right Femur should be found. Bone sample IS-0933 (dated approximately 10.900 years B.P.) was collected from this site.

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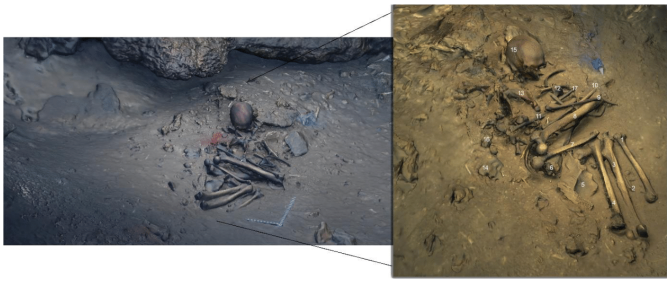

Figure 7 3-dimensional view of Burial Station 4. In the amplified image a detailed description of the bone's positions: 1. Right Humerus; 2. Right Ulna; 3. Right Femur; 4. Right Tibia; 5. Right Ilium; 6. Left Tibia; 7. Left Humerus; 8. Left Femur; 9. Right Radius; 10. Right Fibula; 11. Left Fibula; 12. Left Ischium; 13. Jawbone; 14. Sacrum; 15. Skull; 16. Left Patella; 17. Left Ulna.

Human remains, Station 8 (location: x:125.03, y:89.72, z:-5.22)

This site is marked by a lithic object clearly placed on the top of a speleothem. It marks the entrance for another room, where other skeletons are located, along with a selection of different fauna bones, including tapirs, macaws and shells. On the base of the speleothem where the lithic artifact is located human and animal bones are placed in a small fracture on the floor. Based on the analysis of the model, it was found that adult and child's bones were placed together with different fauna bones, including wild pig tooth and various caiman bones. A full vision of the site can be observed in Figure 8. Bone sample IS-0932 (dated approximately 8.600 years B.P.) was collected from this site.

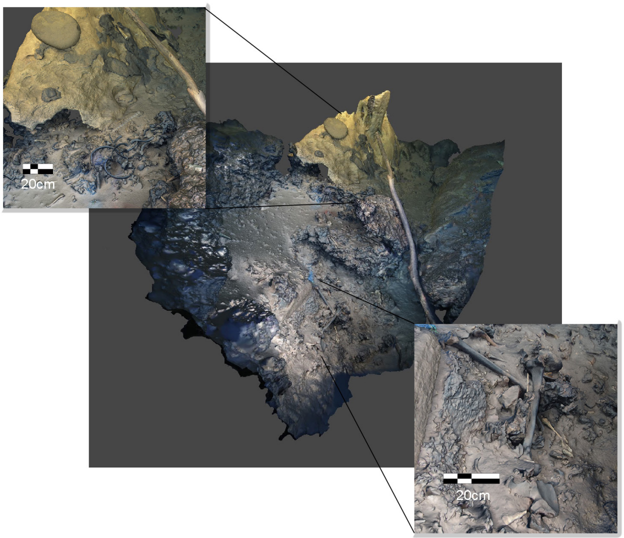

Figure 8 A broader vision of Station 8, along with details of the placement of the bones. In the upper amplified image, it can be seen the lithic object, along with human and fauna bones at the base of the speleothem where it is located. In the lower enlarged photo, two human femurs and a pelvis can be observed. The largest bones probably rolled down due to the slope of the terrain.

Human remains, Station 16 (location: x:112.48, y:89.28, z:-3.19)

In this area the skull of a child is placed, along with a jawbone. Although a preliminary assessment indicates that the estimated age of the child at the moment of death is compatible for the skull and the jawbone, it cannot be affirmed at this point that both bones belonged to the same individual. The skull is folded on itself, probably due to the immaturity of the bone structure. A 3-dimensional image of the artifacts is displayed in Figure 9.

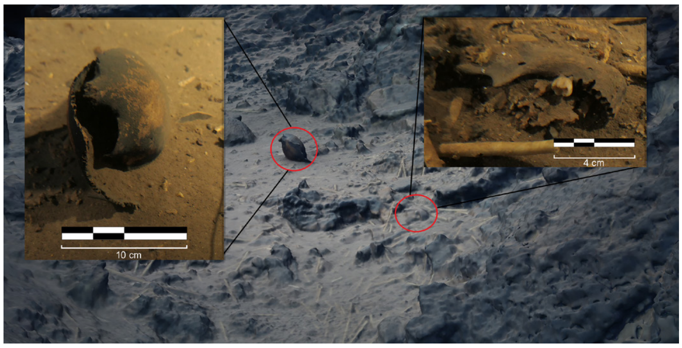

Figure 9 Infant skull and jawbone circled in red and zoomed-in for easier visualization.

Human remains, Station 14 (location: x:116.92.90, y:71.41, z:-8.35)

Located in the far southern end of the same room where Station 8 is located, a human male skull was partially buried. No other human bones are present in the area. Images of the skull in its original location, and after its removal are displayed in Figure 10. A longitudinal cut of the 3-dimensional model of the room where this burial site is located is illustrated in Figure 11. As can be observed, the site is located in the highest area of the room.

Figure 10 Station 14, where a partially buried human male skull was found. In the upper right corner, the enlarged image extracted from the 3-dimensional model produced in the laboratory.

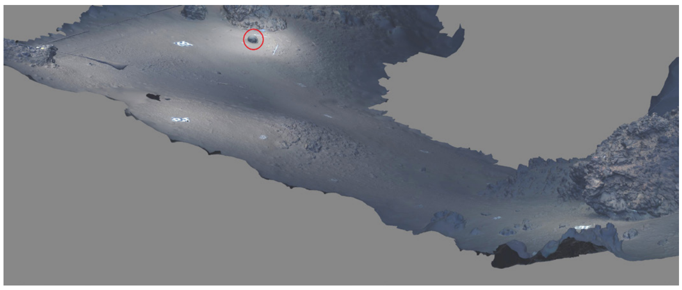

Figure 11 Longitudinal cut of the room where Station 14 is located. The site is marked in red. As it can be observed, the burial site is located in the highest area in the chamber.

Zoo archaeological area 1, Station 7 (location: x:131.32, y:85.399, z:-4.009)

A flat area of approximately 20m^2 in the transaction to the aphotic zone is covered with fauna bones, mostly belonging to deers and tapirs. In the eastern part of this area, a small rock with a partly broken, and potentially burnt, jawbone of a tapir is observed. Further analysis of the bones shall be conducted to determine what kind of manipulation happened to the bones and whether any of the bones is

Citation: Schirato SR, Jardim A, Le Maillot C, et al. Early Holocene human occupation in the lowlands of South America- Gruta Azul de Cocalinho, Brazil. *J His Arch & Anthropol Sci.* 2024;9(2):99‒108. DOI: 10.15406/jhaas.2024.09.00304

human. Additionally, in the same area the only dated megafauna bone was found (part IS-0934, Table 4). An orthomosaic, extracted from the model, is displayed in Figure 12.

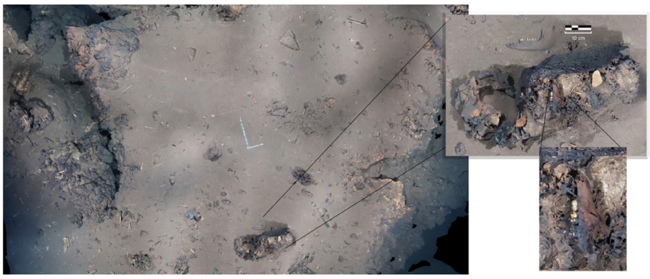

Figure 12 Flat area in the transaction to the aphotic zone where remains from different animals can be observed. In the right side, amplified images of the apparently burnt jawbone placed on the top of a rock.

Zoo archaeological area 2, Station 15 (location: x:105.90, y:99.71, z:-4.85)

Inside the room where sites 3 and 4 were found, located at approximately 2 meters from Staton 14, a flat, elevated, rocky surface is covered with fauna bones. Differently than Zooarchaeological area 1, this area contains the remains of many macaws, also found in other areas of Zone 3, however in smaller quantities. Shells are also present on the site. Human remains, namely one rib and one clavicle were found approximately 1.5 meters to the north of this area. An orthomosaic of the area is displayed in Figure 13.

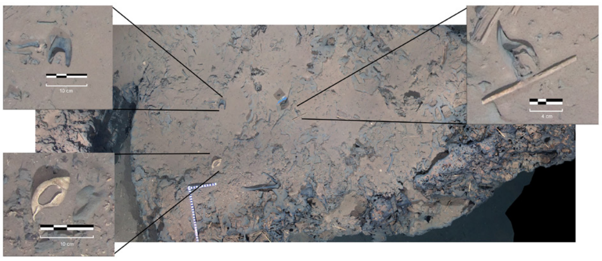

Figure 13 Flat surface where fauna remains can be found. On the bottom left, upper right and upper left conners expanded samples of macaw beaks and shells were highlighted for illustration.

Ceramic Jar

A full ceramic jar was found in Zone 1, in the aphotic zone, at a depth of 27 meters. A model of the jar was made at the location before it was brought to the surface for further analysis. A 3-dimensional view of the jar, extracted from the model, is shown in Figure 14.

Figure 14 The intact ceramic jar partially buried found at a depth of 27meters, in the aphotic zone of the cave.

Discussion

Human remains in submerged caves, in areas that were flooded due to either changes in pluviometric patterns or in ocean level, are observed in different parts of the world. The teenage female skeleton named Naia found in the Sac Actum cave system[28] is one example of a death likely caused by an accident in the aphotic zone of the cave. In other cases, like the skeleton that became known as "Eve of Naharon", found in the On Bel Ha cave system, also located in the Yucatan Peninsula, the human remains in the cave are part of a mortuary ritual.[29] In this sense, Gruta Azul is a peculiar site in South America, where human remains were found, whether related to mortuary rituals or not, in the submerged galleries of a cave that was once dry.

Based on the dates obtained so far, it is possible to theorize that Gruta Azul has been occupied by humans in at least two different time periods, one when the water level was at least 8 meters below its current level, which would extend access to the western area of the cave (Zone 3) up to a total penetration of approximately 150 meters; and a second one, which probably began around 2.000 BP and extended to approximately 500 BP or later. Since the late artifacts (bones and ceramic) are found either in the dry area of the cave (Zone 2), or in the lake shore in an area that remains partially exposed during the dry season, according to the water level measuring device (Zone 1), it is a possibility that the later occupation happened after the groundwater rose its current levels.

This occupation hypothesis would be consistent with the difference between the ages of the bones found in Zone 3 and the bones and ceramic found in Zones 1 and 2. Zone 3 has remarkable differences from Zones 1 and 2. While human remains are found in Zones 1 and 2, they are usually associated with ceramic. Due to the proximity, the type a and dates of artifacts observed, and knowing that there is a variation in the water level of at least 2 meters, that would make possible the transit between them, future research might conclude that there is no reason to distinguish Zones 1 and 2. Zone 3 is deeper into the cave, in the aphotic area and in lower terrain, therefore access to the area would require the water level to be at least 8 meters below the current levels. Interestingly, in Zone 3 human bones are found in association with fauna remains. Whether there is a relationship between human and fauna bones, or even if they are contemporaneous, will require further evaluation.

Broken pieces of ceramic are found in different parts of the lake and, based on their shapes, it is possible to assume that they were parts of jars similar to the one found intact at a depth of 27 meters (Figure 10). The resemblance between the complete ceramic jar found at 27 meters in Zone 1, and other ceramic jar pieces found in Zone 2 (Figures 4 and 14) would provide additional support to the assumption that these zones were used in the same period. Given the location of the intact jar and the slope of the lake, it is reasonable to assume that it rolled down from the surface to the place where it was found, given that there is no direct access to the surface of the lake at that point.

Central Brazil is a confluence zone, a corridor through which different ceramist groups passed over time, coming from different directions. It has been demonstrated the existence of different construction techniques, styles and morphologies for pieces produced in the area in the same period of those observed in Gruta Azul, covering 1.000 years, from 1.500 to 500 years BP, with influences from the Bolivian highlands to the Amazon area.[30] At the current stage, although samples of the ceramic found in Gruta Azul have been dated, a comprehensive study of the construction technique used, and its respective origin has not been made.

Zone 3 has no traces of ceramic, but instead, has a high concentration of fauna bones. While the presence of tapirs and deer bones in the transaction to the aphotic zone could be related to eating

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habits (further analysis shall be conducted to determine what kind of manipulation happened to the bones, if any), or rituals of early humans, or even could be explained by other facts not related to human activity, the great concentration of macaw remains deeper into the cave, in the aphotic zone, where those animals are not expected to occur naturally, all in close proximity with human remains, might configure the testimony of early burial ceremonies, as observed in other sites.[31] In the same manner, the presence of terrestrial mollusks in the same location raises the possibility that they either entered, or were placed, there when the area was dry and, given their presence among human remains, their utilization in burial ceremonies can't be ruled out. Of course, at this stage of the research, the association of human and fauna remains is speculative, since it might be caused by natural processes, without human intervention.

Station 4 is the location where the most complete skeleton was found, so far. As described in the Results Section, an initial observation would presume that the body was left in that place in a flexed position. However, the relative position between the bones could be an attempt to recreate the human anatomy, where large arms and legs bones were placed in wrong positions, opening space for the hypothesis that the skeleton was assembled *post-mortem* in a secondary burial ritual, as observed in other sites in South America.[10] At this point, however, it can't be ruled out that natural factors may have been responsible for the disarticulation and change of position of the bones.

The location of Station 14 is especially interesting, since it is at a depth of 7 meters, on an upward slope, in a room where the maximum depth is approximately 8.5 meters, as demonstrated in Figure 11. This positioning excludes the hypothesis of the skull rolling down from the entrance of the room, where Station 14 is located, providing further support to the idea that such an area was occupied when the water level was much lower, and that the skull was intentionally placed in that location.

Serra do Calcario is located underneath the South Atlantic Convergence Zone (SACZ), a key driver of the South American Monsoon System (SAMS) variability in South America.[32] Recent studies based on speleothems formation have revealed a nearly invariant rainfall trend in the area over the mid-to-late Holocene, suggesting that the SACZ has maintained its latitudinal position and intensity, while the area located to its southeast has shown an abrupt reduction in the humidity during the mid-Holocene, between 9200–5500 years BP.[2,33] While such dryer periods have a significant impact in human occupation in central and eastern Brazil, causing these areas to be depopulated or abandoned,[34] it is unclear whether the Serra do Calcario area was impacted by such dryness, or if the significant changes in the human occupation happened. It is interesting to note, however, that the archeological material found in the Gruta Azul are from the Early Holocene era or from the second millennia and earlier, providing some support for the Archaic Gap theory[34] of reduced human presence in the area.

Terrestrial carbonates can be precisely dated by U-series disequilibrium methods, since they usually show no evidence for open-system behavior,[35] therefore the latest dates obtained from each sample are a good approximation of the moment when their formation stopped. A possible explanation for the speleothem formation interruption would be the increased water level, since, once submerged, speleothem growth will cease. Based on the U-Th dating of the speleothems, combined with the observation that there are no speleothems below the depth of 7 meters, it is possible to assume that this area of the cave has been mostly submerged since its formation, while the area above, located between the depth of 7 meters and the surface, has been mostly submerged for at least the past 5.000 years. Based on observation that the speleothems collected deeper in the water column are older, it is possible to assume that the water rising process started as early as 13.000 BP, when we have the first evidence of ceased growth in one of the collected speleothems (MTCO-03), a period that would coincide with the Younger Dryas, and it related increased humidity.[1]

Besides the speculation about the water level rise given by the speleothem growth cessation mud layers in some of the speleothems indicate periods of rapid increase in the water table possibly resulting from flooding events, followed by low energy periods which led to the accumulation of detrital material above the speleothems. Then, the water column must have decreased in order for the detrital material to become cemented by the carbonate in the drip water solution. U/Th dates before and after the mud layer in MTCO-14C indicate a paleoflood event between 13.142 ± 84 and 11.955 ± 185 years BP coinciding with the Younger Dryas. Other paleofloods might have occurred between 8.000-7.000 and around 5.850 years BP as suggested by speleothems MTCO-21A and MTCO-21B.

The fact that little speleothem growth was observed in the area were water level fluctuation is currently observed (within a 2 meters range) in the last 5.000 years BP, and no speleothem growth was observed in the permanently flooded area of the lake in the last 5.000 years B.P. can provide additional support to the understanding that this humidity in the Serra do Calcario area increased around 5.500 years BP, elevating the water levels approximately to its current configuration. This understanding would be in line with the dates obtained from human remains and ceramic, and would support the initial idea that the cave has been occupied by humans in at least two different time periods, one when the water level was at least 8 meters below its current level, and another, more recent, when the water level was similar to the present.

As for dating processes, four out of the five samples collected were dating using U-series disequilibrium methods, due to the absence of protein for radiocarbon dating. While for speleothems this technique can be very reliable, since they are closed systems, i.e., their U-uptake is negligible, for bones the situation is less obvious. Modern bones and teeth contain low concentrations of U, while archeological samples may contain much higher concentrations.[21] However, bones are not completely closed systems, which means that there might be U-update or leach over time, as well as Th-uptake or leach, causing the ages to be potentially under or over estimated. Since there are no actual observations of Th leach in nature, the most likely scenario, assuming U-uptake, would be an under estimation of the age calculated by this method.[21] In this case, to reduce the uncertainties in the age calculation processes Monte Carlo simulations were used as described in the Materials and Methods section. Nonetheless, the consistency between the dates obtained by U-series disequilibrium and the estimated water level dynamics that would allow access to Zone 3, provides further support to the dates obtained so far.

Finally, Gruta Azul's archaeological site has hundreds of square meters, and each new expedition to the location uncovers new artifacts. To the present moment, no excavation has been made, so the results herein discussed are based on superficially apparent artifacts. Additionally, only a few samples have been collected and dated, while the analysis of the additional material found will certainly shed extra light on the history of human occupation in central South America.

Acknowledgments

The authors would like to thank the Annicchino family, owners of the property where the site is located, for their unconditional support to the research. We also would like to thank the farm manager, João Paulo Massoneto, and the volunteers João Fernandes and Humberto Moran for the logistical support. We are grateful to Global Underwater Explorers for the coordination and execution of all diving operations.

Lastly, we would like to thank Luana Sposito, Dr. Maria Ana Correia, Dr. Waldiane Cossermelli and Manuel Dimitri Gomes for their assistance during the evaluation and excavation of the archaeological site.

Funding

The research was granted with the Explorers Club flagship number 134.

Conflicts of interest and final statements

The authors declare no conflict of interest. The authors have no relevant financial or non-financial interests to disclose. The authors have no competing interests to declare that are relevant to the content of this article. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. The authors have no financial or proprietary interests in any material discussed in this article.

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