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Methodology for the estimation of the age categories of *Hydrochoerus hydrochaeris* (Rodentia, Hydrochoeridae) through the cranial and femur morphometry

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Abstract: The aim of this study is to propose a methodology to estimate the age of individuals of capybaras (*Hydrochoerus hydrochaeris*) using some morphometric measurements of the skull and femur. A sample of 250 individuals from different age categories was analyzed. These categories were estimated based on the ossification degree of the cranial sutures. Out of 28 morphocranial measurements taken, nine of them allow us to adequately determine the degree of bone maturation and the relative age: pre maxillonasal maximum width (MNW), pre maxillonasal minimum width (MNMW), parietal width (PW), intermaxillary width (IMW), maximum width of the 3rd molar (MW), minimum width of the 3rd molar (MMW), 3rd molar length (M3), molar length (ML), and molar 1 and 2 length (M12). In case of the femur, the measurements did not show differences among age categories, thus, we discarded these morphometric magnitudes as possible age indicators. This work proposes new measures to estimate and to analyze the relative age structure of the capybara population, providing a useful tool in the management and conservation of this species, and to evaluate paleontological and archaeological assemblages.

Keywords: age estimation; capybara; morphometric measurements; skull.

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Introduction

The capybara (*Hydrochoerus hydrochaeris* Linnaeus, 1766) is the biggest living rodent in the world (Ojasti 1973, 2011), whose weight in the adults can reach 80 kg (Ojasti 1973, 2011, Mones and Ojasti 1986, Mones 1991). It is a social animal with semiaquatic habits (Herrera 1999, Herrera et al. 2011, Ojasti 2011) and its distribution covers the tropical and subtropical wetlands in South America (Figure 1; Mones and Ojasti 1986). Throughout its distribution, the capybara is a natural resource of social and economic importance because of its good quality meat and leather (Ojasti 1991). The large harvest of this species makes it necessary to conduct measures and regulations that allow its sustainable use (Torres 1992).Therefore, the study of population parameters such as mortality and age structure, are the key factors to analyze and monitor the population state and dynamic and demographic information in order to effectively develop sustainable management initiatives (Rabinovich 1978, Maffei 2001).

Despite the importance of these parameters, age profiles analysis and mortality of this mammal are scarce, probably because no suitable morphological methods have been found to estimate the age of the individuals based on accessible measurements. Although two methods based on morphometric measurements have been developed to that aim, they were not widely used as either requires skulls in good conditions or are not good to estimate the age of the animals. The first one is based on the degree of suture closure of each skull (Ojasti 1973, 2011). Although it is a precise methodology, skulls with the cranial sutures in good conditions are rarely found in the field. The second approach explored the degree

Figure 1: Capybaras' range distribution (*H. hydrochaeris*) in South America and sector where the samples were recollected (Black spot). Figure modified from Campos Krauer (2009).

of ossification of the femoral epiphysis, but no defined pattern to estimate the age has been found (Ojasti 2011).

The capybara has a modified synapsid skull (Kardong 2007), which consists of 34 bones (including the ear bones; Bode et al. 2013). Its growth has a strong correlation with age, following allometric rules (Vassallo 2000, Payán Garrido 2007). Consequently, it might be a useful structure for the adequate estimation of the different age categories. Therefore, the aim of this study is to develop and propose a methodology to estimate the age of individuals using morphometric measurements of the skull. In addition, we also explored different magnitudes of the femur, which have been proved to be suitable measurements to estimate the age categories in other species of rodents (Cardini and O'higgins 2004, Ojasti 2011).

In this study we propose an alternative methodology which enables, based on bones remains, the estimation of the age of the individuals in a simple and practical way in the field. This methodology could be useful, not only in population studies of capybaras, but also on the monitoring carried out in areas of legal hunting and/or poaching, where usually there are "cementeries" or places of exploitation, where the hunters leave parts of corpses from which they do not benefit (skulls, bones, and in some cases skins; Bolkovic et al. 2006, Payán Garrido 2007). Furthermore, this methodology might also be useful to estimate the age profiles of specimens issued from paleontological and archaeological sites. *Hydrochoerus hydrochaeris* is found in archaeological sites along the South American lowlands, not only for feeding purposes, but also related with human symbolic behaviors (Acosta and Mucciolo 2009, Rosa 2010, Schmitz and Ferrasso 2011, Santiago 2012, Loponte et al. 2012, Ottalagano et al. 2015). Such tool of archaeozoological analysis of mortality patterns allows understanding the human behavior in the past (Wing et al. 1992, Steiner 1994, Lyman and Cannon 2004, Wolverton and Lyman 2012).

Materials and methods

Study area

The present samples were collected in Guayaibí Park (28°00′S, 57°18′W), located in the Iberá wetland, province of Corrientes, northeastern Argentina (Figure 1). This wetland is a large continental area with subtropical climate (Neiff 2004). In late winter 2012 and summer 2013, skeletal remains (skulls and femurs) were collected using transects covering an area of 19.500 ha. In this study 250 skulls and 59 femurs were recovered and analyzed. The reason for numerical imbalance found of the different skeletal remains is because the carcasses distributed in the landscape had a different integrity degree, as a result of the action of scavengers. The skeletal remains are curated at the National Institute of Anthropology and Latin American Thought (Buenos Aires) (Supplemental Table S1).

Morphometric measures

As for the collected skulls, the relative age of each animal was estimated based on the ossification degree of basocranials sutures indicated by Ojasti (1973, 2011; Table 1). From each skull, 27 morphometric measures were taken, some of them suggested by Payán Garrido (2007); (FPL2, FPW, PL, ECW, OW, ZW, M3, M12, DL, CE, and JH) and some additional (FPL1, MNL, MNW, MNMW, PW, ECL, IMW, MW, MMW, ML, PMW, PBW, OH, OD, FJH, TL; Table 2, Figures 2 and 3). Furthermore, the number of prims or enamel rods of third molar were counted (#primsM3). Measures of up to 150 mm were taken with a digital caliper, and for those of larger sizes, a vernier caliper was used.

Table 1: Age ranges that correspond to the age categories based on the basocranials sutures (ACBS) (Ojasti 1973, Ojasti 2011).

Ecological age	ACBS	Basocranials sutures	Age range
Offspring	I	Suture between the parietals but not completely ossified.	Days before birth–4 months
Young	II	Ossified suture between the parietals and the basioccipital-condyle suture open.	4 months–1 year
Subadult	III	Ossified suture between the basioccipital-condyle, presfenoides-basisphenoid suture open.	1 year–1 year and a half.
Adult	IV–V	Ossified suture between the presfenoides-basisphenoid, ossified suture between supraoccipital-exoccipita halfway or completely, suture between basisphenoid-basioccipital open.	1 year and a half–2 years
Adult	VI	Ossified suture between exoccipital-supraoccipital, suture between basisphenoid-basioccipital open.	2 years–4 years
Adult	VII	All named sutures ossified.	4 years and up

Table 2: Skulls percentage with the corresponding measurement taken the error between the two measurements of the skulls of all the categories and the name of each measurement.

Variable	Measurements	% measured	Error
FPL1	Fronto-parietal length I	71.6	0.53
FPL2	Fronto-parietal length II	72.4	0.84
FPW	Fonto-parietal width	78.4	0.42
MNL	Premaxillonasal length	90.4	0.60
MNM	Premaxillonasal maximum width	96.4	0.16
MNMW	Premaxillonasal minimum width	97.2	0.68
PL	Parietal length	82.4	0.96
PW	Parietal width	92	0.45
ECW	Exoccipital condyle width	74	0.27
ECL	Exoccipital condyle length	71.6	0.55
OW	Occipital width	67.2	0.22
ZW	Zygomatic width	82.4	0.86
IMW	Intermaxillary width	98	0.21
MW	M3 maximum width	99.6	0.10
MMW	M3 minimum width	99.6	0.22
M3	3rd molar length	99.2	0.38
#prismsM3	Number of prisms of 3rd molar	99.2	0.00
ML	Molars length	99.6	0.51
M12	1st and 2nd molar length	99.6	0.28
DL	Diastema length	76.8	0.66
PMW	Premolar width	76.4	0.27
PBW	Basal premolar width	76	0.11
CE	Braincase height	74	0.27
JH	Jugal height	87.6	0.43
OH	Orbit height	92	0.37
OD	Diagonal orbit height	92.4	1.04
FJH	Frontal-jugal height	82.4	1.18
TL (cm)	Total length	61.6	0.14

The age categories used were those considered by Ojasti (2011): offspring (O=Category I), young (Y=Category II), subadults (SA=Category III), and adults (A=Categories IV, V, VI, and VII). In adult category, classes IV and V were considered as a single category because V has duration of a few months, so it is unlikely to find variations in a bone measurement in such a short time.

Additional measurements of the femur from each of the individuals previously analyzed were also conducted. In the case of the femur, seven measurements were taken (Figure 4).

Each measure was performed two times (X1, X2) by the same person. The accuracy of each measure was determined by the error between the two measurements (X1, X2). The error was calculated using the following formula: Error=$\sum(X_1-X_2)^2$/number of measured skulls.

Data analysis

From all the measurements taken in each category, those that could be successfully measured at least in 70% of the collected skulls were preselected for the statistical analysis. Regarding the offspring category, in which many measures could not be performed because most of the skulls were found broken, the preselection criterion was reduced to 60%. With the preselected measurements, an Analysis of Variance (ANOVA, significance p corrected with Bonferroni method) was conducted to compare the age categories O–Y, Y–SA, and SA–A. In the case of the adults, a Principal Components analysis was made to reduce the number of variables to those that better explained the variance among the four age categories. Subsequently, an analysis of variance (ANOVA, significance p corrected by Bonferroni method) was carried out.

The measurements taken in the femur were compared by using an analysis of variance (ANOVA, significance p corrected with Bonferroni method).

Results

To differentiate offspring (Category I) from young (Category II), nine measurements out of 28 were preselected,

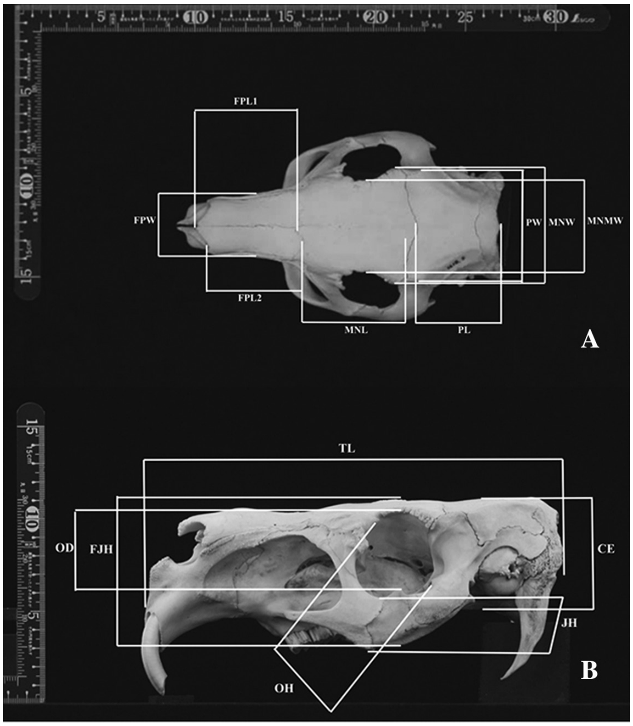

Figure 2: Capybara's skull (*H. hydrochaeris*) and the undertaken morphometric measurements. (A) Dorsal view. (B) Lateral view. See Table 2 for definitions of abbreviations of measurements.

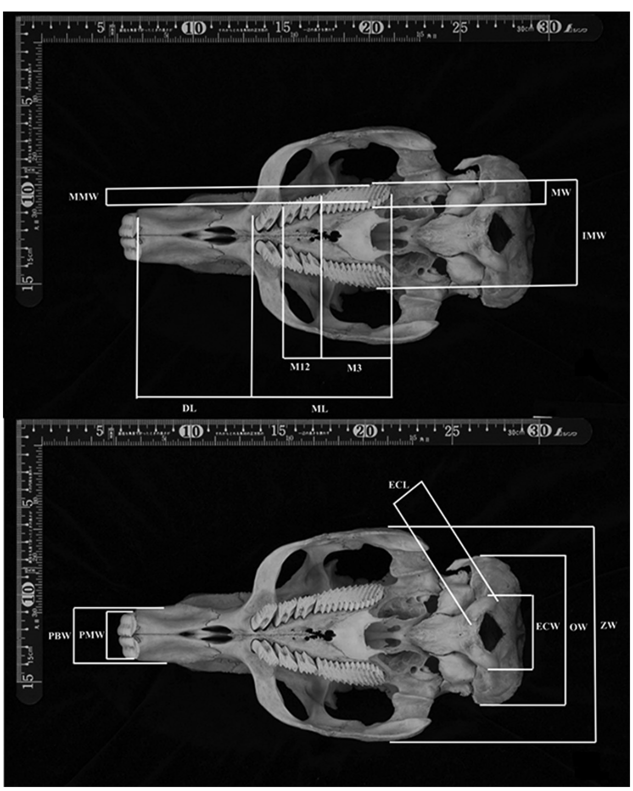

Figure 3: Capybara's skull (*H. hydrochaeris*) and the undertaken morphometric measurements (ventral view). See Table 2 for definitions of abbreviations of measurements.

Figure 4: Capybara's femur (*H. hydrochaeris*) and the undertaken morphometric measurement. DW, Distal femur maximum width; PW, proximal femur maximum width; TW, total maximum width; IW, internal width; HW, femur's head width; CEH, external condyle maximum height; CIH, internal condyle maximum height.

following the criteria described in data analysis. All of them turned out to be useful to differentiate these two categories (Bonferroni correction, $p<0.0055$; Table 3a). In the case of the young (Category II) and subadults (Category III), 14 measurements were preselected and all were appropriate to distinguish these categories (Bonferroni correction, $p<0.0035$; Table 3b). Finally, to discriminate subadults (Category III) from adults (Categories IV–V, VI, and VII together), 16 measurements were preselected and found suitable to distinguish SA and A (Bonferroni correction, $p<0.0031$; Table 3c).

Depending on all the measured skulls (all categories) the percentage of skulls with the corresponding measurement taken and the error between the two measurements was calculated (Table 2).

From all the studied measurements, nine of them (MNW, MNMW, PW, IMW, MW, MMW, M3, ML, and M12) are enabled to differentiate all age categories (O, Y, SA, and A). Among adults (Categories IV–V, VI, and VII of Ojasti 1973, 2011), 18 measures were preselected and submitted to Principal Component Analysis. The result of the analysis suggested that the first axis is associated with the age category and body size of individuals (Supplemental Figure S1), explaining the 75% of the variance. From this axis, 11 variables, which showed the greatest contribution to the first component (greater variance, eigenvector >0.25; Supplemental Table S2), were extracted. The ANOVA revealed significant differences between categories in just five measures (MNW, MNMW, ZW, IMW, and FJH). None of them were capable for separating the three adult categories (Bonferroni's correction, $p<0.0045$; Table 4). Four of them (MNW, ZW, IMW, and FJH) enabled to discriminate

Table 3: Mean values in millimeters (MV), confidence intervals (CI) and the results of analysis of variance for the different measurements made between categories.

Variable	MV	CI	MV	CI	F	p-Value
a)		O		Y		O/Y
MNW[a]	50.98	49.04–52.91	58.59	53.94–63.23	25.1	<0.0001
MNMW[a]	40.19	38.22–42.16	48.54	44.92–52.15	31.84	<0.0001
PW[a]	50.89	48.65–53.14	55.71	50.14–61.29	23.84	<0.0001
IMW[a]	36.34	33.76–38.92	45.86	42.77–48.96	36.29	<0.0001
MW[a]	8.16	7.3–9.02	10.87	10.06–11.68	37.81	<0.0001
MMW[a]	6.05	5.39–6.71	8.35	7.75–8.95	38.99	<0.0001
M3[a]	23.51	21.32–25.7	30.84	28.53–33.15	30.48	<0.0001
ML[a]	45.6	42.29–48.91	57.81	53.67–61.95	24.2	0.0001
M12[a]	13.37	12.37–14.38	17.09	15.99–18.18	27.17	<0.0001
b)		Y		SA		Y/SA
MNW[a]	58.59	53.94–63.23	73.96	69.71–78.2	44.33	<0.0001
MNMW[a]	48.54	44.92–52.12	61.42	57.92–64.93	48.65	<0.0001
PW[a]	55.71	50.14–61.29	69.14	65.85–72.43	43.6	<0.0001
ZW[a]	98.07	89.38–106.77	115.08	106.94–123.22	26.62	0.0001
IMW[a]	45.86	42.77–48.96	56.44	53.28–59.61	29.93	<0.0001
MW[a]	10.87	10.06–11.68	13.54	12.5–14.58	22.8	0.0001
MMW[a]	8.35	7.75–8.95	10.65	9.75–11.54	23.9	0.0001
M3[a]	30.84	28.53–33.15	37.94	35.46–40.42	27.84	<0.0001
ML[a]	57.81	53.67–61.95	70.31	66.01–74.61	19.61	0.0001
M12[a]	17.09	15.99–18.18	20.95	19.65–22.25	24.5	0.0001
JW[a]	21.14	18.67–23.6	28.89	26.47–31.31	25.52	0.0001
OH[a]	38.2	35.82–40.58	46.59	44.15–49.03	27.56	<0.0001
OD[a]	36.19	34.48–37.9	41.12	39.33–42.9	18.24	0.0004
FJH[a]	57.82	52.6–63.05	76.12	71.01–81.24	30.28	<0.0001
c)		SA		A		SA/A
MNL[a]	64.66	60.97–68.35	71.01	70.52–71.51	28.85	<0.0001
MNW[a]	73.96	69.71–78.2	83.16	82.57–83.74	44.01	<0.0001
MNMW[a]	61.42	57.92–64.93	67.9	67.4–68.41	28	<0.0001
PL[a]	56.64	54.85–58.43	61.02	60.55– 61.48	18.72	<0.0001
PW[a]	69.14	65.85–72.43	73.87	73.35–74.39	14.69	0.0002
ZW[a]	115.08	106.94–123.22	132.89	131.99–133.79	76.63	<0.0001
IMW[a]	56.44	53.28–59.61	63.09	62.65–63.52	39.73	<0.0001
MW[a]	13.54	12.5–14.58	15.04	14.88–15.19	17.09	0.0001
MMW[a]	10.65	9.75–11.54	12.18	12.03–12.32	20.45	<0.0001
M3[a]	37.94	35.46–40.42	42.41	42.05–42.77	26.83	<0.0001
ML[a]	70.31	66.01–74.61	78.44	77.82–79.05	36.5	<0.0001
M12[a]	20.95	19.65–22.25	23.1	22.9–23.31	27.64	<0.0001
JW[a]	28.89	26.47–31.31	34.33	33.92–34.74	42.4	<0.0001
OH[a]	46.59	44.15–49.03	51.16	50.79–51.53	30.11	<0.0001
OD[a]	41.12	39.33–42.9	45.44	45.06–45.82	30.33	<0.0001
FJH[a]	76.12	71.01–81.24	85.62	84.9–86.34	41.53	<0.0001

F, Statistical value. The abbreviations of the variables are listed in Table 2. a) Between offspring (O) and young (Y); Bonferroni's correction, $p<0.0055$; b) between young (Y) and subadults (SA); Bonferroni's correction, $p<0.0035$; c) between Sub adults (SA) and Adults (A). Bonferroni's correction, $p<0.0031$. [a]Indicates significant differences between categories.

the category VII from the other categories. Finally, MNMW did not show differences in the multiple comparisons.

It is worthy to mention that the skulls corresponding to different age categories exhibit the same number of prims. Thus, #PrimsM3 was not included in the statistical analysis

Out of the 59 femur we found, 3.4% corresponded to the category of age II, 3.4% corresponded to the category III, 3.4% corresponded to the category IV–V, 18.6% to the category VI, and 71.2% to the category VII. Thus we could only compare statistically the femur corresponding to category

Table 4: Mean values in millimeters (MV), confidence intervals (CI) and the results of analysis of variance for different measurements taken between categories IV–V, VI, and VII.

	IV–V		VI		VII		F	p-Value
	MV	CI	MV	CI	MV	CI		
MNW[a]	78.91	76.52–81.31	80.93	79.79–82.08	84.32	83.71–84.93	13.01	<0.0001
MNMW[a]	64.92	62.58–67.25	66.51	65.55–67.47	68.68	68.11–69.24	6.35	0.0022
ZW[a]	127.75	121.99–133.5	128.9	127.37–130.47	134.7	133.83–135.62	19.25	<0.0001
ECL	26.58	25.42–27.75	39.74	39.14–40.35	26.6	26.27–26.93	0.01	0.9944
IMW[a]	61.07	58.78–63.37	61.4	60.5–62.3	63.86	63.41–64.3	11.28	<0.0001
MW	14.25	13.6–14.9	14.68	14.32–15.04	15.24	15.08–15.41	5.54	0.0047
MMW	11.48	10.78–12.18	11.97	11.65–12.28	12.32	12.15–12.49	4.87	0.0088
M12	22.39	21.5–23.28	22.79	22.37–23.22	23.28	23.05–23.52	1.92	0.1502
JW	32.22	30.15–34.29	33.44	32.74–34.14	34.85	34.37–35.33	4.66	0.0109
OH	50.24	48.59–51.9	50.41	49.67–51.14	51.51	51.07–51.94	3.81	0.0242
FJH[a]	82.08	75.5–85.03	83.82	82.65–84.98	86.88	86.2–87.56	12.34	<0.0001

F, Statistical value. Bonferroni's correction, p<0.0045. The abbreviations of the variables are listed in Table 2. [a]Indicates significant differences among categories.

VI and VII which showed no differences for any of the measurements (Bonferroni's correction, p<0.007; Table 5).

The mean values of the 28 measurements taken in the skull and the seven measurements measured in the femur by age category were calculated and presented in the Supplemental material (Supplemental Table S3, Supplemental Table S4, and Supplemental Table S5, respectively) together with the standard error, the maximum and minimum value, and the confidence interval.

Discussion

From all the measures evaluated in this study, nine of them (MNW, MNMW, PW, IMW, MW, MMW, M3, ML, and M12) had been proved useful to differentiate all the four age categories (O, Y, SA, and A). The proposed measures were highly accessible in the skulls even though the bones we collected had been exposed to disturbances such as the trampling of cattle and horses, predation by the scavengers plus extreme environmental conditions. All the measures we selected could be successfully taken, at least in 92% of the collected skull. Therefore, these measures correspond to strong bones with a high degree of conservation (the pre maxillonasal bone, the parietal bone, and the area of the palate and the molars). The ease and accuracy with which the measurements could be performed were also relevant to select the most adequate measures. For example, the MNW and the PW are recommended among the measures of the dorsal view of the skull, as they could be taken very precisely and with a very low error between the two measurements. The MNW and the PW were taken where the bones of the skull get wider (the pre maxillonasal in the rostral region of the skull and the parietal bone in the braincase of the skull), creating a clear boundary

Table 5: Mean values in millimeters (MV), confidence intervals (CI) and the results of analysis of variance for the different measurements taken of the femur belonging to categories VI and VII.

Variable	Category VI		Category VII		VI/VII	
	MV	CI	MV	CI	F	p-Value
PW	51.03	49.22–52. 83	52.37	51.37–53.37	2.77	0.1044
IW	7.47	6.45–8.49	7.63	7.3–7.97	1.75	0.1942
DW	42.11	41.34–42.89	43.26	42.47–44.04	2.44	0.1267
CEH	50.76	47.33–54.18	51.4	49.94–52.87	6.20E–04	0.9803
CIH	53.38	52.31–54.46	54.1	53–55.2	0.17	0.6859
HW	23.6	21.94–25.26	23.53	22.9–24.15	0.07	0.7924
TW	27.89	25.9–29.88	29.9	29.3–30.51	4.09	0.0503

The abbreviations of the variables are listed in Table 2. F, Statistical value. Bonferroni's correction, p<0.007.

for positioning the gauge. In the case of the MNMW, the error was higher than the error measured in the MNW and PW. It might be due to the morphological irregularities of the bone, which made the measurements less precise.

The relevance of the measures taken from the palate (i.e., IMW, MW, MMW, M3, ML, and M12) is due to the integrity of this region in most of the skulls from the offspring. Although a few whole skulls from offspring were found, probably because of their high fragility due to not fully ossified basocranial sutures, the palatal region was well preserved and accessible for measuring in more than 98% of the skulls. In addition, all these measurements could be accurately taken, as boundaries were very clear.

In the case of the adults, there is no measurement that may allow us to differentiate the three adult categories (IV–V, VI, and VII), despite having found differences in some measures (MNW, MNMW, ZW, IMW, and FJH). Some of these measures (MNW, ZW, and FJH) could only differentiate the last category (VII) from the rest. The absence of variation would imply a poorly differentiated growth or a more continuous one. Yet, these measurements allowed us to differentiate individuals over the age of 4 years (VII category). Being able to differentiate, the latter category has important implications in population ecology. Individuals older than 4 years of age help to estimate the reproductive success of the population, its biological viability, and/or the hunting pressure (Ojasti 1973, 2011, Federico and Canziani 2005).

The morphological variables of the crania of one geographical region often have some limitations that restrain their use in other areas (Garduño 2000, Molinari 2007). Thus, to extrapolate this methodology to the populations of other regions, updated samples are required to validate the measures we proposed. Many of the mean values of the measurements taken in Colombia (Payán Garrido 2007) are below the confidence interval obtained in this work. Only the mean values of some measures for certain age categories were within the confidence interval. For juveniles we had no values of all measures used in both studies, and in the Colombia's study, the offspring individuals have no data for comparison. It is likely that the lowest values found in the measures presented by Payán Garrido (2007) corresponding to category VII are associated to the fact that the skulls came from commercial hunted animals and therefore, they did not show such advanced ages as the ones you can get in populations without hunting pressure. The absence of hunting and big predators in the study area, could allow the individuals to reach more advanced ages, thus, bigger sizes. However, the lowest values were also observed in categories SA, AIV, AV, and AVI, what may be showing a geographic variation in body size of the species according to latitude (Ashton et al. 2000).

For the femur, although a trend is observed in the size related to the age, the magnitudes obtained did not allow us to distinguish the different age categories of adults considered in this work.

Regarding the results obtained in this study, we could conclude that the selected cranial measurements are adequate to perform a clear and precise skull classification according to the broad age categories (offspring, young, subadult, and adult), while the femur measurements are not recommended. We suggest MNW, IMW, M3, MW, MMW, ML, and M12 as morphometric distances criteria to estimate the age of a population of capybaras. Having done this study in a hunting-pressure-free area allowed us to extend the range of measurements to adult over the 4 years of age.

According to the above and taking into account that the capybara is a species with high cynegetic pressure, the proposed measures, easily measurable even for people without technical training, permit to identify precisely the age structure of individuals predated or hunted, which is key information to the management decisions related to the control and/or the conservation of the species.

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