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# Population affinities in pre-colonial West Africa: The case of the burial cave Iroungou (Gabon, 14th–15th century CE)

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## Abstract

**Introduction:** Our knowledge of the populations of sub-Saharan Africa in the periods before European colonization is limited. Few archeological sites containing human remains have been identified, and written sources for these periods are rare. The discovery in 2018 of the Iroungou sepulchral cave (Gabon), whose use predates the arrival of the Portuguese (14th–15th centuries CE), is an exceptional source of information: at least 28 individuals associated with numerous metal artifacts were found there. The anthropobiological remains were left in situ, but the eight best preserved crania were digitized.

**Objectives:** This study focuses on the population affinities of these crania, whose morphology was described using 237 landmarks.

**Materials and Methods:** Geometric morphometric analyses were used to compare the eight Iroungou specimens with 154 individuals representing 12 well-defined African populations. After alignment (Generalized Procrustes Analysis), morphological affinity was assessed using Euclidean and Mahalanobis distances, and posterior probabilities of population membership (discriminant analysis).

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**Results:** Results indicate that the eight Iroungou crania have, on average, more affinity with Bayaka Pygmy, followed by Central African Bantu. Nevertheless, individually, the Iroungou specimens show an important morphological variation and the eight crania can be separated into different affinity groups: Bayaka and Central African Bantu, KhoeSan, and East-African Bantu. Finally, one individual presents strong affinity with Somalis.

**Conclusion:** This phenetic mapping of the Iroungou sample raises questions about the profile of the individuals deposited in the cave in a geographical area known for the Loango pre-colonial kingdom, which ruling class seemed to have had privileged relationships with the Pygmy populations.

#### KEYWORDS

cranial morphology, geometric morphometrics, phenetic affinities, pre-colonial Central Africa

#### Résumé

**Introduction:** Nos connaissances sur les populations d'Afrique sub-saharienne des périodes précédant la colonisation européenne sont limitées. Peu de sites archéologiques contenant des restes humains ont été identifiés, et les sources écrites pour ces périodes sont rares. La découverte en 2018 de la grotte sépulcrale d'Iroungou (Gabon), dont l'utilisation remonte au XIV<sup>e</sup>-XV<sup>e</sup> siècles de notre ère, avant l'arrivée des Portugais, constitue une source d'information exceptionnelle: au moins 28 individus associés à de nombreux artefacts métalliques y ont été retrouvés. Les restes anthropobiologiques ont été laissés in situ, néanmoins, les huit crânes les mieux préservés ont été numérisés.

**Objectifs:** Cette étude s'intéresse aux affinités populationnelles de ces crânes, dont la morphologie a été décrite à l'aide de 237 points-repères répartis sur le massif facial supérieur et le calvarium.

**Matériel et méthodes:** Des analyses par morphométrie géométrique ont permis de comparer les spécimens d'Iroungou avec 154 individus représentant 12 populations africaines bien définies. Après alignement des conformations (analyse Procrustes généralisée), l'affinité morphologique a été évaluée à l'aide des distances procrustes, euclidienne et mahalanobis, ainsi que des probabilités postérieures d'appartenance à une population (analyse discriminante).

**Résultats:** Les résultats indiquent que les huit crânes d'Iroungou présentent en moyenne plus d'affinités avec les populations pygmées Bayaka, suivies des Bantus d'Afrique centrale.

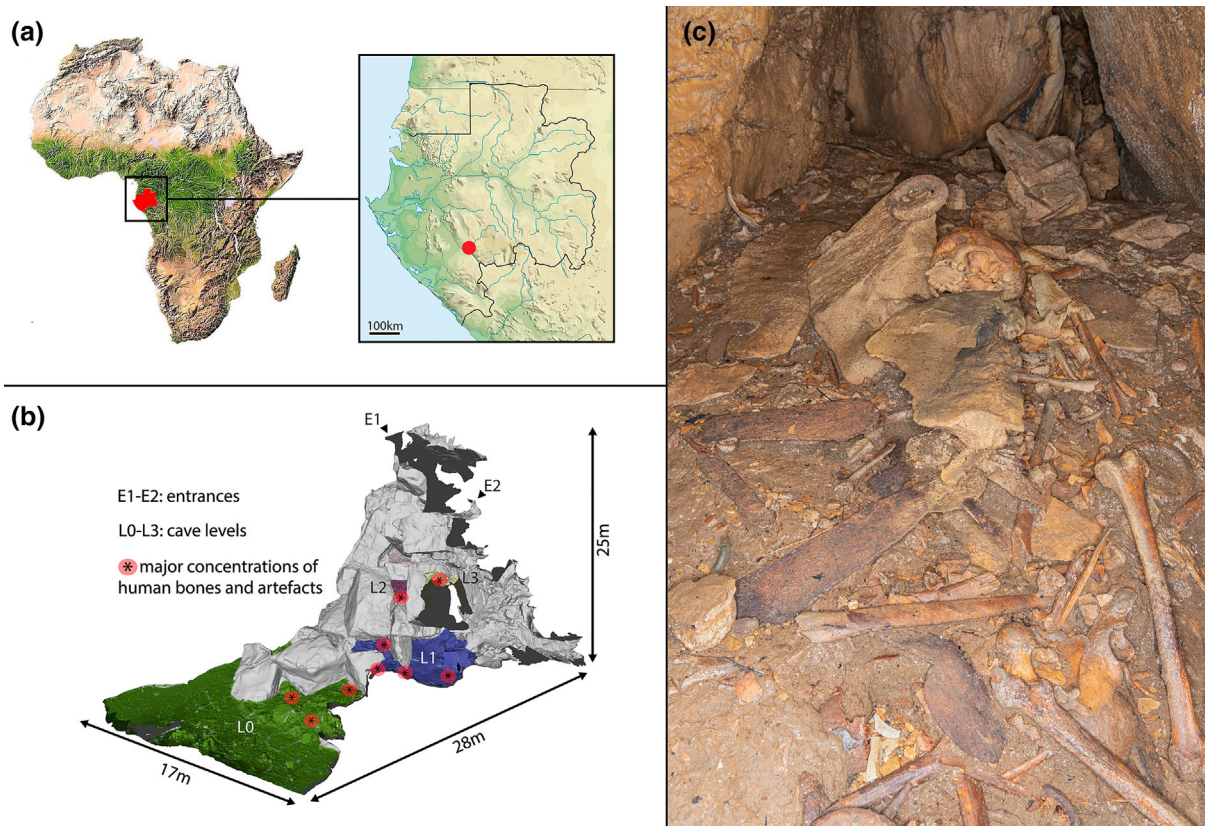
**Discussion:** Cette affinité moyenne recouvre une réalité complexe: la population d'Iroungou est la plus hétérogène de notre échantillon et les huit crânes peuvent être séparés en différents groupes d'affinités: avec les Bayakas et les Bantus d'Afrique Centrale, les KhoeSan, et avec les Bantus d'Afrique de l'est. Enfin, un individu présente une affinité très forte avec les Somalis de notre échantillon. Cette cartographie phénétique de la population d'Iroungou interroge sur le profil des individus déposés dans la grotte dans une zone géographique connue le royaume précolonial Loango dont la classe dirigeante semble avoir eu des relations privilégiées avec les populations pygmées.

## 1 | INTRODUCTION

Human remains from the Iron Age (2500 BP–1500 AD) of West Central Africa (WCA) are extremely rare in the archeological record (Froment, 1998). In this context, the discovery of the Iroungou Cave (Ngounié Province, Gabon, Figure 1a), in 1992 and its exploration from 2018 to 2020, brings much needed information regarding the anthropological characteristics and mortuary behavior of the pre-colonial inhabitants of WCA (Villotte et al., 2021). The cave is connected to the surface through two openings in its ceiling, making it accessible only by abseiling. It is 25 m deep at most, and consists of four main levels, covering a total area of ca. 2000 m<sup>3</sup> (Figure 1b). The four exploration missions of the cave revealed the presence of thousands of scattered human remains and numerous artifacts including 512 metallic objects, 127 marine shellfish and 39 performed carnivores teeth (Villotte et al., 2021). Radiocarbon dates on human bone samples ( $n = 10$ ) indicate that the cavity had been used on two or more occasions during the 14th and 15th centuries AD, that is, just prior to contact with Portuguese at the end of the 15th century (Villotte et al., 2021).

The human remains and most of the artifacts found in the cave were left in situ in their original locations in order to ensure long-term preservations of the bones. Nevertheless, anthropometric data were collected on the accessible remains on the surface to establish an

anthropobiological profile of the population which used the cave. The minimum number of individuals has been estimated at 28, among which four are juveniles (<15 years old), three are females and seven males. There is no sign of ante-mortem bone trauma on the specimens and systematic evulsion of the four upper incisors were observed on the 22 identifiable maxillary fragments. In contrast, none of the 19 mandibular fragments presented sign of incisor evulsion (Villotte et al., 2021). Evulsion of the anterior teeth is a well-known practice in many parts of the world, including Africa (Friedling, 2003), where it is documented as early as the Upper Paleolithic in Northern Africa (De Groote & Humphrey, 2016). Such practice may have spread towards Sub-Saharan Africa during the Upper Paleolithic (Stojanowski et al., 2014) before disappearing during the Neolithic (De Groote & Humphrey, 2017). From 4000 to 3000 BP, dental modifications appear again in the Sub-Saharan African record, stemming from West Africa and seemingly following the Bantu expansion (4000–3000 BP) (Irish, 2017). According to Irish (2017) dental modifications are performed on both sex and the six most common reasons for it are ornamentation, tribal or ethnic affiliation marker, initiation ceremony into adulthood, punishment, pain endurance and tradition. Less common, health needs and diplomacy are also documented (Irish, 2017). Regarding the Iroungou Cave, the systematic evulsion of the four upper incisors could indicate cultural or tribal homogeneity within the individuals recovered in the burial (Villotte et al., 2021). This could be



**FIGURE 1** (a) Map of Africa and Congo showing the location of Iroungou. (b) Three-dimensional map of the Iroungou cave showing the entrances to the cave and the three levels where artifacts and human remains were discovered. (c) Human remains accumulation on the floor of the cave.



further supported by the fact that this particular dental modification practice (evulsion of the four upper but not of the inferior incisors) is rather uncommon: it has been reported by 19th and early 20th centuries ethnographers for West Central African populations (Lalouel, 1950; van Rippen, 1918) and it is also known from contemporary South African populations from the Cape (Friedling & Morris, 2007).

West Central African Iron Age burials have been described previously, some of which presenting rich iron artifacts (Meister & Eggert, 2008), but such an accumulation of bones of a large number of individuals associated with hundreds of metallic artifacts, which can be found at Iroungou Cave, is rather unique in the archeological record of the region. The only other known similar site is a 13th-century mass burial discovered in a former cistern in Benin City (Nigeria), where over 40 individuals were recovered and which has been interpreted as an evidence of ritual sacrifice indicative of centralized authority (Connah, 1975).

The preliminary study of the Iroungou Cave did not bring enough information to fully decipher the context of usage of the burial. Nevertheless, the richness of the described material associated to the human remains seem to indicate that the burial was not used for common funerary practices. Moreover, the demographic profile of the human assemblage and the absence of ante-mortem trauma does not advocate either for a catastrophic or warfare deposit. On the contrary, Iroungou Cave may have been used as a burial site for important individuals (Villotte et al., 2021).

While more archeological excavations are necessary to further test hypotheses regarding the interpretation of the burial, some additional evidence are available and could help understanding better the site. Indeed, during the 2020 exploration missions, some of the human remains accessible on the floor of the cave were digitized using 3D photogrammetry. Among those remains, 8 well-preserved crania were photographed and high definition 3D models were computed from the pictures (Figures 2, S1, and S2).

The present study aims at characterizing the populational and phenetic affinities of the Iroungou assemblage through the morphometric study of those eight crania. Particularly we aim at understanding whether: (1) the Iroungou individuals represent a single coherent population and (2) to which of the 12 current African populations forming the comparative sample of the study are they morphologically and phenetically closer. To do so, the Iroungou specimens' morphologies are described and compare to the comparative sample and 3D geometric morphometric analyses (i.e., GMM) are used to compute both phenetic distances between Iroungou and the comparative sample and phenetic affinity profiles for the Iroungou samples.

## 2 | MATERIALS AND METHODS

### 2.1 | Samples description

The study sample is composed of eight cranial specimens (Tables 1 and S1, Figure 3) which were digitized in situ in the cave through

photogrammetry. Those are the most well-preserved specimens for which digitization was possible given that the cave was not excavated. To study the phenetic affinities of the Iroungou specimens, they were compared to a sample composed of 141 specimens from the 19th and early 20th centuries and 13 specimens dated ~6000 BP (i.e., the Pre-Dynastic specimens), representing 12 well-defined African populations from all over the continent (Figure 2a). Thirty eight specimens represent three South African populations (i.e., San  $n = 12$ , Khoikoi  $n = 14$ , and South African Bantus  $n = 12$ ); 28 specimens are from two eastern African populations (i.e., Haya  $n = 14$  and Somali  $n = 14$ ); 27 individuals represent two North-African populations (i.e., Berber  $n = 14$ , Pre-Dynastic  $n = 13$ ), 14 specimens represent West African Bantus, and Central African populations are represented by 47 individuals from four populations (i.e., Bayaka Pygmies  $n = 14$ , Kongo  $n = 11$ , Téké  $n = 13$  and Ngounié  $n = 9$ ) (Figure 3a and Tables 1 and S1). which appears to have been present in the region at the time when the Iroungou Cave was used. The Fang people which constitutes one of the largest ethnic group in present day's Gabon were not included in the sample as they reach the area in the 19th century (Alexandre, 1965). As much as possible, groups were made following the ethnical origin of each specimens. For three populations of the comparative sample, geographical groups were used and the populations are composed of several regional Bantu ethnic groups: Susu and Mandenka for West Africa, Nguni, Sotho, Xhosa, and Zoulou for South Africa, and Tsogo and Myéné for the people living near the Ngounié river in Gabon (a few kilometers away from the Iroungou Cave).

### 2.2 | 3D model buildings and landmarking

We used three procedures to obtain the 162 crania 3D models (Tables 1 and S1): (1) Photogrammetry for 94 models, including the eight Iroungou specimens, using the Metashape software (Agisoft, v1.8.4); (2) Medical computed tomographic scans for 56 models (voxel size between 0.449219 and 0.488281 mm) processed in Amira (v5.5, FEI); and (3) Surface scanning for 12 specimens using an optical scanner (HDI Advance, 45  $\mu$  accuracy, LMI) and the FlexScan 3D software (v.3.3, LMI). The three procedures yields accurate and comparable 3D models (Katz & Friess, 2014; Slizewski et al., 2010) which morphology is described by landmarks collected by one of us (AM) with the Landmark software, IDAV (Wiley et al., 2005) following the procedure implemented in (Mounier et al., 2020): 95 landmarks on the face and 142 on the calvarium ( $n$  total = 237, the prosthion and alveolare could not be collected due to the systematic evulsion of the four upper incisors in the Iroungou specimens). Intraobserver tests were run (i.e., repeated measures ANOVA run on PC1 to PC10 after generalized Procrustes analysis (GPA) and principal component analysis (PCA) of 24 individuals which landmarks were collected three times) and the results indicate good repeatability (see Tables S17 and S18 and Figure S10). Out of the 237 landmarks, 182 are curved semi-landmarks (two degrees of freedom, which are allowed to slide) distributed along the cranial sutures and curves joining craniometric



**FIGURE 2** *Norma frontalis* and *norma lateralis* of the photogrammetric 3D models of the eight Iroungou crania. (a) L0-004; (b) L0-005; (c) L0-007; (d) L0-012; (e) L1-002; (f) L1-004; (g) L1-009; (h) L1-010. Specimens L0-004, L0-012, and L1-004 were virtually reconstructed through mirroring, the original 3D models are displayed in Figure S1. Additional views of the specimens can also be found in Figures S1 and S2.

points (Figure 3b and Table S2). This data aims at capturing as much as possible of the phenetic signal from the cranium. Missing landmarks were estimated by mirroring and, where mirroring was impossible, by a thin-plate-spline (i.e., TPS) interpolation using the available landmarks (Bookstein, 1989) of each of the groups forming the sample. This approach was applied to 13 specimens from the comparative sample, none of which had over 7.2% of missing data and to five of the eight Iroungou specimens: L0-004, L0-005, L0-012, L1-004, and L1-009 with respectively 1.3%, 8.0%, 38.4%, 10.6%, and 2.1% of missing landmarks after mirroring (Table S1). Three specimen's 3D models, L0-004, L0-005, and L1-004 were first partially reconstructed using

mirroring directly on the 3D models (see, Figures 2, S1, S2, and Video S1).

### 3 | METHOD

#### 3.1 | Morphological description

The morphology and state of preservation of each Iroungou specimen was briefly described and some prominent morphological characteristics were confronted to the comparative sample.



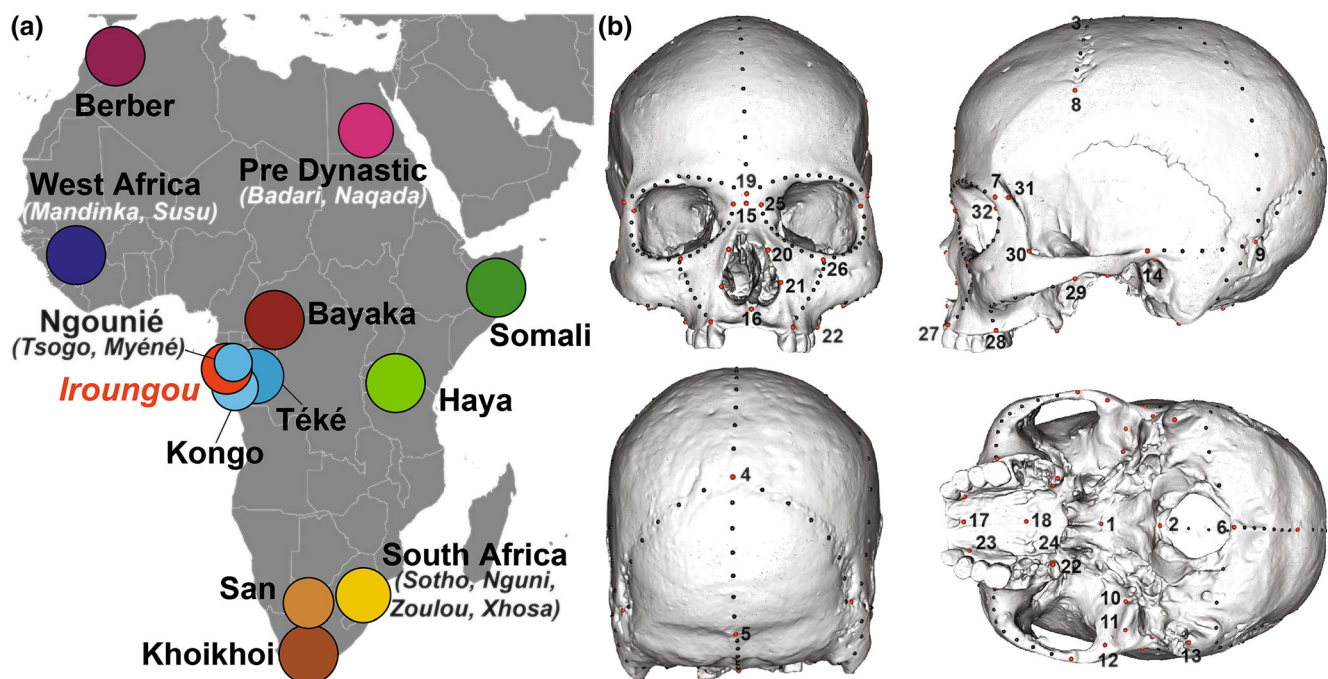
**TABLE 1** Study and comparative samples description (see Table S1).

Sample id	n	Sex <sup>a</sup>	Chrono.	Country	Ethny/site	3D <sup>b</sup>	Instit. <sup>c</sup>	Reference
Study sample								
Iroungou	8	-	14th–15th c.	Gabon	Iroungou	PH	IRC	Villotte et al. (2021)
Comparative sample								
San	12	0.67	19–20th c.	Republic of South Africa	San	CT/PH	DC/MH	
Khoikhoi	14	0.50	19–20th c.	Republic of South Africa	Khoikhoi	CT/PH	DC/MH	
Bayaka	14	0.36	19–20th c.	Central African Republic	Bayaka	PH	MH	
Kongo	11	0.27	19–20th c.	Congo	Kongo	CT/PH	DC/MH	
Téké	13	0.46	19–20th c.	Congo	Téké	PH	MH	
Ogooué	9	0.67	19–20th c.	Gabon	Tsogo	PH	MH	
Haya	14	0.43	19–20th c.	Tanzania	Haya	CT	DC	
Somali	14	0.00	19–20th c.	Somalia	Daarod, Ainhao	CT	DC	
West Africa	14	0.57	19–20th c.	Guinea	Susu, Mandenka	CT/PH	DC/MH	
South Africa	12	0.17	19–20th c.	Republic of South Africa	Nguni, Sotho, Xhosa, Zoulou	CT/PH	DC/MH	
Pre-Dynastic	13	0.46	6.3–4.9 ka	Egypt	Badari, Naqada	OP/CT	DC	Hendrickx (1999)
Berber	14	0.50	19–20th c.	Morocco	Berber	PH	MH	

<sup>a</sup>Ratio of number of female divided by the total number of specimens in each population.

<sup>b</sup>3D models were obtained through the use of medical computed tomographic scans (CT), 3D surface scanning with an optical scanner (OP) and photogrammetry (PH).

<sup>c</sup>DC: Duckworth Laboratory (Cambridge, United-Kingdom); MH: Musée de l'Homme (Paris, France); IRC: Iroungou Cave, Gabon.

**FIGURE 3** (a) Map of Africa showing the provenance and size of each sample analyzed in the study. (b) Landmarks and semi-landmarks used in the geometric morphometrics analyses shown on Iroungou specimen L0-007.

### 3.2 | Geometric morphometrics analyses

The landmark data set was superimposed using a GPA (i.e., see Gower, 1975; Rohlf & Slice, 1990), removing the information relating to

scale, position and orientation of the specimens, enabling the analysis to focus on the shape component (i.e., Procrustes shape coordinates) only. To correct for bilateral asymmetry, the symmetrized landmark configuration were used for subsequent analyses (Klingenberg et al., 2002).

### 3.3 | Centroid size, allometry and Procrustes distances

Centroid size of each specimen was extracted from the results of the GPA and were displayed using boxplots to visualize the difference in size between the crania of the sample (Figure S3). To reduce the dimensionality of our data, we used the Procrustes residuals from the GPA to compute a PCA (i.e., Table S3, Figure S4) which PC scores were then used to perform subsequent analyses. Allometry (i.e., the impact of size on shape) was explored through linear regressions of the shape variables (PC scores) against the size (i.e., log(centroid size); Table S3 and Figure S5A). Shape variation explained by allometry was represented through a TPS-warped current modern human individual which were used to compute color maps enhancing the morphological differences between large and small specimens (see Mounier & Mirazón Lahr, 2019; Figure S5B). Given that allometry was found, we used the residuals of the regression to perform the subsequent analysis on shape only. Results of analyses led on the uncorrected data are available in Figures S4B, S6–S8 and Tables S11–S16.

### 3.4 | Canonical variate analyses

To explore the Iroungou specimens populational affinity, we performed three canonical variate analyses (i.e., CVA) using the PC coordinates as variables. The number of PCs used for each CVA must be lower than the number of individuals present in the smallest group of the analysis, which for the first and third CVAs (i.e., CVA Full and CVA Comparative) is eight and for the second CVA (i.e., CVA Reduced) is seven. CVA Full (Tables S5 and S6) and CVA Reduced (Tables S7 and S8) explore the phenetic affinity and profile of the Iroungou sample. CVA Full is calculated from the entire study and comparative samples described by the coordinates of the first seven PCs (representing 60.0% of the total variation of the data, Table S3). CVA Reduced is computed from the full comparative sample, and from only seven specimens from the Iroungou sample, described by the first six PCs (55.9% of the total variation of the data, Table S4). Based on CVA Full and Reduced results, pairwise Mahalanobis, Euclidean distances among all possible pairs of groups (group centroid) were computed and associated *p*-values were calculated by permutation test (10,000 rounds) (Tables 2 and 3). Finally, CVA Comparative (Tables S9 and S10) explores the phenetic affinities and profiles of each specimen of the Iroungou sample separately. It is computed from the comparative sample only and the Iroungou specimens are later projected on the discriminated morphospace. We chose to run CVA Comparative with the first seven PCs in order to facilitate comparisons of results with CVA Full. Multivariate normality (i.e., Henz-Zickler test) and homoscedasticity (Box's M test) were tested before each analysis (Tables S5, S7, and S9).

For each CVA, predictions of group belonging were computed for each individual specimen of the study and comparative sample (Tables S6, S8, and S10). Those values were used to compute phenetic affinity profiles of each population (CVA Full and Reduced, Tables 4

and 5) and to compute the phenetic affinity profile for each specimen of the Iroungou sample (CVA Comparative, Table 6). The profiles are presented as stacked barplot (see Zinger, 2021 and Figures 4–6).

## 4 | RESULTS

### 4.1 | Morphology and state of preservation of the Iroungou specimens

On average, the Iroungou crania are relatively gracile showing no strong development of ectocranial structures in the supra-orbital, occipital and nuchal regions. They show rounded neurocrania with high frontal bones in *norma lateralis* and relatively small upper faces, despite the fact that some degree of protrusion of the lower maxilla may be noted in some specimens. The squared orbits are large compared to the size of the upper face and the intra-orbital space is well-developed. Each of the Iroungou crania presents a variably developed *tuberculum marginale* on the posterior rim of the zygomatic bone, just below the frontal process. The eight specimens present an evulsion of the central and lateral incisors (Villotte et al., 2021).

Four specimens were found on the lowest level of the cave floor (L0): L0-004 is an almost complete cranium. Nevertheless, the specimen's right side was strongly embedded in the cave floor sediment and the 3D digitization process could not be fully completed. The resultant 3D model used in the present study lacks part of the right maxilla, zygomatic, frontal, parietal and occipital bones, as well as the full right temporal bone (Figure S1). The missing parts were reconstructed using mirroring on the cranium itself (Figure 2a) before collecting the 3D landmarks. In *norma frontalis*, the cranial vault is high and laterally narrow with a small medial ridge on the frontal and on the biparietal suture. The small upper face present large squared orbits. In *norma lateralis* the frontal bone is almost vertical and the preserved nasal bones are weakly-projected. Both the *tuberculum marginale* and the mastoid process are small.

L0-005 (Figures 2b and S1) is less complete, missing most of the occipital bone and part of the right temporal bone. In *norma frontalis*, the calvarium is high, showing a medial ridge on the posterior part of the frontal and on the sagittal suture, with a small upper face displaying large squared orbits. The parietal eminences are well-developed laterally. In *norma lateralis*, the rounded frontal bone is less vertical than in L0-004. The lower maxilla and the nasal bones are projected anteriorly. The *tuberculum marginale* on the zygomatic is large on the left and small on the right. The *processus zygomaticus ossis temporalis*'s morphology could result from pathological deformation and is showing an oversized *tuberculum zygomaticus anterius*. The mastoid process is small.

Cranium L0-007 (Figures 2c and S1) is complete, but show a degree of bilateral dissymmetry especially visible on the maxilla, but which affects the entire calvarium. The specimen shows slightly projecting *arcus supraciliaris* along with marked nuchal lines and frontal lines which form a small crest on the parietal. In *norma frontalis*, the upper face is smaller than most of the other Iroungou specimens with



TABLE 2 Mahalanobis and Euclidean distances from CVA Full.

	Irongou	San	Khoi	Bayaka	Haya	Somali	Kongo	Téké	Ngounié	W. Africa	S. Africa	P. Dynastic	Berber
Euclidean distances													
Irongou	-	0.028	0.032	<b>0.011</b>	<b>0.015</b>	0.027	<b>0.015</b>	0.022	<b>0.018</b>	<b>0.020</b>	<b>0.018</b>	0.030	0.039
San	2.262	-	<b>0.014</b>	0.030	0.024	0.026	0.031	0.030	0.033	0.021	<b>0.013</b>	<b>0.015</b>	0.032
Khoi	2.572	<b>1.258</b>	-	0.031	0.026	0.028	0.029	0.024	0.030	0.018	0.020	<b>0.019</b>	0.034
Bayaka	<b>1.217</b>	2.429	2.311	-	<b>0.011</b>	0.029	<b>0.012</b>		0.021	<b>0.018</b>	0.020	0.031	0.044
Haya	<b>1.590</b>	1.991	1.898	<b>1.101</b>	-	0.022	<b>0.011</b>	<b>0.015</b>	0.022	<b>0.011</b>	<b>0.013</b>	0.023	0.036
Somali	3.119	2.936	3.322	3.457	2.682	-	0.027	0.028	0.033	0.022	<b>0.018</b>	<b>0.013</b>	0.021
Kongo	<b>1.565</b>	2.486	2.155	<b>1.071</b>	<b>0.971</b>	3.187	-	<b>0.014</b>	<b>0.016</b>	<b>0.017</b>	0.020	0.029	0.039
Téké	2.158	2.667	1.985	<b>1.519</b>	<b>1.260</b>	3.297	<b>1.142</b>	-	<b>0.017</b>	<b>0.012</b>	0.024	0.028	0.037
Ngounié	<b>1.612</b>	2.849	2.513	<b>1.633</b>	1.998	3.882	<b>1.418</b>	<b>1.698</b>	-	<b>0.020</b>	0.027	0.034	0.038
W. Africa	<b>1.875</b>	1.874	<b>1.491</b>	<b>1.393</b>	<b>0.818</b>	2.796	<b>1.341</b>	<b>1.021</b>	1.900	-	<b>0.015</b>	0.019	0.031
S. Africa	<b>1.674</b>	<b>1.059</b>	<b>1.671</b>	1.860	<b>1.197</b>	2.294	1.801	2.187	2.468	<b>1.415</b>	-	<b>0.015</b>	0.031
P. Dynastic	2.844	1.899	2.305	3.050	2.200	<b>1.236</b>	2.851	2.839	3.539	2.162	<b>1.555</b>	-	0.023
Berber	4.023	3.705	4.099	4.556	3.766	1.699	4.181	4.178	4.474	3.690	3.270	2.258	-

Note: Values in bold indicate non-significant distances between groups (i.e. groups are not statistically different, see Table S5 for the *p* values).

TABLE 3 Average predictions based on CVA Full.

	San	Khoi	S. Africa	W. Africa	Kongo	Ngounié	Téké	Bayaka	Haya	Somali	P. Dynastic	Berber	Irongou
San	<b>25.33%</b>	20.17%	12.50%	6.76%	1.71%	1.85%	0.95%	2.93%	3.84%	1.20%	7.91%	0.66%	5.01%
Khoi	20.73%	<b>30.68%</b>	8.93%	10.03%	5.70%	2.25%	6.86%	5.06%	4.95%	0.43%	4.95%	0.27%	2.33%
S. Africa	13.20%	6.36%	<b>15.55%</b>	6.28%	4.55%	3.47%	2.52%	4.47%	9.26%	4.78%	8.85%	1.62%	9.01%
W. Africa	6.50%	8.38%	8.41%	<b>17.05%</b>	10.11%	7.20%	15.83%	10.36%	14.28%	2.19%	5.89%	0.98%	7.89%
Kongo	2.28%	3.56%	5.66%	7.52%	<b>19.49%</b>	11.12%	13.25%	11.79%	10.39%	0.75%	1.32%	0.14%	6.40%
Ngounié	0.98%	1.91%	3.82%	7.50%	9.38%	<b>39.76%</b>	5.44%	6.59%	4.49%	0.17%	0.25%	0.12%	11.93%
Téké	3.29%	9.70%	4.03%	12.38%	12.41%	8.60%	<b>25.96%</b>	9.00%	10.40%	0.58%	2.64%	0.24%	4.03%
Bayaka	5.83%	6.24%	5.77%	7.36%	13.74%	7.53%	12.05%	<b>27.07%</b>	12.62%	0.72%	1.08%	0.07%	<b>16.66%</b>
Haya	7.15%	5.89%	9.82%	12.84%	13.32%	6.09%	13.08%	12.53%	<b>15.49%</b>	3.05%	3.95%	0.59%	9.06%
Somali	1.70%	0.84%	6.04%	2.48%	0.51%	1.48%	0.49%	1.02%	4.62%	<b>45.72%</b>	22.47%	20.22%	6.22%
P. Dynastic	6.93%	4.47%	10.32%	5.97%	0.93%	4.36%	1.08%	1.04%	4.90%	21.84%	<b>30.64%</b>	8.83%	3.85%
Berber	2.46%	0.78%	2.51%	0.12%	0.07%	0.18%	0.05%	0.06%	0.59%	16.36%	9.29%	<b>66.00%</b>	1.50%
Irongou	3.62%	1.01%	6.63%	3.73%	8.08%	6.11%	2.43%	8.07%	4.17%	2.23%	0.78%	0.26%	16.10%

Note: Mean percentage of chances to belong to one of the groups of the model per population. The highest percentage to belong to a group for each population is highlighted in bold.

TABLE 4 Mahalanobis and Euclidean distances from CVA Reduced.

Euclidean distances	Irongou	San	Khoi	Bayaka	Haya	Somali	Kongo	Téké	Ngounié	W. Africa	S. Africa	P. Dynastic	Berber
Mahalanobis distances	-	0.029	0.030	<b>0.008</b>	<b>0.012</b>	0.029	<b>0.011</b>	<b>0.020</b>	<b>0.017</b>	<b>0.018</b>	<b>0.019</b>	<b>0.031</b>	0.041
Irongou													
San	<b>2.331</b>	-	<b>0.013</b>	0.030	0.024	0.025	0.030	0.030	0.033	0.020	<b>0.013</b>	<b>0.015</b>	0.032
Khoi	2.315	<b>1.056</b>	-	0.031	0.026	0.027	0.029	0.025	0.030	0.018	0.020	<b>0.018</b>	0.033
Bayaka	<b>0.725</b>	2.413	2.282	-	<b>0.010</b>	0.029	<b>0.011</b>	<b>0.018</b>	0.021	<b>0.017</b>	0.020	0.031	0.043
Haya	<b>1.349</b>	1.896	1.895	<b>1.102</b>	-	0.021	<b>0.011</b>	<b>0.015</b>	0.021	<b>0.011</b>	<b>0.013</b>	0.022	0.035
Somali	3.479	2.865	3.048	3.352	2.376	-	0.026	0.027	0.033	0.021	<b>0.018</b>	<b>0.013</b>	0.021
Kongo	<b>1.043</b>	2.379	2.149	<b>1.014</b>	<b>0.968</b>	2.897	-	<b>0.014</b>	<b>0.015</b>	<b>0.017</b>	0.020	0.029	0.039
Téké	<b>1.753</b>	2.532	1.978	<b>1.454</b>	<b>1.235</b>	2.946	<b>1.132</b>	-	<b>0.016</b>	<b>0.012</b>	0.024	0.027	0.036
Ngounié	<b>1.247</b>	2.835	2.479	<b>1.627</b>	1.985	3.790	<b>1.358</b>	<b>1.627</b>	-	<b>0.020</b>	0.027	0.034	0.038
W. Africa	<b>1.590</b>	1.757	<b>1.486</b>	<b>1.371</b>	<b>0.818</b>	2.484	<b>1.337</b>	<b>1.005</b>	1.872	-	<b>0.015</b>	0.019	0.031
S. Africa	<b>1.827</b>	<b>1.059</b>	<b>1.560</b>	1.868	<b>1.074</b>	2.175	<b>1.689</b>	2.049	2.471	<b>1.298</b>	-	<b>0.015</b>	0.030
P. Dynastic	3.150	1.884	2.107	3.022	2.023	<b>1.174</b>	2.687	2.617	3.513	1.965	<b>1.518</b>	-	0.023
Berber	4.371	3.605	3.800	4.429	3.469	1.681	3.886	3.818	4.349	3.372	3.130	2.174	-

Note: Values in bold indicate non-significant distances between groups (i.e., groups are not statistically different, see Table S7 for the *p* values).

TABLE 5 Average predictions based on CVA Reduced.

	San	Khoi	S. Africa	W. Africa	Kongo	Ngounié	Téké	Bayaka	Haya	Somali	P. Dynastic	Berber	Irongou
San	<b>26.84%</b>	20.99%	12.68%	6.40%	3.80%	1.76%	1.16%	3.10%	4.12%	1.07%	8.18%	0.72%	4.93%
Khoi	19.93%	<b>28.98%</b>	8.51%	9.92%	3.71%	2.53%	6.67%	4.83%	4.62%	1.02%	6.86%	0.45%	3.73%
S. Africa	13.35%	6.69%	<b>15.98%</b>	6.60%	6.20%	3.40%	3.41%	4.68%	9.28%	4.61%	9.64%	2.05%	7.72%
W. Africa	6.78%	8.11%	8.91%	<b>15.76%</b>	9.65%	7.82%	14.90%	10.22%	13.42%	3.85%	6.27%	1.72%	9.72%
Kongo	2.58%	3.26%	6.03%	7.10%	<b>18.82%</b>	10.63%	11.79%	11.25%	9.83%	1.70%	1.35%	0.40%	10.09%
Ngounié	0.93%	2.30%	3.64%	7.35%	9.23%	<b>36.99%</b>	5.35%	6.13%	4.46%	0.19%	0.20%	0.17%	12.90%
Téké	3.85%	8.17%	3.90%	11.16%	12.32%	9.68%	<b>24.65%</b>	10.10%	10.18%	2.28%	2.28%	0.60%	6.53%
Bayaka	5.80%	7.04%	6.03%	7.78%	13.67%	7.25%	11.44%	<b>25.60%</b>	12.44%	0.91%	1.31%	0.10%	<b>18.39%</b>
Haya	7.81%	5.68%	10.83%	12.16%	12.96%	6.36%	12.43%	11.81%	<b>14.25%</b>	4.95%	4.93%	1.16%	11.02%
Somali	1.45%	0.92%	6.75%	4.12%	0.76%	1.23%	1.95%	0.70%	6.35%	<b>42.14%</b>	20.26%	20.03%	0.92%
P. Dynastic	6.31%	5.05%	10.05%	7.57%	1.69%	3.57%	1.87%	0.99%	5.19%	20.27%	<b>25.84%</b>	9.08%	1.21%
Berber	1.81%	0.92%	3.27%	0.37%	0.16%	0.17%	0.37%	0.03%	0.92%	16.70%	12.49%	<b>63.47%</b>	0.02%
Irongou	2.56%	1.88%	3.41%	3.73%	7.03%	8.62%	4.01%	10.56%	4.94%	0.30%	0.39%	0.07%	12.81%

Note: Mean percentage of chances to belong to one of the groups of the model per population. The highest percentage to belong to a group for each population is highlighted in bold.

**TABLE 6** Predictions for the Iroungou specimens (projection—CVA Comparative).

	L0-004	L0-005	L0-007	L0-012	L1-002	L1-004	L1-009	L1-010	Iroungou avg
San	1.20%	0.27%	1.82%	<b>39.37%</b>	0.02%	2.09%	21.69%	1.78%	8.53%
Khoi	0.77%	0.72%	2.98%	1.19%	0.07%	0.29%	15.53%	0.89%	2.81%
S. Africa	7.25%	0.87%	3.70%	38.75%	0.10%	12.47%	<b>22.80%</b>	11.05%	12.12%
W. Africa	15.03%	1.84%	<b>28.19%</b>	0.37%	1.48%	1.75%	3.90%	18.96%	8.94%
Kongo	8.02%	17.84%	5.58%	3.96%	7.50%	1.07%	12.30%	3.08%	7.42%
Ngounié	2.22%	<b>56.53%</b>	18.18%	7.42%	26.90%	0.17%	5.81%	0.17%	14.67%
Téké	5.76%	8.36%	7.41%	0.03%	7.95%	0.44%	2.05%	3.69%	4.46%
Bayaka	<b>34.94%</b>	10.17%	22.16%	6.55%	<b>53.62%</b>	0.79%	5.72%	17.33%	<b>18.91%</b>
Haya	22.75%	3.39%	9.60%	2.17%	2.35%	4.44%	7.16%	<b>28.78%</b>	10.08%
Somali	0.92%	0.00%	0.10%	0.03%	0.00%	<b>43.20%</b>	0.54%	6.51%	6.41%
P. Dynastic	1.13%	0.01%	0.29%	0.15%	0.00%	21.13%	2.48%	7.71%	4.11%
Berber	0.02%	0.00%	0.01%	0.00%	0.00%	12.17%	0.03%	0.06%	1.54%

Note: Percentage of chances to be classified as a member of one of the groups of the model per Iroungou individual. The highest percentage to belong to a group for each specimen is highlighted in bold.

a vertically short maxilla, large squared orbits and a wide inter-orbital space. In *norma lateralis* the frontal is high and the calvarium is very rounded especially around the occipital. The posterior rim of the right zygomatic presents a strongly developed *tuberculum marginale*. The mastoid process is strongly developed. The nasal bones are slightly more projecting than the other specimens of the sample.

L0-012 (Figures 2d and S1) is the least well-preserved specimen of the Iroungou sample analyzed in this study and the smallest in size (see Figure S3). The posterior parts of the right parietal and the temporal are missing along with the occipital. The left parietal, temporal, and zygomatic are missing. The specimen appears gracile, but frontal lines are visible on the frontal bone and the *arcus supraciliaris* are slightly projecting anteriorly. In *norma frontalis* L0-012 presents a vertically short, but damaged, maxilla, large squared orbits and a large inter-orbital space similar to what can be observed in L0-004 and L0-007. In *norma lateralis*, the frontal bone is rounded, but not vertical. The left zygomatic displays a strongly developed *tuberculum marginale* on the posterior border of the zygomatic. The nasal bones and lower maxilla are weakly-projected anteriorly.

Four additional individuals were captured on the second level of the cave (L1): L1-002 (Figures 2e and S2) is extremely well-preserved. It is the most robust specimen of the Iroungou sample, and the largest (see Figure S3), showing a small but clear occipital torus, projecting *arcus supraciliaris* along with well-developed mastoid processes. In *norma frontalis*, the calvarium appears relatively narrow, the frontal eminence is strongly projecting forward. The face is small, but the maxilla extends more vertically than what is observed in the rest of the sample, making the squared orbits appear smaller. The inter-orbital space is large. In *norma lateralis*, the calvarium is well-rounded, the occipital is slightly projecting posteriorly. The lower maxilla is strongly projected forward while the projection of the nasal bones is weak. The left zygomatic display a strong *tuberculum marginale*.

Only the right side of the specimen L1-004 (Figures 2f and S2) is preserved, and most of the frontal is damaged. The specimen appears

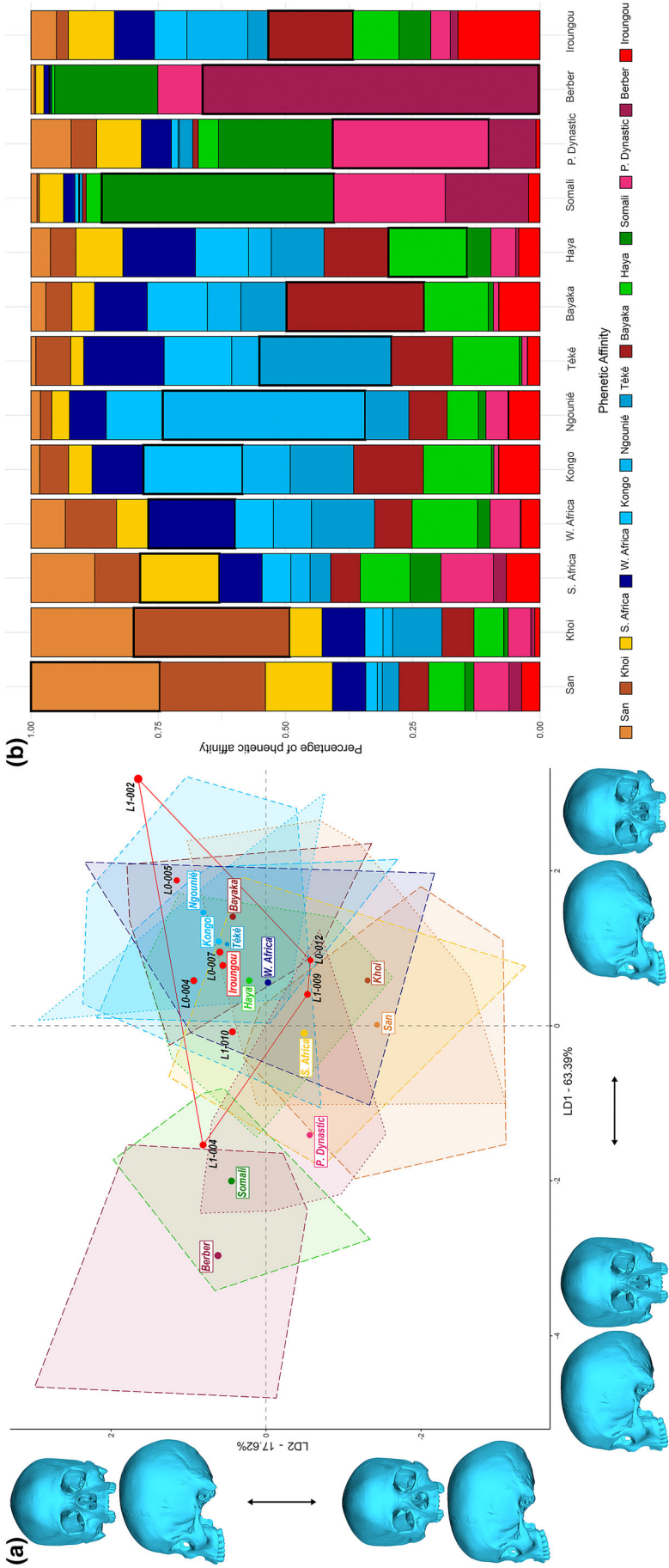
relatively robust with a small occipital torus, a clearly visible frontal line and a strongly-developed mastoid process, but the *arcus supraciliaris* are barely visible. In *norma frontalis*, the calvarium is relatively high and the upper face vertical development is among the largest of the Iroungou sample, making the squared orbits appear smaller. In *norma lateralis*, the frontal bone does not seem to have been particularly vertical, the nasal bones are missing and the lower maxilla projects slightly anteriorly. A small *tuberculum marginale* could be present on the posterior border of the zygomatic, but some matrix on the bone makes this observation difficult.

L1-009 (Figures 2g and S2) is almost complete to exception of the right half of the upper face. The surface of the bone is covered in matrix making part of the morphology difficult to describe. In *norma frontalis*, the calvarium present well-developed parietal eminences, a small face with squared and large orbits with slightly projecting *arcus supraciliaris*. The inter-orbital space is wide. In *norma lateralis*, the frontal bone is vertical, a well-developed *tuberculum marginale* is present on the zygomatic and the mastoid process is small.

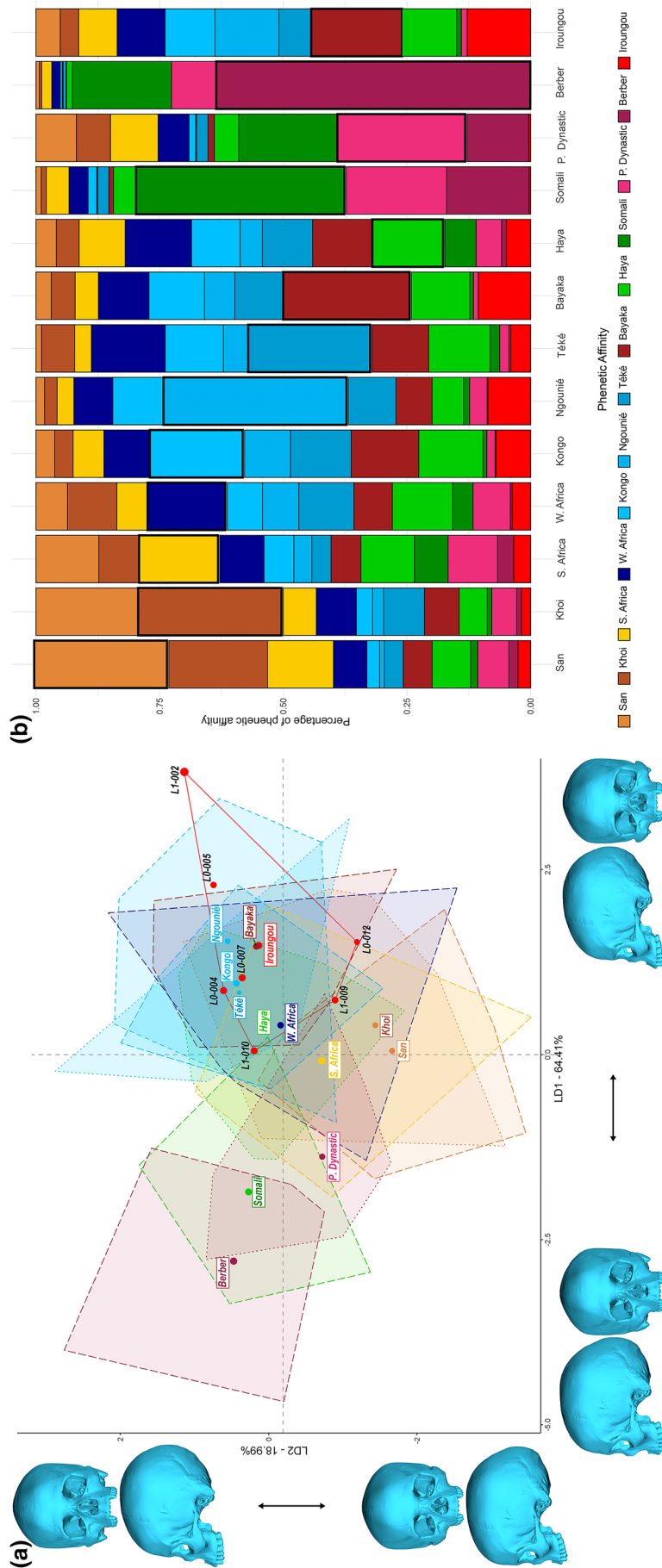
L1-010 (Figures 2h and S2) is a relatively robust specimen presenting slightly projecting *arcus supraciliaris*, frontal lines on the parietal bone, and a very faint occipital torus. In *norma frontalis*, the calvarium is high, with well-marked parietal eminence. The inter-orbital space is relatively well-developed and the squared orbits are large. The upper face is small with a vertically short maxilla. In *norma lateralis*, the frontal bone is slightly receding, and the lower part of the maxilla does not project anteriorly. There is a small *tuberculum marginale* on the left zygomatic (absent on the right) and small mastoid process.

The Iroungou specimens appear relatively larger than the other African populations of our sample. When looking at the centroid sizes, only the South African Bantu present a larger median size (Figure S3). The population showing the closest centroid size distribution to the Iroungou size are the Somalis (but this sample is composed exclusively of male individuals) and the Haya. Most of the Iroungou specimens

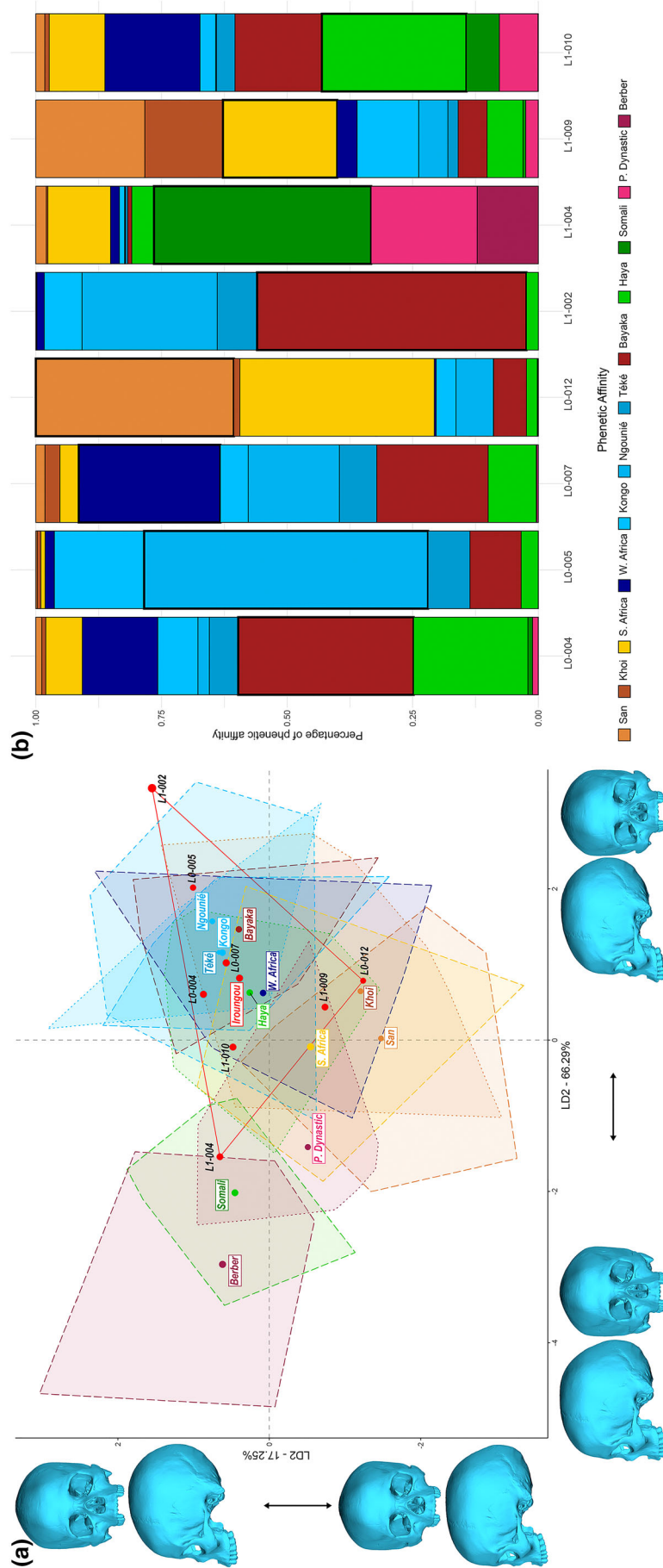




**FIGURE 4** (a) Morphospace formed by LD1 (17.62% of the variance) and LD2 (63.39%) from CVA Full. (b) Phenetic affinity for each population showing the percentage of chance to be classified in one of the groups analyzed in the study. Individuals from the comparative sample groups present the highest percentage of chance to be classified within their group of origin. Individual from the Irongou group present the highest percentage of chance to be classified as Bayaka.



**FIGURE 5** (a) Morphospace formed by LD1 (18.99% of the variance) and LD2 (64.41%) from CVA Reduced. (b) Phenetic affinity for each population showing the percentage of chance to be classified in one of the groups analyzed in the study. Individuals from the comparative sample groups present the highest percentage of chance to be classified within their group of origin. Individual from the Iroungou group present the highest percentage of chance to be classified as Bayaka.



**FIGURE 6** (a) Morphospace formed by LD1 (17.25% of the variance) and LD2 (66.29%) from CVA Comparative. (b) Phenetic affinity for each Iroungou specimen showing the percentage of chance to be classified in one of the other groups analyzed in the study. The Iroungou crania shows varied patterns of phenetic affinities, characterized, for most of the specimens, by strong affinities with Bantu populations and with the Bayaka pygmies. L1-004 and L1-009 present a phenetic profile showing strong affinities with southern Africans (San and South African Bantus) and as a strong outlier showing strong affinities with Somalis.



present relatively similar centroid size values, to the exception of L1-002 which appears much larger.

Finally, and apart from the Iroungou crania, evulsion of the four upper incisors was only observed in the Bayaka Pygmy population of the comparative sample (5 specimens out of 14, Figure S9). No specimen from the other groups of the comparative sample present such dental modification. Evulsion of the two upper central incisors was nevertheless observed within the comparative sample: one Bayaka specimen (Py-23641), one Kongo individual (AfC-6989), five Téké specimens (AfC-20501, AfC-20504, AfC-20508, AfC-20510 and AfC-20515), one cranium from the Ngounié group—a Tsogo (AfC-18642), one west-African—a Susu (AfW-9861) and one Bantu from South-Africa—a Nguni (AfC-1734) whom also shows teeth loss with resorption of the alveolar bone on the right side of the maxilla (both incisors, the canine and P3; see Table S1).

## 4.2 | Geometric morphometrics

### 4.2.1 | Allometry

After GPA alignment of the data two PCAs were run: one on the full comparative and Iroungou samples (PCA Full) and one on the full comparative sample but on a reduced Iroungou sample (removal of L1-004, PCA Reduced). Allometry was tested on the shape data from both PCAs represented by the PC coordinates. The two analyses yielded similar results, identifying a significant impact of size on shape for PC2, 4, and 5 (Tables S3, S4 and Figure S5) with ~9% of the shape variation displayed by PC2, ~3% of this variation on PC4 and ~6% on PC5 being explained by the effect of size on shape in both cases (Tables S3 and S4). The results presented in the rest of the manuscript are based on the allometry-corrected data (see Tables S11–S16 and Figures S6–S8 for results from original shape data).

## 4.3 | Phenetic affinity of the Iroungou sample

Figure S4 presents the morphospaces formed by PC1 and PC2 (representing 27% of the variance) from the full sample Analysis (PCA Full, Figure S4A,B presents the morphospace based on data uncorrected for allometry). There is little discrimination between the groups but we can note that non-Bantu populations are on average positioned towards PC1 positive values, while Bantu populations tends to present more negative values on PC1. The Bayaka pygmies are the only non-Bantu individuals clustering towards negative PC1 values.

Results from the discriminant analysis based on the entire sample (CVA1, Henz-Zirkler multivariate normality test = 0.951,  $p = 0.432$ ; Box's M homoscedasticity of covariance matrices:  $\chi^2 = 322.46$ ,  $p = 0.693$ , Table S5) show, as expected, a better discrimination within the morphospace (Figure 4a), with non-Bantu North-African (i.e., Berber and Pre-Dynastic) and East-African (i.e., Somali) populations clustering around negative values on LD1 (63.39% of the variance) and non-Bantu South-African populations (i.e., San and Khoi)

clustering towards negative LD2 (17.62%) values. The Bayaka pygmies and the Bantu populations, to the exception of South-African Bantus cluster towards the positive values on both LD1 and LD2. The Iroungou sample show a rather large variation within the morphospace, but tends to cluster along with the Bantu and Bayaka groups. This large variation is partly explained by two specimens: L1-002, which is the largest specimen of the Iroungou sample (Figure S3), pots outside of the variation of the Bantu populations and appears as an extreme of the general Bantu morphology, and L1-004 which patterns with the North-African and Somali populations.

The Euclidean and Mahalanobis distances calculated between the different populations (Tables 2 and S5) indicate that the Iroungou group, on average, can be statistically distinguished from six populations of the comparative sample all of them but one (Téké) non-Bantu groups: the San and Khoikhoi from Southern Africa, the Somali and the Berber and Pre-Dynastic from North Africa. The Iroungou sample present on average the lowest Mahalanobis and Euclidean distances with the Bayka pygmies (respectively 1.217 and 0.011) followed by the Kongo (1.565 and 0.015) and the Haya (1.590 and 0.015).

CVA1 overall prediction accuracy is low with only 43.21% of the specimens being classified within their group of origin (Table S6, 43.83% when the data is uncorrected for allometry, Table S12). Nevertheless, each group of the comparative sample presents a relative majority of its member classified in their group of origin, with considerable variation: from 66% of accurate classification for the Berber to 15.49% for the Haya (Tables 3 and S6 and Figure 4b). Only 16.1% of the Iroungou specimens are classified by CVA1 within the Iroungou group, and 16.66% of them are classified as Bayaka Pygmies. Overall, the Iroungou sample appears to present the most diverse phenetic profile of the analyzed groups (Figure 4b).

Similar patterns are observed when considering the allometry uncorrected data (Figure S6, Tables S11 and S12).

To investigate whether the high level of phenetic variation observed within the Iroungou sample could be due to the presence of an outlier in the group, we ran a second CVA from which L1-004 was removed. Results from CVA2 (Henz-Zirkler multivariate normality test = 0.959,  $p = 0.214$ ; Box's M homoscedasticity of covariance matrices:  $\chi^2 = 238.15$ ,  $p = 0.725$ , Table S7) yielded a very similar morphospace (Figure 5a) where the Iroungou sample's average plots much closer to the Bayaka sample's average. This shift is clearly noticeable when looking at the average Euclidean and Mahalanobis distances (Table 4). Only five populations are now statistically different from the Iroungou sample as the Téké can no longer be distinguished from the Iroungou specimens. The Iroungou sample is on average closer to the Bayaka (respectively 0.725 and 0.008) followed by the Kongo (1.043 and 0.011), the Ngounié (1.247 and 0.017) and the Haya (1.349 and 0.012).

CVA2 overall prediction accuracy is slightly higher with 44.72% of the specimens being classified in their group of origin (Table S8, 42.87% when the data is uncorrected for allometry, Table S14). Again, each group presents a majority of individuals being classified in their group of origin, with variation from 63.47% (Berber) and 14.25% (Haya) (Tables 5 and S8, Figure 5b). The Iroungou sample only present

12.81% of its sample being classified within its population of origin, while 18.39% of the sample present more chances to be classified as a Bayaka Pygmy (Table 5, Figure 5b).

As was observed for CVA1, the analysis of the allometry uncorrected data yielded congruent results (Figure S7, Tables S13 and S14).

#### 4.4 | Phenetic affinity within the Iroungou sample

To better understand the phenetic affinity variation within the Iroungou sample we ran a third discriminant analysis on the comparative sample only, before projecting the Iroungou specimens on the calculated discrimination (CVA3, Henz-Zirkler multivariate normality test = 0.954,  $p = 0.385$ ; Box's M homoscedasticity of covariance matrices:  $\chi^2 = 283.57$ ,  $p = 0.838$ , Table S9). The resulting morphospace (Figure 6a) is similar to the one obtained after CVA1 (Figure 4a) and Iroungou occupies a more central position within the Bantu populations than in CVA2 (Figure 5a).

CVA3 overall prediction accuracy is also slightly higher with 45.45% of the specimens being classified in their group of origin (Table S10, 45.45% with the data uncorrected for allometry, Table S15). The Iroungou specimens show a high degree of variation regarding their classifications to the comparative sample (Table 6). Two individuals express strongest affinities with the Bayaka (L0-004, 39.94%; L1-002, 53.62%), three with Bantu populations from Central and West Africa: Ngounié (L0-005, 56.53%), West Africa (L0-007, 28.19%), Haya (L1-010, 28.78%), one with the South-African Bantus (L1-009), and one with South-African Sans (L0-012). Finally, the specimen which was identified as an outlier in the first analysis, L1-004, is classified as a Somali (43.20%) and present almost no affinity with Bantu or Pygmy population, but instead with North African groups (Pred-Dynastic, 21.13% and Berber, 12.17%).

On average, the Iroungou specimens present the most affinities with the Bayaka (18.91%) followed by the Ngounié (14.67%), South-African Bantus (12.12%), and Haya (10.08%).

The results from the analysis of the uncorrected data yielded congruent results (Figure S8, Tables S15 and S16).

## 5 | DISCUSSION

### 5.1 | Allometry

The results from both the allometry-corrected and uncorrected data are extremely congruent, showing that a stable captured phenetic signal from our database. Indeed, the removal of the shape variation that can be explained by size did not impact strongly the results of the analyses.

### 5.2 | Morphology of the Iroungou crania

The morphology of the cranium has been found to be partly influenced by adaptive and/or functional factors (Bastir & Rosas, 2013;

Menegaz et al., 2010), this is especially true for the mid-face and the upper jaw (Smith, 2009). Nevertheless, the morphology of the cranium still strongly correlates with population history (Eyquem et al., 2019; Harvati & Weaver, 2006; Katz et al., 2017) and can therefore be used to assess phenetic affinities between human populations.

The eight Iroungou specimens are globally well-preserved and their morphology can be carefully observed and discussed. Additionally, they do not display morphological modification that could be link to adaptative/functional factors nor do they present strong morphological alteration due to pathologies. A striking aspect of the Iroungou sample remains the evulsion of the four maxillary incisors which led to maxillary alveolar bone resorption. Analyses presented in this study took this important aspect in consideration and the antero-inferior aspect of the maxilla was not taken into consideration in the morphological analyses but is discussed regarding its possible cultural significance (see *infra*).

Morphological description shows a relative homogeneity within the Iroungou sample: the specimens are gracile with a very rounded calvarium, high forehead and relatively small upper faces. The systematic evulsion of the four upper incisors may visually contributes to perception of the antero-posterior length of the upper face. Nevertheless, such short upper face is matched by most of the comparative sample but the Berber, Somali and Pre-Dynastic which appears to have more supero-inferiorly developed upper face along with less anteriorly projecting lower maxilla. The Iroungou calvaria are also relatively short antero-posteriorly and high supero-inferiorly with only the North African Berbers presenting higher cranial vault (see Figures 4 and 5). The Iroungou sample presents, on average, morphological affinities with sub-Saharan populations originating from West and Central Africa: the Bayaka, the Kongo, the West-African Bantus, and the Haya which currently occupies the eastern part of the central tropical forest in Africa. They are, however, less similar to the Téké and the Ngounié samples (Tsogo and Myéné) which currently lives a few kilometers away from the Iroungou cave.

When considering the shape of the eight Iroungou crania, differences arise within the sample. Four main phenetic profiles can be identified within the sample. One individual appears clearly as an outlier: L1-004 present strong affinities with the Somali and North-Africans of the comparative sample. L1-004 is the specimen of the sample that required the most reconstruction, through mirroring, as most of the right side of the skull is missing (Figures 3 and S2). The reconstruction may have had an impact on the overall morphology of the specimen and could partly explain the obtained phenetic profile. Two specimens are most similar to South-African populations: L0-012 is most similar to the Sans and presents an overall percentage of chances to belong to one of the three South-African populations (i.e., San, Khoi, and South-African Bantus) which reaches over 79%; and L1-009 for which this percentage is above 45% (with over 22% of chances to be classified as a South-African Bantu). The third phenetic profile represented in the group shows affinities with West and Central Africa Bantu populations (i.e., West-Africa, Kongo, Téké, Ngounié, Haya). L0-005 (56.53% Ngounié and 87.97% with the entire West Central African populations), L0-007 (28.19% West Africa and 68.49%), L1-010 (28.78% Haya and 54.67%), L0-004 (22.75% Haya

and 53.77%), and L1-002 (26.9% Ngounié and 46.19%). These individuals also express high phenetic affinity with the Bayaka pygmies, especially the last two individuals which main affinity is actually for the Bayaka (i.e., L0-004 39.94% and L1-002 53.62%). This third phenetic profile is congruent with geography as the Bantu and Bayaka populations are located, as is the Iroungou Cave, around and through the tropical rain forest.

Nevertheless, the eight Iroungou crania could represent more than one population. Such a phenetic profile for eight crania recovered from a unique burial place that was in use for a maximum of 200 years, according to the C14 dating (Villotte et al., 2021), is puzzling, especially given that all eight crania display the systematic evulsion of the four upper incisors. This kind of body modification practices have been historically documented in Africa (Lalouel, 1950; van Rippen, 1918) and have recently been reported as a dominant dental modification practice, in both males and females, in the Northern Suburbs of Cape Town in South Africa (Friedling & Morris, 2007). Dental modification practices are generally believed to be triggered either by cultural/social or medical reasons (Irish, 2017). Regarding cultural and/or social reasons for modifying the denture, ornamentation and ethnic/tribal identity have been identified as the most common (Irish, 2017). The latter seems to have been replaced by “gangsterism,” indicating ones belonging to an informal group, and peer-pressure among the current inhabitants of the Northern Suburbs of Cape Town where belonging to a tribe has been strongly undermined by urbanization and past apartheid policies, while “fashion” remains a strong reason to perform dental modification (Friedling & Morris, 2007). Other reasons have been put forward such as “diplomacy,” when members of a tribe adopt the dental modification style of a more powerful neighboring group (Irish, 2017), and providing additional social markers, a form of tribal and intra-tribal class identification, beyond the traditional ethnic identity (Gould et al., 1984).

Looking more closely at Pygmy populations, with whom the Iroungou specimens share the strongest phenetic affinities in the present study, Lalouel (1950) notes that evulsion of the maxillary incisors, only the central or both central and lateral incisors, is common among Babinga Pygmies. This is contradictory with reports by Irish (2017) as he indicates that the ablation of the incisors was almost never observed among Aka Pygmies and rarely seen within the Babinga Pygmies. This statement seem confusing as Babinga (and Bayaka as well) is a generic term which encompasses Aka populations (Lalouel, 1950). Nevertheless, out of the fourteen 19th century Bayaka Pygmies included in the comparative sample of the present study, 5 specimens (Py-9880, Py-17762, Py-18446, Py-18448, and Py-18449) present an evulsion of the four upper incisors (Figure S9). No specimen in the other populations of the comparative sample used in the present study show the evulsion of the four upper incisors (see Table S1). Answering the question of the meaning of this dental modification would help to characterize the phenetic profile of the Iroungou sample.

Finally, the strong (and highest on average within the sample) Pygmy phenetic signal within the Iroungou sample raise the question

of the relationships of pre-colonial Bantu populations, which at the time in the area were most likely part of powerful kingdoms, with the local Pygmy populations.

### 5.3 | Relationship of the Pygmies with the pre-colonial Bantu kingdoms of Central Africa

Around the time of use of the Iroungou sepulchral cave (14th to the end of the 15th century) several kingdoms were being formed in Western Central Africa (Vansina & Obenga, 1992). Aside the famous Kongo Kingdom, another political entity was developing on the Western coast of Central Africa: the Loango Kingdom. At the latest, at the beginning of the 16th century, what is now the Ngounié province of Gabon where the Iroungou cave is located, was part of the Loango Kingdom territory (see fig. 19.3 in Vansina & Obenga, 1992). It is therefore a possibility that the population which used the Iroungou sepulchral cave was part of this kingdom or at least part of the political entity which prefigured the formation of the Loango Kingdom.

The strongest phenetic affinities shown by the eight crania from the Iroungou cave are with the Pygmy population not with the Bantu populations of the comparative sample from the same geographical area (Ngounié, Kongo, Téké groups). An interesting line of thought comes from the numerous historical sources reporting on the interactions between the powerful Bantu kingdoms of the time and the “autochthones” of the forested areas of central Africa: the Pygmies (Moïse, 2014). While Pygmy populations are now marginalized by their Bantu neighbors, linguistic (Bahuchet, 1993) and genetic evidence (Verdu et al., 2009) would indicate that this was not always the case and that numerous exchanges took place between the populations and from the time of the Bantu expansion until as late as 1500 CE the Pygmy populations were regarded as equal or at least as complementary to the immigrant populations (Moïse, 2014). Nevertheless, and despite the change in social status generally associated to the Pygmies, additional historical evidence from the early 17th century reports on the Pygmy relations to the royal elites of Loango Kingdom. The local Pygmies are described as bypassing local Bantu in establishing alliance and trading relations with provincial rulers (Klieman, 2003; Moïse, 2014). Thus, the possibility of close ties between local elite of the time and Pygmy populations cannot be discarded.

What does this tell us about the populational affinity of the people who were buried in the Iroungou Cave? Main results of the present analysis indicate that the Iroungou specimen may represent a phenetically varied population, which could represent different source populations. They also indicate that there may have been a certain cultural homogeneity within this group. In both cases possible links to Pygmy populations arise. A tentative explanation would be that the people buried at Iroungou were from both Bantu and Pygmy origin, while sharing a common social background which could be reflected in the style of dental modification which they are all displaying.

Additional evidence is nevertheless needed to decipher fully the human history behind the Iroungou Cave. Proper excavation of



the cave as well as genomic analyses should bring more light into this fascinating case of pre-colonial burial practice in Western Central Africa.

## AUTHOR CONTRIBUTIONS

**Aurélien Mounier:** Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); writing – original draft (lead); writing – review and editing (lead). **Sébastien Villotte:** Conceptualization (equal); investigation (equal); methodology (supporting); project administration (equal); writing – original draft (supporting); writing – review and editing (supporting). **Sacha Kacki:** Conceptualization (equal); investigation (equal); methodology (supporting); project administration (equal); writing – original draft (supporting); writing – review and editing (supporting). **Pascal Mora:** Data curation (equal). **Loic Espinasse:** Data curation (supporting). **Jules Zamke Dempawo:** Project administration (supporting). **Christian Gerin:** Project administration (equal); writing – review and editing (supporting). **Quentin Meunier:** Project administration (equal); resources (equal); writing – review and editing (supporting). **Richard Oslisly:** Funding acquisition (lead); project administration (lead); resources (lead); writing – original draft (supporting); writing – review and editing (supporting).

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## DATA AVAILABILITY STATEMENT

The analyzed data in the present study (landmarks coordinates) is available in Supplementary Materials and is being published along with the study. The 3D models of the analyzed individuals can be accessed through request to the competent curating authority (see Table S1 for details about the institutions curating each of the individual).

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## SUPPORTING INFORMATION

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