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Brief Communication: Ancient Remains and the First Peopling of the Americas: Reassessing the Hoyo Negro Skull

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ABSTRACT *OBJECTIVE:* A noticeably well-preserved ~12.500 years-old skeleton from the Hoyo Negro cave, Yucatán, México, was recently reported, along with its archaeological, genetic and skeletal characteristics. Based exclusively on an anatomical description of the skull (HN5/48), Chatters and colleagues stated that this specimen can be assigned to a set of ancient remains that differ from modern Native Americans, the so called "Paleoamericans". Here, we aim to further explore the morphological affinities of this specimen with a set of comparative cranial samples covering ancient and modern periods from Asia and the Americas.

METHODS: Images published in the original article were analyzed using geometric morphometrics methods. Shape variables were used to perform Principal Component and Discriminant analysis against the reference samples.

RESULTS: Even thought the Principal Component Analysis suggests that the Hoyo Negro skull falls in a subregion of the morphospace occupied by both "Paleoamericans" and some modern Native Americans, the Discriminant analyses suggest greater affinity with a modern Native American sample.

DISCUSSION: These results reinforce the idea that the original population that first occupied the New World carried high levels of within-group variation, which we have suggested previously on a synthetic model for the settlement of the Americas. Our results also highlight the importance of developing formal classificatory test before deriving settlement hypothesis purely based on macroscopic descriptions. Am J Phys Anthropol 158:514–521, 2015. © 2015 Wiley Periodicals, Inc.

After decades of intense research, the subject of the settlement of the New World continues to be highly controversial. Although it is widely recognized that America was the last continent to be populated, probably from Asia through Beringia during the last glaciations at the end of the Pleistocene, researchers' views on various aspects of this process (e.g., from where, by whom, how many times the continent has been populated) differ significantly (Lahr, 1996; Bonatto and Salzano, 1997; Santos et al., 1999; Tarazona-Santos and Santos, 2002; Bortolini et al., 2003; Schurr, 2004; Neves and Hubbe, 2005; Goebel et al., 2003; Fagundes et al., 2008; González-José et al., 2008; Marangoni et al., 2013; Raghavan et al., 2014a, 2014b, Rasmussen et al. 2014; Dixon 2013, among others). This is probably due to the fact that insights into the peopling of the Americas comes from a variety of disciplines including geology, paleoecology, archaeology, skeletal biology, and genetics, yet the models that intend to explain such different lines of evidence are often centered on only one specific data type, sometimes disregarding potentially complementary interpretations of other traits. Good examples of this problem are the contrasting views or hypotheses that have emerged from gene or craniofacial evidence during the last decades. For instance, apparently mutually exclusive hypotheses have emerged from the analysis of skull morphology and molecular genetics

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Additional Supporting Information may be found in the online version of this article.

Abbreviations: GPA, Generalized Procrustes Analysis; HN, Hoyo Negro; PCA, principal component analysis; PD, Procrustes distance

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TABLE 1. Sample composition

Code	Group	Population	Chronological range	*n*
ACA	NA	California, USA	1000	49
AIN	AS	Ainu, Japan	1000	10
ARA	NA	Araucano, Argentina	1000	43
AUS	AUS	Aborigines, Australian	1000	36
BCS	NA	Baja California Sur, Mexico	1000	23
BOL	NA	Aymara, Bolivia	1000	18
BUR	AS	Buriats, Siberia	1000	10
CAL	NA	Calama, Chile	1000	24
CHA	NA	Chaco, Argentina	1000	10
ECU	NA	Paltacalo, Ecuador	1000	53
EOW	EOW	Late Pleistocene (Early) Old World	30-11 kyr	13
ESK	ESK	Eskimos, Greenland	1000	46
FUE	NA	Fuegians, Chile and Argentina	1000	10
LS	PA	Paleoamericans from Brazil	11-7.5 kyr	11
MAP	NA	Mapure, Venezuela	1000	38
NPA	NA	North Patagonians, Argentina	1000	18
OUR	AS	Ourgas, Siberia	1000	18
PAM	PA	North Paleoamericans	10000	6
PAT	NA	Central Patagonians, Argentina	1000	38
PER	NA	Ancon, Peru	1000	37
PG	NA	Pampa Grande, Salta, Argentina	1000	25
TCH	AS	Tchouktchi, Siberia	1000	14
TLA	NA	Aztecs from Tlatelolco, Mexico	1000	26

Series included in analysis of lateral view of the skull. See also González-José et al. (2008 Table 1).
Populations were grouped following a geographic and chronological criterion.
AS: Asiatic, NA: modern Native Americans, AUS: Australians, EOW: Early Old World, ESK: Eskimos, PA: Paleoamericans.

(e.g. Single Wave versus Two Components/Stocks models, see a discussion in González-José et al., 2008).

The intense debate concerning the pre-Columbian peopling of the Americas is being constantly fed by new discoveries. A central concern in this debate is how to explain the craniofacial shape and size variation observed among earliest and modern Native Americans from different regions, and to interpret this magnitude and pattern of variation in combination with other lines of biological and non-biological evidence. Of particular interest to this issue is the recent publication made by Chatters et al. (2014), who reported the archaeological, craniofacial, and genetic characteristics of the Hoyo Negro skull (HN5/48), a very well-preserved specimen from a submerged cave in Yucatan, dated ~12.500 years ago. This specimen adds to the recent discoveries and subsequent analyses of the 24,000-year-old Mal'ta individual (MA-1) from Siberia (Raghavan et al., 2014a), and the ~12,600-year-old Anzick-1 specimen from Montana, USA (Rasmussen et al. 2014), whose genomic data fuelled a new round of discussions about the affinities and dispersal patterns of the first Americans.

The paleogenetic analysis of the Hoyo Negro skeleton indicates that it carries a Beringian-derived mitochondrial DNA (haplogroup D1). Moreover, according to the authors, the HN5/48 "cranial and dental characteristics are comparable to those of other, less complete pre–10-ka Paleoamerican skeletons," thus suggesting that this specimen is "among the small group of Paleoamerican skeletons that are morphologically distinct from Native Americans." Based on these statements, Chatters et al. (2014) conclude that the differences between Paleoamericans and Native Americans probably resulted from in situ evolution rather than separate ancestry. However, such conclusions are based only on a macroscopic anatomical description of the skull, with a formal statistical and morphometric analysis still lacking.

Besides (and because of) the relevance of any well-preserved specimen of the chronological and geographical characteristics that Hoyo Negro exhibits, a formal test aimed to estimate the morphological affinities of HN5/48 in relation to other craniofacial series from early and modern horizons from the Americas and the Old World is necessary. In this context, the use of geometric morphometric methods and comparison with large comparative series can provide information that complements any macroscopically based affiliation. Here, we present a formal geometric morphometric statistical analysis aimed to explore the morphological affinities of the Hoyo Negro skull with existing craniofacial databases including both ancient and modern skulls from Asia and the Americas. A wide spectrum of comparative samples is mandatory, since previous research is congruent in reporting the high levels of between-populations (within-continent) craniofacial diversity observed in the Americas, not only in geographical, but also in chronological terms (Relethford, 2002; González-Jose et al., 2001, 2008).

After obtaining quantitative estimators of affinities and/or membership of HN5/48, we attempt to interpret the relevance of this skull in the context of the existing models proposed to explain the early human settlement of the Americas.

MATERIALS AND METHODS

We performed a geometric morphometric analysis of the HN5/48 skull plus modern and ancient Asian and New World reference samples (see Table 1), whose craniofacial affinities were discussed elsewhere (González-José et al., 2003, 2008; de Azevedo et al., 2011; Bortolini et al., 2014). The reference samples covered the entire continuum of variation in shape found in Asia and the Americas. This sample included 576 complete adult skulls of both sexes representing modern series from Australia and Asia, early and late series from South and

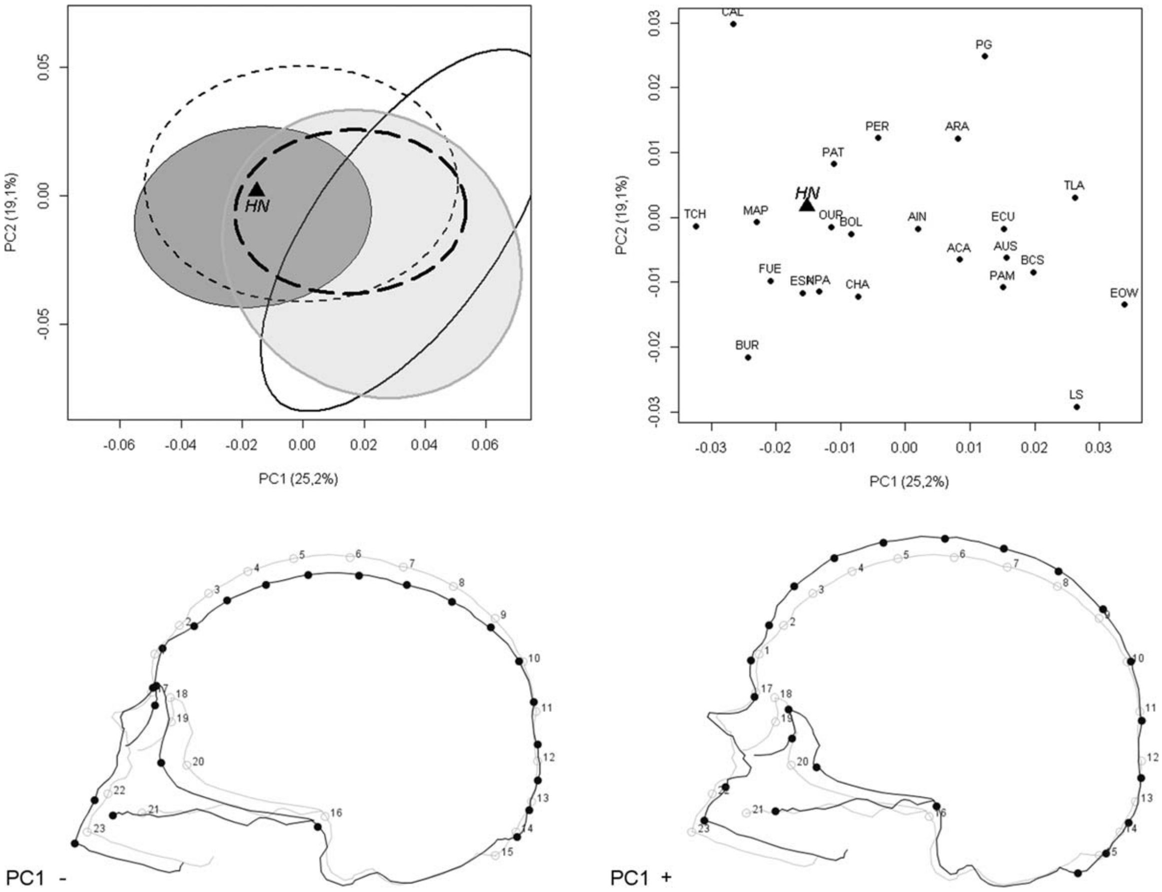

Fig. 1. Shape affinities of the Hoyo Negro specimen in lateral view of the skull: PCA. First two principal components explaining 25.2% and 19.1% (respectively) of the total variation. Upper left: Individual scores plot with 90% confidence ellipses for classifier "group" (see Table 1). AS plus ESK: dark gray filled ellipse; EOW: empty ellipse with black solid line; PA: light gray filled ellipse; NA: empty ellipse with dotted line; AUS: empty ellipse with long-dash line. Upper right: Population centroids across PC1 and PC2 together with Hoyo Negro (HN) individual scores. Individual scores for the HN5/48 skull are plotted as a black triangle. Bellow: The general shape in lateral view is represented by 23 landmarks and semilandmarks (see González-José et al., 2008, Fig. 1). Shape variation explained by the first principal component (PC1) is represented as a deformation of an outline drawing using the thin-plate spline function, depicting variation as displacements from consensus (light gray line) to the positive (right graph) and negative (left graph) extreme values (black lines). Note that shape changes occurring between landmarks 22 and 17 should not be taken into account since there are no landmarks or semilandmarks covering that region and capturing the shape of the nasal profile.

North America, and a composite series of late Pleistocene Old World specimens (Table 1).

To recover the general shape of the skull in lateral view, individual lateral photographs were used to digitize 23 landmarks and semilandmarks (see Fig. 1 in González-José et al., 2008). To include the Hoyo Negro individual to our reference sample, we used the HN5/48 skull photograph in lateral view presented by Chatters et al. (2014) in Supporting Information Figure S8, in order to digitize the same configuration of landmarks (González-José et al., 2008, Fig. 1). The authors also present a photograph of the HN5/48 skull in lateral view in Supporting Information Figure S7, however the skull is not well oriented (note the lack of plane of reference in the frontal view counterpart, for instance), thus avoiding any solid geometric morphometric comparison because of lack of coplanarity. However, althought Chatters et al. (2014) didn't inform the protocol they used to photograph and orient the skull, we consider Supporting Information Figure S8 is a better representation of the HN5/48 skull since it consists of a screen grab of a digital 3D model, which is presented on a (probably) automatic orientation in the sagittal plane. Further validation of S8 based on measurements reported in the Supporting Information presented by Chatters et al. was not possible because, unfortunately, the classical measurements presented by the authors in Supporting Information (page 18), consist of craniofacial indexes that are useless for the required validation of our analysis in lateral view (e.g., no possible comparison can be derived among indexes obtained on the S8 photographic specimen and the presented indexes). In the case of the frontal view, only orbital and nasal indexes can be computed but this would be useless to statistically compare S7 vs. S8 due to the deficient orientation of the S7 specimen. Considering all the above, we digitized upon S8 the configuration of 23 landmarks and semi-landmarks used in previous papers (see González-José et al., 2008, Fig. 1). Replicated analyses made exclusively on S7 resulted on highly coincident results.

The reference photographs dataset used here (Table 1) and elsewhere (González-José et al., 2008) were obtained according to the recommendations made by Zelditch

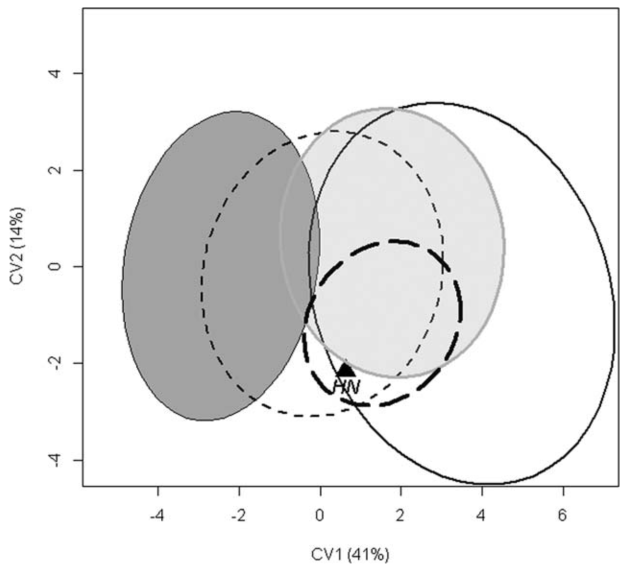

Fig. 2. Shape affinities of the Hoyo Negro specimen: discriminant analysis (CVA). First two canonical variables explaining 41% and 14% (respectively) of morphological variation. Individual scores plotted with 90% confidence ellipses for classifier "group" (see Table 1). AS plus ESK: dark gray filled ellipse; EOW: empty ellipse with black solid line; PA: light gray filled ellipse; NA: empty ellipse with dotted line; AUS: empty ellipse with long-dash line. Individual scores predictions for the HN5/48 skull are plotted as black triangle.

et al. (2004). Before being photographed, each specimen was oriented according to the Frankfurt plane, and the prosthion-inion line defining the sagittal plane was positioned orthogonal to the camera objective. Parallax (e.g., rainbow) effects were controlled by situating the skull in the centre so that its image did not extend into the distorted region of the visual field. Landmarks and semi-landmarks were digitized, scaled, and processed using TPSDig and TPSRelW software (Rohlf, 2003, 2004). Original configurations from all the series including the HN5/48 specimen were superimposed using the generalized procrustes analysis (GPA, Rohlf and Slice, 1990) to remove the effects of translation, rotation, and scaling. Sliding semi-landmarks placed along the contour of the cranial vault were relaxed following the minimum bending energy criterion using the TPSRelW routine (Rohlf, 2004). During superimposition, a measure of size is estimated as the *centroid size*, which is computed as the square root of the summed distances between each landmark coordinate and the centroid (mean x, y, z, landmark for the configuration). At the scaling step of the superimposition, all configurations are scaled to a *centroid size* equal to unit, thus size information is discarded (to be studied independently) and shape information is condensed in the aligned specimens' configurations. Thus, although images presented by Chatters et al. (2014) in Supporting Information lacks of a scaling factor, this doesn't avoid the analysis of pure shape. Since a size estimator cannot be derived by the materials published by Chatters et al., the allometry effects (shape variation that is related to size) cannot be studied. Previous work (Lahr and Wright, 1996; Rosas and Bastir, 2002) have revealed that there exist significant association between robusticity and cranial size in modern humans, by which the larger the size of the skull the greater the development of the cranial superstructures. However, we previously demonstrate that the analysis made after removal of allometric effects on Native American groups showed a very similar pattern of differences and affinities between groups (see Figs. 2 and 3 in González-José et al., 2008).

The aligned shape coordinates obtained after Procrustes superimposition were subjected to a standardization of sex on female values (female adjusted value = female value + [male average − female average]) to avoid the potential effect of sex. Sex-corrected data were imported into MorphoJ (Klingenberg, 2011) to perform a principal component analysis (PCA). Furthermore, principal component scores obtained in MorphoJ were brought to R (R Development Core Team, 2015) and used to run a discriminant analysis (CVA) using the known reference population samples as the classification criterion. To reduce the input number of variables for the discriminant analysis, we took only the first 11 principal components explaining 90% of the total variance in the sample. The discriminant function was then used to estimate the posterior probability (the probability based on our knowledge of the values of other variables that the respective case belongs to a particular group) for the representation of the HN5/48 individual. However, a known disadvantage of performing a CVA on shape coordinates is that geometric morphometric methods typically produce a large number of variables, and when the number of variables is close to the number of individuals, groups appear separated in a CVA plot even if they are samples from the same population (see Mitteroecker and Bookstein, 2011). Hence, as an alternative to CVA analysis we performed a between-group PCA (the projection of the data onto the principal components of the group averages (Mitteroecker and Bookstein, 2011) using MorphoJ (Klingenberg, 2011). Finally, as an alternative way to assess the morphological affinities of the Hoyo Negro skulll in relation to the reference samples, we computed Procrustes distances between individual Procrustes coordinates of HN5/48 and the population means using code written in R (R Development Core Team, 2015).

Additionally, we repeated all the analysis using frontal view of Hoyo Negro skull presented by Chatters et al. (2014) in Supporting Information Figure S8. However, because these lead to same conclusion as the analysis in lateral view of the skull, analysis and results corresponding to the frontal norm are presented as Supporting Information (for a list of series used in the frontal view analysis see Supporting Information Table S1; for a list of the landmarks digitized in frontal view see Supporting Information Table S2).

RESULTS

PCA analysis performed on the lateral views of Hoyo Negro and reference samples showed that, when considering a wide range of geographic (from Australia to southern South America) and chronological (from late Pleistocene specimens to modern series) variation, craniofacial phenotypes are not arranged into discrete units, as suggested by Chatters et al. (2014) and other authors using labels such as "Amerindian" or "Paleoamerican" to describe this complex pattern of variation. Conversely, the craniofacial variation was clearly arranged in a continuous spectrum of samples in both, lateral view (Fig. 1) and frontal view (Supporting Information Fig. 1) analyses. For example, ancient groups such as the Lagoa Santa series, or modern groups from Baja California or

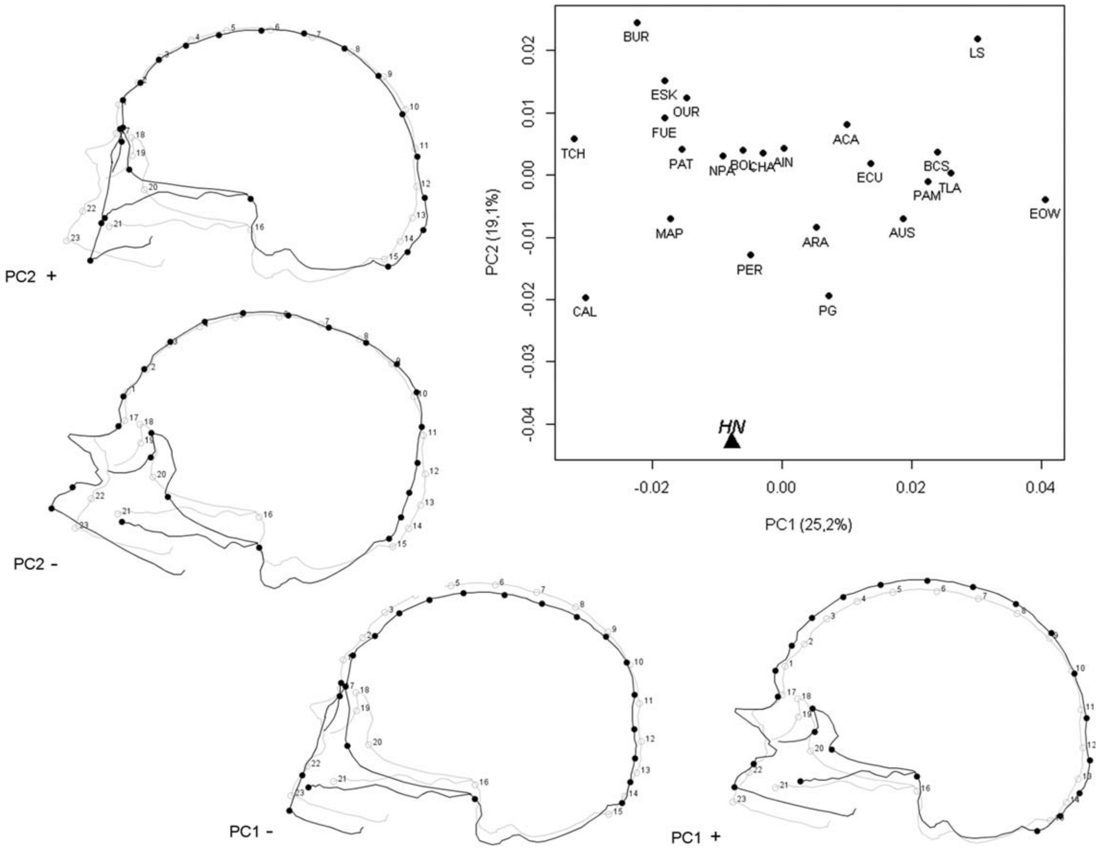

Fig. 3. Shape affinities of the Hoyo Negro specimen: Between-group PCA. Population scores across PC1 and PC2 together with Hoyo Negro individual scores (black triangle) obtained after a between PCA. The general shape in lateral view is represented by 23 landmarks and semilandmarks (see González-José et al., 2008, Fig. 1). Shape variation explained by the first principal component (PC1) is represented as a deformation of an outline drawing using the thin-plate spline function, depicting variation as displacements from consensus (light gray line) to the positive (right graph) and negative (left graph) extreme values (black lines). Note that shape changes occurring between landmarks 22 and 17 should not be taken into account since there are no landmarks or semilandmarks covering that region and capturing the shape of the nasal profile.

Tlatelolcans, were placed on an extreme of variation that is closely associated with early Old World specimens (Fig. 1). These samples are characterized by their low and projected faces, subnasal prognatism, long vaults, retracted zygomatics, and low noses (Fig. 1). Since Old World early specimens are placed in this extreme of variation, we could consider this shape as an ancestral, generalized craniofacial pattern ubiquitously distributed across the Old World during the Late Pleistocene (Lahr, 1996). Conversely, some Native American groups like Eskimos and Northeastern Asian groups show a derived morphological pattern characterized by high and flat retracted faces, short vaults, massive, anterior-projected and high zygomatics, and high noses (Fig. 1). The Hoyo Negro specimen fall well between both extremes (Fig. 1), thus suggesting that HN5/48 is an undifferentiated skull sharing affinities with some *Paleoamericans* but also with many Native Americans. See also an intermediate position of HN5/48 along PC1 in Supporting Information Fig. 1. Furthermore, HN5/48 occupies an extreme position along PC2 in frontal view (Supporting Information Fig. 1) with a greater orbital width, and a narrowing of the mid and lower face, characteristics that are shared with modern populations from South America, more than with old series like Lagoa Santa (or South *Paleoamericans*).

Results from discriminant analysis are presented in Table 2 and Figure 2 (Supporting Information Table 3, and Supporting Information Fig. 2 for analysis in frontal view). The lateral view discriminant analysis assigned the HN5/48 skull to a modern population from Northeastern Argentina (Chaco), whereas the corresponding frontal view analysis resulted in California as the nearest sample to Hoyo Negro. In both approaches, the next nearest sample corresponds to another modern group from Central Argentina (Araucanians). Figure 2 and Supporting Information Fig. 2 show the first two canonical variables obtained from discriminant analysis (with the individual scores predictions for the HN5/48 skull) performed on the lateral and frontal views, respectively. As in the PCA analyses, the CVA evaluation placed HN5/48 on an intermediate position among the generalized and derived extremes of skull variation. Note that, even when the discriminant analysis results would place the Hoyo Negro cranium closer to Chaco population from Argentina (Table 2), the shape affinities of the Hoyo Negro specimen as seen in the PCA plot (Figure 1) reveals similarities to other South American and Siberian mean population scores. Note however, that the discriminant analysis was performed on PC scores accounting for near 90% of the total variation in the sample, whereas Figure 1 only show the first two principal axes of variation, explaining just 25.2% and 19.1% (respectively) of the total variation. In such cases, lack of coincidence is an expected result. Whereas the first

TABLE 2. Discriminant analysis for series included in analysis of lateral view of the skull

POP	HN
CHA	0.6298
ARA	0.1601
NPA	0.1140
AUS	0.0552
PG	0.0227
EOW	0.0076
PER	0.0058
ECU	0.0034
BOL	0.0006
MAP	0.0004
FUE	0.000084
CAL	0.000081
LS	0.000079
TCH	0.000049
ESK	0.000022
BUR	0.000021
PAM	0.000021
TLA	0.000007
PAT	0.000005
BCS	0.000005
AIN	0.000005
OUR	0.000002
ACA	0.000001

Posterior probabilities for the Hoyo Negro (HN) skull in relation to the reference samples (POP) are shown in decreasing order. *Paleoamericans* series are remarked in shading gray.

TABLE 3. Procrustes Distances for series included in analysis of lateral view of the skull

POP	PD
CHA	0.0563
MAP	0.0564
AUS	0.0575
NPA	0.0587
PER	0.0609
FUE	0.0633
BOL	0.0656
TCH	0.0656
ECU	0.0656
ARA	0.0661
PAM	0.0674
CAL	0.0675
PG	0.0680
AIN	0.0686
BCS	0.0694
OUR	0.0699
ESK	0.0701
EOW	0.0703
ACA	0.0713
PAT	0.0715
BUR	0.0736
TLA	0.0766
LS	0.0810

Procrustes distances (PD) between the Hoyo Negro skull and population means of the reference samples (POP) are shown in increasing order.
Paleoamericans series are remarked in shading gray.

PCs are useful to depict the general patterns of shape variation, discriminant analyses are more powerful to assign the specimen under study to a reference sample, by means of posterior probabilities derived from almost 100% of variation.

Figure 3 and Supporting Information Fig. S3 show the between-group first two principal components depicting population average shape changes. As in the classical PCA presented above, between-group PC1 also depicts an ancestral-derived axis of variation in human's populations. Along these axes, HN5/48 individual has clearly an intermediate position. Furthermore, along the second (PC2) axis of variation (Fig. 3 and Supporting Information Fig. S3), the HN5/48 skull occupies a more extreme loading characterized by a prognatic lower face, a shortening of posterior neucranium and retracted and lower zygomatics (lateral view, Fig. 3) and a greater orbital width, and a narrowing of the mid and lower face (frontal view, Supporting Information Fig. S3). Intriguingly, HN5/48 skull shows some differentiation from the rest of the sample along PC2 both in lateral and frontal view and do not cluster with *Paleoamericans* (nor any other particular series). Since the HN specimen can be well far of his own population centroid, this can be an artefact of the between-group PCA analysis.

Finally, Table 3 and Supporting Information Table 4 show the Procrustes distances computed between HN5/48 individual against the population means of the reference sample. Again, the closest morphological relationship of HN5/48 skull is with a modern population from Northeastern Argentina (Chaco), followed by a modern population from north of South America (Maipure from Venezuela). In the frontal view-analyses, the greater affinity of Californians found by discriminate analysis (Supporting Information Table 3) is corroborated by the Procrustes distances analysis (Supporting Information Table 4). Note that, as well as in the case of posterior probabilities computed from discriminant function, the oldest series from the Americas (or *Paleoamericans*) occupy distant positions from HN5/48 skull (e.g., the 11th and 23rd position for PAM and LS, respectively, in Table 3).

DISCUSSION

Chatters et al. (2014) affirm that HN5/48 "is among the small group of Paleoamerican skeletons" and that "the oldest skeletal remains from the Americas consistently fail to group morphometrically with modern Native Americans…." However, the complete morphological description and discussion of the HN5/48 morphological affinities made by Chatters et al. (2014) is based on typological labels such as "Paleoamericans" or "Native Americans" thus frustrating any possibility of discussing the skull's characteristics on a population-based framework (e.g. considering within-group variability, distances, generalized versus derived traits, etc.). Then, they turn to typological labels to suggest a scenario in which "the differences in craniofacial form between Native Americans and their Paleoamerican predecessors are best explained as evolutionary changes that postdate the divergence of Beringians from their Siberian ancestors". Of course, some degree of *in situ* micro evolution of craniofacial shape is expected, but the use of discrete categories to explain the early, basal pattern of Paleo- and Native American variation disregards the fact that large portions of cranial shape variation observed in *Paleoamericans* significantly overlaps with some modern Native American populations (González-José et al., 2008; de Azevedo et al., 2011; Bortolini et al., 2014).

Analyses presented here, as well as other previous results (Ray et al., 2009; Achilli et al., 2013, Rasmussen et al., 2010, 2014; Raghavan et al., 2014a, 2014b) reinforce a model that we published earlier (González-José et al., 2008; de Azevedo et al., 2011; Bortolini et al., 2014). This models suggests that an initial major dispersal took place after the Last Glacial Maximum (21,000 years ago), when a founder population in Beringia was carrying Asian-derived Y-chromosome and mitochondrial lineages as well as high levels of within-group craniofacial heterogeneity. These results are of key importance in demonstrating that a generalized (non-derived) skull shape pattern was probably dispersed worldwide in Late Pleistocene-Early Holocene human populations (Lahr, 1996; González-José et al., 2008; de Azevedo et al., 2011; Bortolini et al., 2014).We also suggested that the biological and cultural characteristics of the first Americans that emerged, in part, during a standstill period in Beringia (first proposed in Bonatto and Salzano, 1997; see also Tamm et al., 2007; Mulligan et al., 2008; Kitchen, et al. 2008), were reshaped mainly in the Holocene by recurrent trans- Beringian/circum-Arctic gene flow and local population dynamics. This probably enabled the evolution of the extremely derived Arctic craniofacial pattern. Interestingly, a recent report on ancient and modern Arctic genomes (Raghavan et al., 2014b) revealed that a very strong population structure found in the past was gradually disrupted by gene flow, which is expected according to our model (González-José et al., 2008).

Since the publication of our Recurrent-Gene-Flow model (González-José et al., 2008), a wide array of evidence including Bayesian modeling of uniparental markers (Ray et al., 2009), mitogenomes (Achilli et al., 2013), ancient genomes (Rasmussen et al., 2010, 2014; Raghavan et al., 2014a, 2014b), and newly reported ancient skulls (Chatters et al., 2014) offered remarkable additional support. Evidence from linguistics, archaeology, and paleoecology appears to be fully compatible (González-José et al., 2008), but new approaches in ancient genomics of humans and paleoenvironment should also bring additional evidence to build a broader and consensual scenario for the first settlement of Americas.

CONCLUSION

Our analyses based on geometric morphometric methods and multivariate analyses performed on the lateral and frontal aspects of the Hoyo Negro skull indicate that it exhibits a generalized, non-derived morphology that resembles many other human remains from Late Pleistocene horizons. As observed in other *Paleoamerican* specimens, the HN5/48 occupies a region in the morphospace where some early remains and some modern Native Americans overlap, which in no way constitutes an anomaly, but a regular pattern already observed in several regions of the New World. As a whole, genomic and phenotypic information presented here and elsewhere support a model that includes an ancestral population presenting high levels of internal generalized phenotypic variation, and a standstill period in Beringia followed by recurrent trans-Beringian/circum-Arctic gene flow that reshaped the biological signature of Native Americans.

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