Investigating cranial morphological variation of early human skeletal remains from Chile: A 3D geometric morphometric approach

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Abstract

Objectives: Archaeological and genetic research has demonstrated that the Pacific Coast was a key route in the early colonization of South America. Research examining South American skeletons >8000 cal BP has revealed differences in cranial morphology between early and late Holocene populations, which may reflect distinct migration events and/or populations. However, genetic, cultural, and some skeletal data contradict this model. Given these discrepancies, this study examines ~9000 years of prehistory to test the hypothesis that Early skeletons have a distinct cranial morphology from later skeletons.

Materials and Methods: Using 3D digital models, craniofacial landmarks, and geometric morphometric analyses, we compared Early Holocene crania (n = 4) to later Chilean samples (n = 90) frequently absent in continental assessments of craniofacial variation. PCA, Mahalanobis distances, posterior and typicality probabilities were used to examine variation.

Results: Two of the earliest skeletons from northern Chile show clear affinities to individuals from later sites in the same region. However, the hypothesis cannot be rejected as one Early individual from northern Chile and one individual from inland Patagonia did not always show clear affinities to coastal populations.

Discussion: Biological affinities among northern populations and other regions of Chile align with genetic and archaeological data, supporting cultural and biological continuity along the Pacific Coast. In Patagonia, archaeological data are in accordance with skeletal differences between the Early inland steppe individual and coastal populations. This study incorporates 3D methods and skeletal datasets not widely used in assessments of biological affinity, thus contributing to a critical body of research examining the ancient population history of western South America.

Keywords
ancient human migration, bioarchaeology, Early American crania, South America
1 | INTRODUCTION

The colonization of the Americas has been the subject of intense scholarly debate for more than a century (Chatters, 2010; Chatters et al., 2014; Erlandson, Braje, and Graham, 2008, 2015; Goebel, Waters, and O’rourke, 2008; Hrdlička, 1907; Hrdlička et al., 1912; Hubbe, Neves, and Harvati, 2015; Jackson, Méndez, de Saint Pierre, Aspillaga, and Politis, 2015; Jantz and Owsley, 2001; Meltzer, 2009; Pedersen et al., 2016; Rasmussen et al., 2014; Stanford and Bradley, 2012). Several new discoveries have aided in a deeper understanding of American origins (Chatters et al., 2014; Raghavan et al., 2014; Rasmussen et al., 2015; Reich et al., 2012) and early cultural adaptations of North and South American populations (Rademaker et al., 2014; Strauss, Hubbe, Neves, Bernardo, and Atui, 2015), yet there remains much to be completed to further our understanding the population history and evolutionary adaptations of South America’s prehistoric populations. Archaeological excavations on the western side of the continent supports an early human presence along the coast, inland valleys, and highlands (Dillehay et al., 2008, 2012; Jackson, Méndez, Seguel, Malдонado, and Vargas, 2007; Jackson, Méndez, & Aspillaga, 2012; Méndez, 2015; Rademaker, Bromley, and Sandweiss, 2013; Reyes, Méndez, Mena, and Moraga, 2012; Ständen and Santoro, 2004; Stothert, 1985). This increased attention to the discovery and documentation of early western South American sites is due, at least in part, to evidence of an early and rapid human colonization event that occurred, possibly by boat, along the Pacific coast of the Americas during the initial peopling of the continents. This model gained credibility with data showing that an early maritime adaption occurred at least as early as 13,000 cal BP at sites along the Pacific coast, which predate several North American archaeological sites (Dillehay et al., 2008, 2012, 2015; Erlandson et al., 2008, Sandweiss et al. 1998). Archaeological discoveries in Patagonia (Reyes et al., 2012), the semi-arid coast of northern Chile (Jackson et al., 2015), and the Atacama Desert (Latorre et al., 2013) indicate that human populations inhabited the area during terminal Pleistocene and into the early Holocene period (Arriaza, Auferheide, & Muñoz, 1993; see also Rademaker et al., 2014).

While archaeological data from Chile and other regions of South America have provided strong support for an early human coastal presence, there is a dearth of direct evidence—namely, well-preserved human coprolites and complete skeletons—that date to the terminal-Pleistocene and early Holocene periods. Despite their scarcity, skeletons that are >8000 cal BP provide the only direct information about the biological composition of the continent’s first inhabitants. Much of the research focused on these early inhabitants of South America has been on skull morphology to address biological affinities of continental and extra-continental populations. Some assessments have revealed that early South Americans have a distinct cranial morphology that is more similar to skeletons outside South America (Hubbe, Neves, Licurgo do Amaral, and Guidon, 2007; Strauss et al., 2015), while others have argued that early and late South American Holocene cranial differences may be less pronounced, with some regional similarities (de Azevedo et al., 2011, 2015; González-José, Bortolini, Santos, & Bonatto, 2008; Kuzminsky, 2013; Kuzminsky, Coonerty, and Fehren-Schmitz, 2017; Manríquez et al., 2011; Menéndez et al., 2015).

There are numerous studies examining the earliest skeletons from the eastern regions of the continent (see Hubbe et al., 2007; Neves and Hubbe, 2005; Powell and Neves, 1999 for examples), and some that have focused on Argentina and the Pacific Coast (deAzevedo et al., 2011; Gonzalez-José et al., 2008; Kuzminsky, 2013; Kuzminsky et al., 2017; Manríquez et al., 2011; Menéndez et al., 2015). In Chile, fewer studies have assessed the Early skeletal series, and instead, much of the cranial research has addressed microevolution, the effects of climate, and regional variation of later-period prehistoric populations. The majority of this work has utilized traditional, nonmetric, or 2D assessments (Martí and Rothhammer, 1987; Menéndez et al., 2015; Rothhammer, Quevedo, Cocilovo, and Llop, 1984; Rothhammer and Silva, 1990; Sutter, 2005; Torres-Rouff, Knudson, & Hubbe, 2013; Varela, Cocilovo, Fuchs, and O’Brien, 2014). Although researchers continue to debate the number of migratory waves responsible for peopling the continent through assessments of cranial variation (von-Cramon-Taubadel et al., 2017), genetic data have shown that there is biological continuity over a 9000-year period in the Americas, with the initial dispersal having occurred along the Pacific coast (Chatters et al., 2014; Fagundes et al., 2008; Fehren-Schmitz et al., 2015; Skoglund et al., 2015; Tamm et al., 2007; see also Raghavan et al., 2015).

In the current study, we test the hypothesis that Early crania (>8000 cal BP) are distinct from later Holocene populations from the same region because it has been documented in other areas of the continent (Hubbe et al., 2015; Kuzminsky et al., 2017; Strauss et al., 2015). The alternative hypothesis is that there are similarities in craniofacial morphology among the Early skeletons and populations from the same region supporting cultural and genetic continuity that existed over several millennia in Chile, and western South America more broadly (Arriaza, Standen, Cassman, and Santoro, 2008; Fehren-Schmitz et al., 2015; Llamas et al., 2016; Standen and Santoro 2004). By utilizing 3D methods that have not been widely applied to questions of cranial variation in Chile, and a skeletal series that differs from most used in South American cranial assessments, our goal is to gain new insights into the population history of prehistoric Chilean populations.

2 | MATERIALS AND METHODS

Ninety-four crania from Chilean coast, inland valleys, and highland sites are included in this study (Figure 1 and Table 1). The skeletal series includes four adult skeletons that predate 8000 years BP (8800 cal BP, and referred to as Early crania) and 90 male and female adult crania dating from ~5000 years BP through the historic period. Chronological sequences vary greatly throughout western South America, but they are roughly divided into three time periods relevant to this study: the Early period (~11,000–3500 BP, 1300–3700 cal BP), categorized by small, semi-sedentary, or nomadic communities who engaged in hunting, gathering, and fishing food procurement activities in different proportions and intensities; the Middle or Formativ
period, which begins at ~3500 cal BP and is characterized by incipient agriculture, larger settled communities, and ceramic technology; and the Late period (postdating ~2000 BP, 2000 cal BP), with increased social complexity, large-scale architecture and agricultural communities in some regions (see Bruhns, 1994 for more detailed overviews of time periods). Incipient agriculture, relatively more sedentism, and animal domestication appear in the semiarid zone no earlier than 2000 BP, 2000 cal BP, while in the Patagonia region, these features were never present and the population remained as hunter-gatherers during the Holocene.

Because some of the collections used in this study have not been recently dated, date ranges are shown in radiocarbon and calibrated years BP. In addition to a diachronic sequence spanning ~9000 years of prehistory, the sample varies by region, ranging from the extreme
north of the Atacama Desert (the Acha, Morro 1, and Playa Miller 8 samples; 18° S), the highlands of the Atacama Desert (Coyo Oriente sample; 23° S), coastal Norte Chico, semi-arid desert region (Punta Teatinos, Guanaqueros, and La Herradura sample; 30° S), continental (Baño Nuevo 1; 45° S), and coastal Patagonia (Chonos archipelago [43–45° S] and the Patagonian general sample) (Figure 1).

The Acha skeletons were excavated from an area ~5.4 km from the coast in the extreme north of Chile in the Atacama Desert, and have been described as having a dolichocephalic cranial shape (Arriaza et al., 1993). Research suggests cultural continuity among the burials of Acha 2 and Acha 3, both of which appear to have relied heavily on marine subsistence. There have also been reported similarities between the earliest Acha sites and those from later Chinchorro archaeological sites, including the Morro 1 site, which is part of this skeletal series (Arriaza et al., 1993; Standen & Santoro, 2004).

The Baño Nuevo 1 skeleton (continental Patagonia) was excavated from a cave site in the Aysén region of southern Chile, and the cranium, although described as dolichocephalic, has also been characterized as also having morphological affinities to later-period Native American crania (Mena, Reyes, Stafford, & Southon, 2003). While it is not the earliest skeleton in Chile (Jackson et al., 2015), it is one of the few complete crania recovered from this region of South America. Investigations at Baño Nuevo 1 cave indicate that humans subsisted as broad-spectrum hunter-gatherers who relied on terrestrial hunting and C3 plants, and used the site as a transitory shelter while occupying the steppe plains (Méndez, Barberena, Reyes, & Nuevo Delaunay, 2014).

The comparative sample from the extreme north of Chile includes individuals from the site of Morro 1, a preceramic/preagricultural site located in the modern city of Arica. Approximate dates for this site place it within the Chinchorro cultural tradition (~5400–3700 BP, 6150–3990 cal BP), most commonly associated with elaborate mumification practices at Morro 1 and at several sites scattered along the coast of the Atacama Desert. Playa Miller 8 (4090 BP, 4500 cal BP), a coastal site, is also associated with the Chinchorro Tradition.
Paleopathological research suggests that the Chinchorro were afflicted with a variety of diseases more commonly found among sedentary groups. Dietary research suggests a heavy reliance on marine resources, with a smaller component of the diet composed of terrestrial resources.

The coastal sites of La Herradura, Guanaqueros, and Punta Teatinos date to the Early period (~4000–3000 BP, 4460–3120 cal BP) and were excavated in the Semi-arid North zone. This region lies adjacent to the Atacama Desert and shows slightly higher levels of precipitation. Archaeological research from excavations at these sites has produced artifacts and faunal remains associated with a marine economy with some supplemental vegetal resources incorporated into the diet (Schiappacasse and Niemeyer, 1986), though no stable isotope information is available for these groups.

The archaeological site of Coyo Oriente was excavated in the highlands of San Pedro de Atacama in northern Chile. Dating to the Middle Period (1310–1040 cal BP), it has been argued that the inhabitants lived in a socially-stratified society associated with agriculture, animal husbandry, textile production, and ceramic technology (Oakland, 1992). Analyses conducted on the skeletons and associated artifacts from the site have suggested that there were external influences from neighboring polities in Bolivia, northwest Argentina, and the Pacific coast (Costa, Liagostera, and Cocilovo, 2008). However, recent bioarchaeological investigations have shown that the influence of migrants from external polities (e.g., Tiwanaku) was not as significant during this period as initially thought (Knudson, 2007).

Complete crania from coastal Patagonia (southernmost Chile) are difficult to obtain owing to constant environmental fluctuations in sea levels, tectonic activities, and wet conditions that negatively impact the preservation of human bone at archaeological sites (Reyes et al., 2015). Despite these impediments, skeletal samples were obtained from two sources: the first is comprised of a skeletal series from recent excavations and surface collections conducted in the Chonos archipelago, and the second from a late-period (historic) collection housed in the American Museum of Natural History (AMNH). The Chonos sample dates from roughly 2030–770 years BP, 2300–150 cal BP, and is comprised of skeletons from several islands within the archipelago. Archaeological data (mainly stone tool and faunal assemblages) as well as chemical bone analyses indicate that precontact individuals had a diet and lifestyle heavily influenced by marine resources in the archipelago, with a small component of terrestrial resources supplementing it, while postcontact individuals had a more varied diet reflecting the introduction of new food sources following Spanish colonialism in the region (Reyes et al., 2015). The Patagonia sample obtained from AMNH has not been systematically dated, and although many of the collections housed in this museum lack good contextual data, the names written on the skull vaults indicate that they are also from late prehistoric and/or historic periods along the coast of western Patagonia.

Sex and age estimations were based on morphology, fusion of the sphen-occipital synchondrosis, cranial sutures, and dental eruption following the procedures outlined in Buikstra and Ubelaker (1994). Because the cultural practice of cranial vault modification (CVM) was prevalent throughout the prehistoric period in Chile, special care was taken to exclude any individuals with modified vaults, including those with minor modification on the frontal and lambdoidal region to avoid any effects that the modification could have on craniofacial assessments of biological affinity (Boston, Smith, Ubeda, Chandia, and Gonzalez, 2015; Rhode and Ariaza 2006; but see Varela et al., 2014). As such, the exclusion of intentional or nonintentional modifications hindered our ability to increase the skeletal sample in every region of Chile. To circumvent this issue, we pooled males and females for the analysis. A preliminary regression analysis of males and females detected a statistically-significant sex difference (3.71% of size on shape; \( p < .001 \) using 10,000 permutations) so a second analysis was conducted of the adult males to avoid any potential issues with sexual dimorphism and allometry (Jungers, Falsetti, and Wall, 1995). As potential shape differences between males and females could skew the results of our pooled analyses, we report both analyses, but place a heavier emphasis on results of the male sample for all of the tests conducted. The crania were scanned with a HD NextEngine laser scanner following the protocol outlined in Kuzminsky (2013) and Kuzminsky and Gardiner (2012). Crania were placed on a turntable connected to the scanner and laptop computer and a total of 18 individual scans for each cranium were merged together to form a complete digital model (Figure 2).

Locating complete, unmodified crania also presented challenges for the landmark selection and acquisition phase of the study. To maximize the sample, 16 landmarks for the face and vault were digitized for each cranium (Table 2). We based this choice on our ability to acquire landmarks and maximize the sample because many crania had broken cranial bases and parietals, thus hindering our ability to add neurocranial landmarks and ensure that the number of individuals was at least three to four times greater than the total number of landmarks to increase the statistical power of the analyses. The final selection of landmarks for the face and vault follow those outlined in Buikstra and Ubelaker (1994) and are similar to those used in previous studies (vonCramon-Taubadel, 2009). All crania were digitized twice using Stratovan Checkpoint Software. Using the replicates for the series of 94 crania, the effects of measurement error were tested with a Procrustes ANOVA (Supplement 1). Results showed that measurement error was minimal given that the mean squares (MS) for individual variation exceeded the Measurement Error. We followed analyses commonly used in studies that employ geometric morphometric approaches that analyze small datasets and larger comparative skeletal collections (Harvati and Hublin, 2012). A Generalized Procrustes Analysis (GPA) that superimposed the landmark configurations by eliminating variation in coordinate positions due to isometric size, orientation, and location of the original scan data was completed, followed by a principal components analysis (PCA) to examine the overall morphological variation among the skeletal series (Hubbe, Neves, and Harvati, 2010; Zelditch, Swiderski, and Sheets, 2012).

Morphological distances were computed with the Mahalanobis’ distance (Mahalanobis, 1936) and defined as the squared distances between two means divided by the pooled sample variance-covariance matrix and are shown as cluster dendrograms to visualize biological
affinities among the skeletal series. We use the Mahalanobis instead of Procrustes distance because there is a heavier emphasis on facial rather than neurocranial landmarks for the skeletal series, which accounts for the covariance between variables and does not give undue weight to regions of the cranium that have a greater number of landmarks. In this way, the Mahalanobis distance ensures that the cranial vault landmarks are weighted into the final distance, despite being fewer in number. Moreover, repeated analyses using both the Procrustes and Mahalanobis distances did not show significant differences in the results, which was supported by a Mantel test that showed high correlation values \( r = .89 \) for the pooled sample, and \( r = .62 \) for males. The posterior probability assignment was used to assign Early crania to the rest of the skeletal series. Probabilities with .70 or higher suggest that they are likely to be members of the group to which they are assigned. Typical-\( \text{ities were used to measure the likelihood than an individual belongs to any of the groups represented in the reference samples, when assum-} \)

\[ \text{ing that the original individual's group may not be part of the analysis (Ousley and Jantz, 2005). Finally, classification matrices were produced to examine the affinities among the comparative sample. All analyses were conducted using MorphoJ (Klingenberg, 2011) and R software} \]

**TABLE 2** Cranial landmarks used in the study

<table>
<thead>
<tr>
<th>Landmark</th>
<th>Paired/unpaired</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial landmarks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Nasion</td>
<td>Midline</td>
<td>Midline intersection of the frontonasal suture and mid-sagittal plane.</td>
</tr>
<tr>
<td>(2,3) Alare</td>
<td>Left, right</td>
<td>Instrumentally determined as the most lateral points on the nasal aperture in a transverse plane.</td>
</tr>
<tr>
<td>(4,5) Zygoorbitale</td>
<td>Left, right</td>
<td>Eye Orbit: point of intersection between zygomatic suture and orbit border.</td>
</tr>
<tr>
<td>(6,7) Upper orbital border</td>
<td>Left, right</td>
<td>Upper border of the Eye Orbit: measured as the maximum height from the upper to the lower orbital borders perpendicular to the horizontal axis of the orbit and using the middle and inferior border as a fixed point.</td>
</tr>
<tr>
<td>(8,9) Ectoconchion</td>
<td>Left, right</td>
<td>Eye Orbit: intersection of the most anterior surface of lateral border and imaginary horizontal line bisecting the orbit. Determined by making a length primitive to divide the eye orbit into two equal halves, then placed landmark point at the midpoint bisecting the orbit.</td>
</tr>
<tr>
<td>(10,11) Zygomaixillare</td>
<td>Left, right</td>
<td>Intersection of the zygomaixillary suture and most medial masseter muscle attachment.</td>
</tr>
<tr>
<td>Vault landmarks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12) Glabella</td>
<td>Midline</td>
<td>Most projecting midline point on the frontal bone above the frontonasal suture.</td>
</tr>
<tr>
<td>(13) Lambda</td>
<td>Midline</td>
<td>Point where the sagittal and lambdoidal sutures meet.</td>
</tr>
<tr>
<td>(14) Bregma</td>
<td>Midline</td>
<td>Point where the sagittal and coronal sutures intersect.</td>
</tr>
<tr>
<td>(15, 16) Asterion</td>
<td>Left, right</td>
<td>Intersection of parietal, temporal, and occipital bones.</td>
</tr>
</tbody>
</table>
with the Geomorph statistical package (Adams, Collyer, Kaliontzopoulou, & Sherratt, 2016; R Core Team, 2014).

3 | RESULTS

Figure 3a displays the shape changes associated with the first two principal components of the PCA with wireframes for the extreme positive and negative values for each PC for the pooled sample (Figure 3b). Shown in frontal and lateral views, shape differences occur at varying degrees for the face and vault along both axes. PC1 shows slightly greater changes occurring on the vault at lambda and the left and right asterion landmarks, respectively. PC 2 shows greater shape changes on the cranial vault at lambda and asterion, and at the eye orbits and the zygomatic region of the face.

Figure 4a shows the shape changes associated with males in the PCA scatterplot (Figure 4b). Similar to the shape changes shown for the pooled sample, landmark shifts occur at the vault and face on the first PC, primarily with lambda and bregma on the cranial vault, and the upper and lower eye orbits on the face. Positive and negative changes
on PC 2 show somewhat greater changes on the face, and particularly at landmarks corresponding to the eye orbits and zygomatics, but changes at asterion on the vault are also evident.

Figure 3b shows the PCA scatterplot for the first two principal components, which comprises ~35% of the total variation in the sample. The distribution of the complete skeletal series is slightly skewed toward the positive sides of each axis. All four of the Early crania (three from Acha, and one individual from the Baño Nuevo 1 site) fall within the general range of variation among the sample. The Chonos series from southern Chile occupies a central position on the positive sides of PC1 and PC2, while the individuals from the site of Coyo Oriente (northern Chile highlands) occupy both positive and negative sides of the PCs, respectively. Individuals from the sites of Guanaqueros and La Herradura are evenly distributed along the first axis, but they occupy only the negative side of PC2, while the Patagonia, Punta Teatinos, and Playa Miller 8 samples show variation along both axes.

Figure 4b shows the PCA of the males in the skeletal series, which comprises about 34% of the variation in the sample. The Early individuals fall on the negative side of PC1, while the Chonos sample from Patagonia has a somewhat central position on the positive sides of PC1 and PC2.
both axes. Males from the site of Coyo Oriente are primarily located on the positive side of the second axis, while individuals from the Guanaqueros, La Herradura, and Morro 1 sites are distributed along positive and negative sides of both axes. The Patagonia samples also occupy space on both positive and negative sides of the axes. The two individuals from the site of Punta Teatinos fall on the positive side of PC2.

Figures 5 and 6 show the cluster dendrograms derived from the Mahalanobis distances for the pooled sample of both sexes, as well as the sample comprised only of males. As the results indicate, there are biological affinities between the Morro 1 (Chinchorro) sample and the Early skeletons from the Acha site located in the northernmost Atacama Desert of Chile. Punta Teatinos, Guanaqueros, and La Herradura, all from coastal semi-arid northern Chile are clustered together with Playa Miller 8 in coastal Atacama, with coastal Chonos showing closer affinities to the Guanaqueros and La Herradura groups from the semi-arid coast. Coyo Oriente and Patagonia are the last to be added to this cluster, while the Early Baño Nuevo 1 individual does not fit into any of the clusters and is the last to be added to the dendrogram.

When the pooled sample is compared with the dendrogram of males (Figure 6), there is a shift in the positions of the Early Acha 3.2 and Baño Nuevo 1 individuals who now form a separate cluster. Morro 1 and two of the Early Acha individuals form an additional cluster, while the remaining groups that include coastal Chonos, Punta Teatinos, and the Guanaqueros and La Herradura samples show closer affinities to the Coyo Oriente and Patagonia samples. Mahalanobis distances and the associated p values are included in the Supporting Information (Supplement 2).

Table 3 shows the results of the posterior and typicality probabilities, according to the discriminant function analysis used to classify the Early individuals into the closest comparative series. Two tests were conducted with the pooled sample of males and females, and a second with only the males. As shown in the pooled sample, Acha 2 and Acha 3.1 were assigned to the Morro 1 group with high probabilities, exceeding .70. The Acha 3.2 individual was assigned to Patagonia, while the Baño Nuevo 1 individual shows its highest probability assignment to Morro 1. The typicalities for the pooled sample suggest that Acha 2 and Acha 3.2 are too different to belong to any reference group, and thus would likely be considered outliers within the skeletal series. When the sample of males is considered, the Acha 2 and Acha 3.1 individuals are again assigned to the Morro group with high probabilities, while Acha 3.2 was assigned to the Chonos coastal population and Baño Nuevo 1 was assigned to the Coyo Oriente sample from the Atacama highlands. The typicalities for all the early male individuals again suggest that they are outliers with respect to the reference series, supporting the hypothesis that Early crania are distinct from the later period series. For the comparative sample (excluding all four of the Early skeletons), classification matrices (Tables 4 and 5) show large drops in correct classifications using the leave-one-out cross-validation method.

4 | DISCUSSION

The Early Holocene skeletons in this study have been characterized as having dolichocephalic cranial morphology, which, combined with the typicality results presented here, suggest that Early crania are different enough to be considered outliers with respect to the comparative sample (Neves and Hubbe, 2005). These results offer support for the hypothesis tested, which states that Early crania are distinct from crania that date to later time periods. What makes this less definitive, however, are the results of the remaining analyses conducted, all of which support the alternative hypothesis, showing high biological affinities among several Early and late period samples from Chile (Arriaza et al., 1993; Kuzminsky et al., 2017; Manríquez et al., 2011; Mena et al., 2003; Neves and Hubbe, 2005). The PCAs did not show any of the Early crania as being divergent from the comparative skeletal series overall, and instead, they fell within the general range of variation.
among the sample in the morphospace. While the cluster dendrograms showed that the Early individuals more or less fall outside the general range of variation for the comparative sample, Early Acha individuals show clear affinities to the Morro 1 group from the same region. The posterior probability assignments, which were performed based on genetic data that suggest all prehistoric populations in the Americas were derived from the same source population and most likely share a biological relationship to later prehistoric populations (Rasmussen et al., 2015), results showed that they have a high likelihood of having affinities to other coastal groups. The close affinities between the Acha individuals and Morro 1 (Chinchorro) groups are consistent with bioarchaeological data, which indicate that similar cultural and dietary practices occurred for several millennia in the same region (Arriaza et al., 2008; Aufderheide, Muñoz, & Arriaza, 1993; Poulson et al., 2013; Watson, Arriaza, Standen, & Muñoz Ovalle, 2013). Further, genetic continuity (Llamas et al., 2016; Manríquez et al., 2011; Moraga et al., 2005), along with the spread of Chinchorro cultural features, such as elaborate mummification practices, have been well documented along the coast of northern Chile (Arriaza, 1995; Standen 1997; Standen & Santoro, 2004). Researchers have argued that cultural practices at Acha were likely precursors to the social activities that flourished a few thousand years later (Arriaza, 1995; Standen & Santoro, 2004). Therefore, cranial morphological similarities in the extreme north of Chile could be attributed to low genetic diversity, cultural continuity, and limited population movement in this region. Such results align with earlier work by Cocilovo, Varela, Espoueys, and Standen (2001) who found closer affinities between preceramic period coastal groups than those from the later time sequences, indicating that increased gene flow into the northern Chile region may have only begun with immigration from the Andean highlands during the Formative Period.

### Table 3 Closest group assignments for the Early crania and their associated probabilities, according to the multivariate discriminant function analysis with the pooled males and females (left) and the male sample (right)

<table>
<thead>
<tr>
<th>Pooled sample</th>
<th>Group classification</th>
<th>Posterior probability</th>
<th>Typicality</th>
<th>Males</th>
<th>Group classification</th>
<th>Posterior probability</th>
<th>Typicality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acha 2</td>
<td>Morro</td>
<td>0.768</td>
<td>0.009</td>
<td></td>
<td>Morro</td>
<td>0.999</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acha 3.1</td>
<td>Morro</td>
<td>1.000</td>
<td>0.415</td>
<td></td>
<td>Morro</td>
<td>0.815</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acha 3.2</td>
<td>Patagonia</td>
<td>0.930</td>
<td>0.035</td>
<td></td>
<td>Chonos</td>
<td>1.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baño Nuevo</td>
<td>Morro</td>
<td>0.748</td>
<td>0.126</td>
<td></td>
<td>Coyo Oriente</td>
<td>1.000</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 4 Classifications and leave-one-out cross validation showing the assignments for the pooled comparative sample groups

<table>
<thead>
<tr>
<th>Classification Matrix</th>
<th>Chonos</th>
<th>Coyo Oriente</th>
<th>Guanaqueros</th>
<th>Herradura</th>
<th>Morro</th>
<th>Patagonia</th>
<th>PM 8</th>
<th>Pt Teatinos</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chonos</td>
<td>100% (12)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (12)</td>
</tr>
<tr>
<td>Coyo Oriente</td>
<td>0% (0)</td>
<td>100% (18)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (18)</td>
</tr>
<tr>
<td>Guanaqueros</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (6)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Herradura</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (21)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (21)</td>
</tr>
<tr>
<td>Morro</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (10)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (10)</td>
</tr>
<tr>
<td>Patagonia</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (18)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (18)</td>
</tr>
<tr>
<td>PM 8</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (3)</td>
<td>0% (0)</td>
<td>100% (3)</td>
</tr>
<tr>
<td>Pt Teatinos</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (2)</td>
<td>100% (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leave-one-out cross validation</th>
<th>Chonos</th>
<th>Coyo Oriente</th>
<th>Guanaqueros</th>
<th>Herradura</th>
<th>Morro</th>
<th>Patagonia</th>
<th>PM 8</th>
<th>Pt Teatinos</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chonos</td>
<td>50% (6)</td>
<td>16.7% (2)</td>
<td>0% (0)</td>
<td>16.7% (2)</td>
<td>8.3% (1)</td>
<td>8.3% (1)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (12)</td>
</tr>
<tr>
<td>Coyo Oriente</td>
<td>11.1% (2)</td>
<td>55.6% (10)</td>
<td>0% (0)</td>
<td>5.6% (1)</td>
<td>0% (0)</td>
<td>27.8% (5)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (18)</td>
</tr>
<tr>
<td>Guanaqueros</td>
<td>16.7% (1)</td>
<td>0% (0)</td>
<td>16.7% (1)</td>
<td>66.7% (4)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Herradura</td>
<td>14.3% (3)</td>
<td>4.8% (1)</td>
<td>14.3% (3)</td>
<td>52.4% (11)</td>
<td>4.8% (1)</td>
<td>0% (0)</td>
<td>9.5% (2)</td>
<td>0% (0)</td>
<td>100% (21)</td>
</tr>
<tr>
<td>Morro</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>10% (1)</td>
<td>50% (5)</td>
<td>30% (3)</td>
<td>10% (1)</td>
<td>0% (0)</td>
<td>100% (10)</td>
</tr>
<tr>
<td>Patagonia</td>
<td>0% (0)</td>
<td>16.7% (3)</td>
<td>0% (0)</td>
<td>5.6% (1)</td>
<td>11.1% (2)</td>
<td>61.1% (11)</td>
<td>5.6% (1)</td>
<td>0% (0)</td>
<td>100% (18)</td>
</tr>
<tr>
<td>PM 8</td>
<td>33.3% (1)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>33.3% (1)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>33.3% (1)</td>
<td>100% (3)</td>
<td>100% (3)</td>
</tr>
<tr>
<td>Pt Teatinos</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>50% (1)</td>
<td>50% (1)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>100% (2)</td>
</tr>
</tbody>
</table>
The biological affinities between the Baño Nuevo 1 individual and later populations are more difficult to interpret. Based on the Mahalanobis cluster dendrograms, the Baño Nuevo 1 individual is the most distant of all those included in the sample, contrasting assessments that suggested this individual has some morphological similarities to later Native American populations (Mena et al., 2003). The posterior probabilities assigned this Early individual to the Morro 1 (Chinchorro) and the Coyo Oriente groups, suggesting some affinities to coastal and highland populations from later time periods. Its dissimilarity to the geographically and temporally closest group in the same region, Chonos, is intriguing. Archaeological research has demonstrated clear subsistence and cultural differences between the coastal Chonos groups of Patagonia and the highlands aligns with some genetic research, which has shown that the South-Central Andean populations (Coyo Oriente) have a biological relationship to Patagonians (García-Bour et al., 2004).

The variation among the later period samples analyzed in this study can be interpreted in varying ways. The close affinities between the Patagonians and Coyo Oriente sample from the Atacama Desert highlands aligns with some genetic research, which has shown that the close affinities between the Guanaqueros, Punta Teatinos, and La Herradura skeletal series is not surprising, as they were excavated from adjacent archaeological deposits separated by ~20 km from each other. The poor classification results (Tables 4 and 5) show that differences between coastal and highland samples from Chile, irrespective of time, are not strongly defined. Such affinities may be explained by an increase in gene flow and extensive trade networks within and between these populations during the middle and late periods. Based on the biological affinities between the Baño Nuevo 1 individual and other populations, it is difficult to make further inferences about the biological affinities of Early individuals from this region of Chile.

Although we predicted that Playa Miller 8 individuals from northern Chile would show closer affinities to the Morro 1 (Chinchorro) samples given that they are temporally and geographically closer to each other, Playa Miller 8 showed closer affinities to individuals from the Norte Chico region, which is located ~1300 km south along the coast. This may be explained by a closer genetic relationship and gene flow among the coastal populations western South America, more generally (Llamas et al., 2016). The close affinities between the Guanaqueros, Punta Teatinos, and La Herradura skeletal series is not surprising, as they were excavated from adjacent archaeological deposits separated by ~20 km from each other. The poor classification results (Tables 4 and 5) show that differences between coastal and highland samples from Chile, irrespective of time, are not strongly defined. Such affinities may be explained by an increase in gene flow and extensive trade networks within and between these populations during the middle and late periods.
5 | CONCLUSION

The assessments that we have conducted with 3D morphometric data suggest that the earliest crania from Chile, when compared to later populations, tend to fall within the general range of variation among the skeletal series. These results are not definitive, however, as the typicalities and cluster dendrograms we performed in this study suggest that the earliest skeletons of Chile are outliers when compared to several samples from later time periods. Nonetheless, these results differ from studies conducted in other regions of South America, which have tended to show clear differences between the early and late Holocene populations (Delgado, 2016; Neves and Hubbe, 2005). Our results may be partially explained by the choice of sample and analytical methods we used that differ from others conducted elsewhere in South America (also see de Azevedo, Quinto-Sanchez, Paschetta, and Gonzalez José, 2015 for a discussion of datasets). The close affinities between the Acha individuals and the later Chinchorro groups in northern Chile align with archaeological data that suggest cultural and dietary continuity occurred over several millennia. Moreover, genetic data have shown that all western South American populations, including prehistoric groups in Chile, descended from the same source population, and aside from episodes of isolation and gene flow, there was no major external contribution to the gene pool over a 9000-year period (Arriaza et al., 1993; Fehren-Schmitz et al., 2015; Kuzminsky et al. 2017; Llamas et al., 2016).

Given the extreme likelihood that there was an early and rapid Pacific coastal migration along South America, with several of the earliest reported archaeological sites, such as Monte Verde located in southern Chile (Dillehay et al., 2008, 2015), our study offers further insight into the biological affinities among the earliest coastal inhabitants and their descendants in Chile. Although debates focused on migration(s), biological affinities, and microevolutionary processes that may have led to differences in South American cranial morphology will likely continue, this study is unique in that it utilizes archaeological data and a skeletal series that have not been commonly applied to questions of craniofacial variation on a continental scale. Through the integration of 3D geometric morphometric methods and skeletal samples from western South America, this study contributes to an evergrowing body of research that is focused on understanding the population history of prehistoric South Americans through archaeology, biological anthropology, and the Earth Sciences.

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