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Taxonomic differences in deciduous lower first molar crown outlines of *Homo sapiens* and *Homo neanderthalensis*

SE Bailey[1,2],R Sorrentino[3,5], G Mancuso[5], J-J Hublin[2,3], S Benazzi[5,2]

[1]Department of Anthropology, New York University, New York USA, [2] Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, GERMANY, [3]Collège de France, 70005 Paris, France, [4]Department of Biological Geological and Environmental Sciences – BiGeA, University of Bologna, Bologna, ITALY, [5]Deparment of Cultural Heritage, University of Bologna, Ravenna, ITALY

Corresponding author: Shara Bailey, Department of Anthropology, New York University, 25 Waverly Place, New York, NY 10003 sbailey@nyu.edu

1 **Abstract**

2

3   Recent studies have demonstrated that the outline shapes of deciduous upper and lower

4 second molars and the deciduous upper first molar are useful for diagnosing hominin taxa –

5 especially *Homo neanderthalensis* and *H. sapiens*. Building on these studies, we use geometric

6 morphometric methods to assess the taxonomic significance of the crown outline of the lower

7 first deciduous molar (dm1). We test whether the crown shape of the dm1 distinguishes *H.*

8 *neanderthalensis* from *H. sapiens* and explore whether dm1 crown shape can be used to

9 accurately assign individuals to taxa. Our fossil sample includes 3 early *H. sapiens*, 7 Upper

10 Paleolithic *H. sapiens* and 13 *H. neanderthalensis* individuals. Our recent human sample

11 includes 103 individuals from Africa, Australia, Europe, South America and South Asia. Our

12 results indicate that *H. neanderthalensis* dm1s cluster fairly tightly and separate well from those

13 of Upper Paleolithic *H. sapiens*. However, we also found that the range of shapes in the recent

14 human sample completely overlaps the ranges of all fossil samples. Consequently, results of the

15 quadratic discriminant analysis based on the first 8 PCs representing more than 90% of the

16 variation were mixed. Lower dm1s were correctly classified in 87.3% of the individuals: the

17 combined *H. sapiens* sample had greater success (90.2%) in assigning individuals than did the *H.*

18 *neanderthalensis* sample (61.5%). When the analysis was run removing the highly variable

19 recent human sample, accuracy increased to 84.6% for *H. neanderthalensis* and 57.1% of Upper

20 Paleolithic *H. sapiens* were classified correctly by using the first four PCs (70.3%). We conclude

21 that caution is warranted when assigning isolated dm1 crowns to taxa: while an assignment to *H.*

22 *neanderthalensis* has a high probability of being correct, assignment to Upper Paleolithic *H.*

23 *sapiens* is less certain.

24

Key Words: *Homo sapiens*, Neanderthals, Tooth shape, Deciduous molars, Geometric morphometrics

1. Introduction

Before we can test evolutionary hypotheses explaining patterns in, and distribution of, morphological variation in our fossil relatives we must first be able to accurately identify hominin species from a fragmentary fossil record. Recent studies have demonstrated this need by showing the importance of accurately associating a culture with the species that made it (Benazzi et al., 2011a; Benazzi et al., 2015). Correctly identifying isolated dental remains has also shed important light on the timing of dispersals of our species (Benazzi et al., 2011b). The ability to accurately assign isolated skeletal and dental elements to taxa may also result in larger fossil sample sizes, which provide greater power to statistical tests aimed as testing the significance of differences among taxa.

Skeletons recovered from the Late Pleistocene, especially during the European Upper Paleolithic, are often incomplete and fragmentary (Churchill and Smith, 2000). Complicating matters is the fact that fragmentary skeletal elements often are morphologically undiagnostic and may be unusable unless they preserve ancient hominin DNA. Dental elements, on the other hand, are more frequently recovered and, due to their durable enamel, are often complete.

Although tooth size alone is not very informative for diagnosing Late Pleistocene taxa (Bailey and Hublin, 2005), tooth crown and root morphology has proven to be quite useful, especially in distinguishing *Homo neanderthalensis* (hereafter: Neanderthals) from *H. sapiens* during the periods in which they overlapped in time and space (Bailey et al., 2009; Been et al., 2017; Benazzi et al., 2011b, 2014; Fabbri et al., 2016; Hublin et al., 2020; Kupczik and Hublin, 2010; Le Cabec et al., 2013 ; Margherita et al., 2016). When complete dentitions are found and

49 crowns are relatively unworn, assigning specimens to taxa is fairly straightforward because

50 Neanderthals have diagnostic combinations of dental characters (Bailey, 2002a; 2002b, 2006).

51 Even incomplete dentitions can be diagnostic if the appropriate teeth and/or characters are

52 preserved (Bailey et al., 2009). However, while many tooth crowns are found complete, they

53 often suffer from wear that obscures or eliminates minor morphological features on the crown

54 (e.g., occlusal crests and small accessory cusps).

55   Early studies of molar crown shape relied on the position of, and relationships between,

56 cusp tips, which required relatively unworn teeth (Bailey, 2004; Morris, 1981). More recently,

57 methods of assessing crown shape (e.g., Elliptical Fourier Analysis - EFA, semi-landmark-based

58 methods) from crown outlines have allowed for the inclusion of both worn and unworn molar

59 crowns in analyses (Benazzi et al., 2012). Studies using these methods have shown that crown

60 outlies of permanent molars are quite useful for partitioning out variation and assigning

61 specimens to taxa (Bailey and Lynch, 2005; Benazzi et al., 2011a; Gómez-Robles et al., 2007;

62 Gómez-Robles et al., 2008, 2011).

63   The small size and thin enamel of deciduous molars make them especially prone to loss

64 of surface information through attrition, especially in paleoanthropological and archaeological

65 samples that predate the advent of processed food. For this reason, the crown outline is

66 particularly useful for assessing shape differences among groups. Over the past decade several

67 studies have confirmed that the outlines of postcanine deciduous crowns can be used to

68 accurately assign individuals to taxa (Bailey et al., 2014b, 2016; Fornai et al., 2016; Moroni et

69 al., 2018a).

70   In hominins, the deciduous second molar (dm2 or dp4[1]) is remarkably similar to the

71   permanent first molar (M1) in both crown outline and morphology (Fig. 1A). While about 15%

72   smaller in size than the M1 (Bailey et al., 2014a), within individuals the dm2 preserves the same

73   number of primary cusps; and the number and expression of accessory features are highly

74   correlated between the two (Edgar and Lease, 2007; Kieser, 1984; Paul et al., 2017). Because the

75   dm2 forms early during ontogeny (Liversidge and Molleson, 2004) it is presumed to be little

76   influenced by environmental variation. Moreover, studies have shown it to be less variable in

77   size and morphology than the deciduous first molar (Farmer and Townsend, 1993; Liversidge

78   and Molleson, 1999; Margetts and Brown, 1978). Thus, it is perhaps not surprising that just like

79   the M1, the dm2 has proven to discriminate between Neanderthals and *H. sapiens* quite well

80   (Bailey et al., 2014a, 2015; Benazzi, 2012; Moroni et al., 2018a).

81   In contrast to the dm2, the dm1 can be more premolar-like than molar-like in form, at

82   least in later *Homo* (Fig. 1B). The dm1 often preserves fewer cusps, with the distal aspects of

83   both upper and lower dm1 reduced compared to the dm2. The dm[1] may even be bicuspid

84   (preserving only mesial cusps) in some *H. sapiens* groups. Like the dm[1], the distal cusps of the

85   dm1 may be completely missing, preserving only the protoconid and metaconid. This variation in

86   cusp number and expression is reflected in the crown's shape.

87

88   [FIGURE 1A and 1B ABOUT HERE]

89

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[1] Here we follow terminology in the dental anthropological literature, which refers to this tooth as a molar. We are aware that in the paleontological literature this tooth is referred to as a premolar.

90   An earlier study of dm^1 shape of Neanderthals and *H. sapiens* resulted in 96.3% accuracy

91   in separating the two groups (Benazzi et al., 2011b). The current study builds on our previous

92   studies of the diagnostic utility of deciduous molar shape for taxonomic affiliation by examining

93   variation of the dm$_1$ (dp$_3$). We analyze the crown shapes of Neanderthals and early, Upper

94   Paleolithic and recent *H. sapiens,* applying geometric morphometric (GM) methods to crown

95   outlines taken from digital occlusal images. Based on our previous research, we expect that the

96   dm$_1$ will distinguish Neanderthals from *H. sapiens* with a high degree of accuracy (80% or

97   higher). Based on results of our earlier study showing that the dm$_2$ and M$_1$ were slightly less

98   diagnostic than the dm^2 and M^1 (Bailey et al., 2016), we expect this may also to be the case for

99   the dm$_1$. The ability of the dm$_1$ to discriminate among taxa will rely, at least in part, on the

100   amount of variation within each group. At a broader level, knowing the degree of variability

101   within groups may allow us to test hypotheses about the evolutionary forces, or the relaxation of

102   such forces, driving this variation.

103   If the dm$_1$ crown outline proves to discriminate well between Neanderthals and *H.*

104   *sapiens*, it will add to the tools available for assessing isolated teeth and assigning them to fossil

105   taxa. If, unlike the dm^1 (Benazzi et al., 2011b), the dm$_1$ crown outline cannot accurately assign

106   teeth to taxa, future work will focus on exploring the possible reasons why the lower molars are

107   less distinctive than the upper molars.

108

109   **2.0 Materials**

110   *2.1. Samples*

111   The materials used in this study include occlusal photographs of dm$_1$s from 126 recent

112   and fossil *H. sapiens* and Neanderthals (Table 1). Our recent *H. sapiens* (RHS) sample includes

113   103 individuals representing Africa, Australia, Europe, South America and South Asia.

114   Deciduous teeth are scarce in the fossil record and our comparative fossil sample, while small,

115   includes nearly all relevant fossil dm₁s available for study: 3 early *H. sapiens* (EHS), 7 Upper

116   Paleolithic *H. sapiens* (UPHS) and 13 Neanderthals. We assigned specimens to taxa based on

117   assignments made in the published literature. These assignments were based on a combination of

118   criteria including: cranial morphology, age, cultural association, and/or their association with

119   taxonomically diagnostic adult human remains.

120   We included only complete and undamaged crowns in our samples. With one exception

121   (Die Kelders 6291), these crowns ranged in status from unworn to moderately worn (three or

122   more small dentine patches, stages 1–4; Molnar, 1971). Figure 2 illustrates the single crown with

123   stage 5 wear (see Methods below for how worn outlines were reconstructed). Even in moderatly

124   worn crowns it was primarily the distal aspect that required correction.We did not consider sex

125   as a variable in this study due to the difficulty in assigning sex to fossil individuals, especially

126   those represented by isolated teeth.

127

128   [TABLE 1 ABOUT HERE]

129

130   We arbitrarily chose to use the left dm₁ to represent each individual. If the left side was

131   not represented or was damaged, we used the right side and mirror-imaged the crown using

132   Adobe PhotoShop® before the analysis. Although the left and right sides may be asymmetrical

133   in size and/or shape, studies have shown that dental asymmetry occurs randomly with regard to

134   side. This phenomenon is known as fluctuating asymmetry (Van Valen, 1962). To date we know

135   of no study quantifying the differences in crown shapes between left and right antimeres.

136 However, we assume that crown shape asymmetry is randomly distributed — as it is for tooth

137 size and dental nonmetric traits, which influence crown shape (see Scott and Turner, 1997 for

138 review).

139

140 *2.2. Methods of data collection and analysis*

141   All but seven occlusal images were taken using a Canon EOS Rebel XT digital 8 MP

142 camera equipped with a macro lens (see Supplementary Online Material [SOM] Table S1). All

143 images were taken from original skeletal and fossil materials (i.e., no casts were used).

144 Photographic images of the fossils were taken by SEB. Some images of recent humans

145 (primarily the African samples) were taken by Caroline Souday (see acknowledgements) under

146 the supervision of SEB. Individual teeth were oriented so that the cervical border was

147 perpendicular to the camera's optical axis. A bubble device was used to level the camera and

148 each image included a similarly leveled millimeter scale that was placed at approximately the

149 same height as the cusp tips. Bailey et al. (2004) have shown that inter-observer error due to

150 differences in image orientation and camera equipment is low (2.4%–4.5%) and not significantly

151 greater than intra-observer error.

152   In seven cases (SOM Table S1) occlusal images were acquired from

153 microtomographic (μCT) image data of original specimens performed by the Department of

154 Human Evolution of the Max Planck Institute for Evolutionary Anthropology. In those cases,

155 either an industrial μCT system or a desktop system was used, and the subsequent voxel

156 resolutions ranged from 14 to 70 μm. The image stacks of each tooth were filtered to improve

157 tissue grayscale homogeneity and then segmented into enamel and dentine components manually

158 with Avizo® v.9 (Thermo Fisher Scientific). The crown surface was extracted as a 3D digital

159 surface model (.ply format). The models of the μCT scans were opened in Avizo® v.9 and then

160 manipulated in 3D space so that the cervical border was perpendicular to the optical axis in both

161 mesiodistal and buccolingual directions (Benazzi et al., 2009). Aviso® v.9 was used to add an

162 appropriate scale and then a screen shot of the occlusal surface (analogous to taking a digital

163 photograph) was taken and saved as a .jpg file. A recent study has shown that there is no

164 significant difference between crown outlines obtained from photos and 3D digital models (Buti,

165 2013).

166   Screen shots and digital images were imported into Adobe Photoshop®. Backgrounds

167 were removed and image contrast was adjusted to provide a clear distinction between the crown

168 outline and the background. Finally, each image was scaled to approximately the same size and

169 resolution (300 dpi).

170   Even in moderately worn dm_1s, interproximal wear sometimes distorted the distal aspect

171 of the crown outline. Less often, the mesial aspect was also affected. In these cases, the outline

172 was reconstructed by estimating the original mesial and/or distal borders (see Bailey, 2004;

173 Gómez-Robles et al., 2007; Wood and Abbott, 1983; Wood and Engleman, 1988). These

174 estimations were based on the buccolingual extent of the wear facet and the overall contour of

175 the tooth (Fig. 2); all estimations were made by SEB.

176

177   [FIGURE 2 ABOUT HERE]

178

179   The occlusal images of the dm_1s were imported in Rhino 4.0 Beta CAD environment

180 (Robert McNeel & Associates, Seattle, WA), placed on the xy-plane of the Cartesian coordinate

181 system, and rotated along the z-axis to have its lingual aspect parallel to the x-axis. Then, for

182   each tooth the crown outline was manually digitized using the curve function. The outlines were

183   centered on their centroid, and equiangularly spaced radial vectors emanating from their

184   superimposed centroids (the first radius parallel to the y-axis and buccally directed) intersected

185   the outlines. Ultimately 24 pseudolandmarks were identified for each outline (Fig. 3; Benazzi et

186   al., 2011a). Finally, the pseudolandmark configurations were scaled to unit centroid size (i.e.,

187   Procrustes shape coordinates) and variation in crown outline shape was explored by principal

188   components analysis (PCA) of the matrix of shape coordinates (Bailey et al., 2014a, b, 2016;

189   Benazzi et al., 2011b; Benazzi et al., 2012; Lacy et al., 2018; Moroni et al., 2018b).

190

191   [FIGURE 3 ABOUT HERE]

192

193   We conducted two separate PCAs. The first analysis included all samples to examine

194   variation among fossil and recent groups. The second analysis used only the recent *H. sapiens*

195   sample to investigate the role of geographic origin in the variation observed.

196   To identify potentially significant differences in crown shape of the dm_1 between groups,

197   permutation tests ($n = 10,000$) were conducted using the first three PCs. These tests compared

198   the distance between two group means to the distances obtained by random assignment of

199   observations to this groups (using Morpho v. 2.8 in R). Values were considered significant at $p <$

200   0.05. Because Neanderthal molars are, on average, slightly larger than those of *H. sapiens* and

201   because size and shape may be related, we also conducted an analysis examining the relationship

202   between shape variables (PCs) and size allometry (logarithm of crown base area). This analysis

203   was investigated by Procrustes ANOVA with permutation procedures ($n = 1,000$) using the R

204   package geomorph v. 3.2.1 (Adams and Otárola-Castillo, 2013).

205   The Shapiro-Wilks test was used to assess the normality of distribution of Procrustes

206   shape coordinates for each group in the sample (Ghasemi and Zahediasl, 2012). Fligner-Killeen's

207   test was performed to test the homogeneity of variances across the groups, rejecting the null

208   hypothesis H0 (variances homogeneity) if $p < 0.05$. Since both assumption of normality and

209   homogeneity of variance were violated, we used leave-one-out cross-validation Quadratic

210   Discriminant Analysis (QDA) to test how well crown shape discriminates taxa (see Results for

211   details). The QDA used the first eight PCs representing about 90% of the variation in the

212   comparison of *H. sapiens* (fossil and recent) and Neanderthals. Whereas, considering the small

213   sample size of UPHS ($n = 7$), the QDA used the first four PCs (70.3%) in the comparison among

214   recent *H. sapiens,* UPHS and Neanderthals, as well as between UPHS and Neanderthals. The

215   number of PCs used for QDA was chosen in order to find the minimum optimal combination of

216   variables (i.e., PCs) within the sensible cutoff in the range of 70% to 90% of variation (Jolliffe,

217   2002; Sorrentino et al., 2020). Posterior probabilities were calculated using equal prior

218   probability of 0.5. The data were processed and analyzed through software routines written in R

219   v. 3.4.3 (R Core Team, 2017).

220
221
222   **3. Results**

223   *3.1. Principal components analysis*

224   Figure 4 illustrates the results of the PCA. The first three principal components account

225   for about 60% of the variance (PC1 = 31.6%, PC2 = 15.5%, and PC3 = 12.4%; Fig. 4a).

226   Allometry is responsible for only 2.1% of overall crown variation (F = 2.72, $R^2 = 0.021$, df = 1, p

227   < 0.05) considering the whole sample; and it remains similar (2.3%) when excluding EHS (F =

228   2.86, $R^2 = 0.023$, df = 1, $p < 0.05$) in Procrustes ANOVA. The contribution of allometry

229 increases to 10.8% in the comparison of Neanderthals and UPHS (F = 2.19, R^2 = 0.108, df = 1, p

230 > 0.05), but the effects of shape variation due to size allometry are not significant in this case. It

231 is, therefore, unlikely that size is a significant driver of shape differences between the two

232 groups.

233

234 [FIGURE 4 ABOUT HERE]

235 The range of variation in recent humans is wide and spans all four quadrants of the PCA

236 plot. With the exception of two *H. sapiens* individuals (Die Kelders 6291 and La Madeleine) all

237 fossil individuals, regardless of taxon, fall within the RHS range. Recent humans appear to be

238 distributed randomly but it is possible that their distribution reflects the geographic range

239 sampled in this study. The results of a PCA exploring the RHS distribution further by grouping

240 RHS samples by geographic region are provided in Figure 5 and discussed below (3.3 Recent

241 human variation).

242

243 [FIGURE 5 ABOUT HERE]

244

245 In Figures 4b and 5 positive PC1 scores represent a relatively rectangular crown shape,

246 whereas negative PC1 scores reflect a more trapezoidal shape with a mesiobuccal projection

247 related to the tuberculum molare. Along PC2, positive scores reflect an asymmetrical crown with

248 a somewhat reduced trigonid portion and unreduced talonid, while negative PC2 scores are

249 associated with a somewhat triangular shape with a reduction in the talonid portion of the crown.

250

251 *3.2. Fossil hominin variation*

252   The three EHS individuals are variable for PC1. However, none have particularly high

253   negative PC1 scores, indicating the absence of a strong mesiobuccal projection (i.e., tuberculum

254   molare). All three individuals have negative scores for PC2, which reflect relatively large mesial

255   cusps. The three EHS individuals fall closer to the range of Neanderthals than they do to the

256   range of UPHS. All of the UPHS individuals possess negative PC1 scores, which reflect the

257   presence of a prominent tuberculum molare. Along PC2 UPHS individuals have mainly positive

258   scores (or low negative scores), indicating crowns with a relatively wider talonid than trigonid.

259   Neanderthal individuals have both positive and negative PC1 scores and mainly negative PC2

260   scores. Along PC1 the Neanderthal dm_1 scores range from moderately positive to moderately

261   negative, reflecting the observation that some possess a strong tuberculum molare, while others

262   are more rectangular and/or symmetrically shaped. Table 2 presents the results of a permutation

263   test of the significance of differences among groups. Significant differences are obtained

264   between the UPHS sample and all the other groups ($p < 0.05$). Significant differences are also

265   found between Neanderthals and the UPHS and RHS samples ($p < 0.05$), but not between the

266   Neanderthal and the EHS samples ($p > 0.05$).

267

268   [TABLE 2 ABOUT HERE]

269

270   The PCA plots in Figure 4 shows that the UPHS and Neanderthal samples are less

271   variable than the RHS sample despite their wider temporal sampling, although small sample

272   sizes may play a role this result. In fact, the two fossil groups separate quite well in shape space

273   (especially in the 3D plot of the first three PCs: Fig 4a), with only one individual falling in the

274   range of both. Figure 6 provides the mean dm_1 crown shapes of UPHS and Neanderthals. As

275   suggested from the PCA plots, the mean shape of UPHS reflects the marked mesiobuccal

276   projection frequently observed in that sample, whereas the mean shape in Neanderthals reflects

277   the wider range of expression in this feature.

278

279   [FIGURE 6 ABOUT HERE]

280   *3.3. Recent human variation*

281   Figure 5 provides a PCA plot of the geographic subgroups within the RHS sample.

282   Figure 7 illustrates the wide range of shape variation within the subgroups. Procrustes ANOVA

283   showed no significant effects (1.8%) of crown variation due to size allometry in the RHS sample

284   (F = 1.84, R_2 = 0.018, df = 1, $p > 0.05$). Table 3 presents the results of the permutation test of

285   significant differences among recent human subgroups in which the two Australian individuals

286   were not included. With the exception of South America, all subgroups span the four quadrants

287   of the PCA graph. Significant differences were obtained between the Sub Saharan African and

288   European ($p < 0.05$), South American ($p < 0.05$) and South Asian ($p < 0.05$) subgroups.

289   Significant differences were also found between South American and European ($p < 0.05$) and

290   South Asian ($p < 0.05$) subgroups. The North African subsample differs significantly from the

291   European ($p < 0.05$) and South Asia ($p < 0.05$) subgroups. Even though significant differences

292   were found, Figure 5 suggests the geographic patterning to the variation is not very strong.

293   Among the recent geographic subgroups, the South American sample shows the narrowest

294   distribution: individuals have positive and negative PC1 scores but only positive PC2 scores.

295

296   [FIGURE 7 and TABLE 3 ABOUT HERE]

297

298   *3.4. Quadratic Discriminant Functions Analysis*

299   Shapiro-Wilks tests show that the distribution of Procrustes shape coordinates of the RHS

300   violate the assumption of normality (W = 0.945, $p < 0.05$), whereas UPHS (W = 0.879, $p > 0.05$

301   and Neanderthals (W = 0.894, $p > 0.05$) do not. The variances of the groups are not

302   homogeneous ($\chi2 = 555.7$, df = 3, $p < 0.05$), even if EHS are excluded ($\chi2 = 321$, df = 2, $p <$

303   0.05). Furthermore, Fligner-Killeen's test shows different variance between RHS and

304   Neanderthals ($\chi2 = 8.65$, df = 1, $p < 0.05$), RHS and UPHS ($\chi2 = 112.79$, df = 1, $p < 0.05$), and

305   between UPHS and Neanderthals ($\chi2 = 4.8$, df = 1, $p < 0.05$).

306   Results of the QDA are provided in Tables 4 and 5. When grouped according to taxon (*H.*

307   *neanderthalensis* and *H. sapiens*), individuals were correctly assigned 87.3% of the time (Table

308   4). The classification for *H. sapiens* was better (90.2%) than it was for Neanderthals (61.5%).

309   When *Homo sapiens* was separated into fossil and recent groups and reanalyzed, RHS were

310   correctly classified 76.7% of the time, but only 42.9% of the UPHS individuals and 53.8% of the

311   Neanderthals classified correctly (Table 5). EHS was not considered in this second analysis, due

312   to its small sample size.

313   [TABLES 4 and 5 ABOUT HERE]

314

315   To explore the effect of the recent human variation on our results, and because our

316   primary goal was to ascertain whether dm$_1$ shape can accurately distinguish between

317   Neanderthals and fossil *H. sapiens,* we re-ran the QDA focusing only on Neanderthal and UPHS

318   groups. Doing this increased the accuracy substantially (Table 6). Correct assignment to the

319 Neanderthal group rose to 84.6% while correct assignment to UPHS increased to 57.1%, with

320 two Neanderthals (Bruniquel and Roc du Marsal) and three UPHS individuals (Estelas, Isturitz

321 and Solutre) misclassified.

322   [INSERT TABLE 6 ABOUT HERE]

323 **Discussion**

324   Results of the present study are in agreement with previous ones, which have

325 demonstrated that there are significant differences between the deciduous molar crown shapes of

326 UPHS and Neanderthals. As was the case for other deciduous molars, we found that assessment

327 of the dm_1 shape provides a relatively accurate method for identifying Neanderthal individuals.

328 However, and in contrast to our previous studies, the success rate in classifying UPHS based on

329 dm_1 shape is substantially lower. This leads us to conclude that a dm_1 assigned to 'Neanderthal'

330 is very likely to be correct, but a dm_1 assigned to 'UPHS' is less certain to be correct.

331   We are somewhat surprised at the mediocre classification accuracy for the UPHS

332 individuals, especially given that in the PCA the UPHS and Neanderthal samples appear to be

333 well separated in shape space (Fig. 4). We believe that our QDA results reflect, at least in part,

334 the choice of PCs and the variance for the QDA. We chose a number of PCs (4) that was both

335 less than the smallest group size ($n = 7$) and also accounted for at least 70% of the variance. Re-

336 running the QDA with five and six PCs (accounting for a slightly higher amount of variation) did

337 not improve the results. Re-running the QDA with only the first three PCs (which are illustrated

338 in Fig. 4a) led to better classificatory results, but the first three PCs accounted for only 60% of

339 the variance. Therefore, we do not have confidence in those results. Since both number of PCs

340 and variance are affected by the size of samples used, we believe that small sample size is

341 responsible, at least in part, for the lower classification accuracy indistinguishing Neanderthals

342 and UPHS in this study compared to previous ones (e.g., Bailey et al 2016).

343   Results from the present study are consistent with those of our previous studies, which

344 found that lower molars are less powerful in discriminating *H. sapiens* (both fossil and recent)

345 and Neanderthal groups than are the upper molars. The first study using dm^1 crown shape to

346 distinguish Neanderthals from UPHS showed the method to be successful 96% of the time

347 (Benazzi et al 2011a). In the same study, the shape of the dm^2 proved to be 100% accurate at

348 discriminating individuals from these two groups. In a follow-up study that included a wide

349 geographic range of recent *H. sapiens*, the accuracy of the dm^2 was only slightly lower (97%;

350 Bailey et al 2014). Subsequent studies that assessed the lower dentition suggested that dm_2

351 shape was also a powerful discriminator of Neanderthals and UPHS and recent European *H.*

352 *sapiens,* but it was slightly less accurate (92%) than the upper deciduous molars (Benazzi et al.,

353 2012). And in a study comparing dm_2 and M_1 shapes, Bailey et al. (2016) confirmed that both

354 lower molars discriminated between these two species less successfully than the upper molars.

355 The results of the present study show that the dm_1 is the least powerful in terms of discriminating

356 Neanderthals from *H. sapiens.*

357   The mediocre discriminatory power of the dm_1 in the present study is at least somewhat

358 related to the wide range of shape variation in recent humans (more than has been observed in

359 the other deciduous molars) and the greater similarity of EHS dm_1 shape to that of Neanderthals,

360 at least as far as can be determined with this small EHS sample. A previous study of the dm_2 and

361 M_1 (Bailey et al., 2016) also suggested that EHS and Neanderthal dm_2 shapes do not differ

362 significantly. However, in that study EHS specimens plotted well within the variation of both

363 RHS and UPHS groups, which makes the dissimilarity between EHS and UPHS dm_1 shapes

364 found in this study somewhat surprising. The similarities between EHS and Neanderthals may

365 suggest that the lower dentition has undergone less change in EHS than it has in UPHS and RHS.

366 Additional specimens from the Middle Pleistocene would help clarify the polarity of dm_1 crown

367 shapes and confirm that this is the case.

368

369 **Conclusions**

370 Based on the recent series of studies of molar crown shapes, we conclude that the lower

371 deciduous molars and the lower permanent M1 are less reliable than the upper molars for

372 discriminating between Neanderthals and *H. sapiens*. Although our results for the dm_1 are

373 somewhat mediocre over all, from a practical standpoint we can say that crown shape of the dm_1

374 is useful for identifying Neanderthals in a Late Pleistocene European context. Unfortunately, we

375 would hesitate to use the dm_1 to identify *H. sapiens* from the same time period/region because

376 the success rate is not much better than chance. In addition, we would not recommend using the

377 dm_1 crown shape to discriminate between these two groups where they co-occur in the Near

378 East, since the early *H. sapiens* dm_1 crown outline does not differ significantly from that of

379 Neanderthals. In sum, the dm_1 crown shape is only of limited use for assigning isolated teeth to

380 taxa.

381

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17

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389

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526

527

528   Figure Legends

529   Figure 1. Comparison of A) upper and lower left dm2 and M1, and B) upper and lower left dm1
530   and dm2 (all images represent the same recent *H. sapiens* from Peru). In both photos, upper is on
531   the left, lower is on the right. For orientation: B = buccal, L = lingual, M = mesial, D = distal.

532   Figure 2. Illustration showing the most worn crown (stage 5 wear: Molnar, 1971) in our sample
533   and how minor corrections were made to the outline before analysis (Early *H. sapiens* Die
534   Kelders 6291). For orientation: B = buccal, L = lingual, M = mesial, D = distal.

535   Figure 3. Illustration showing methods for acquisition of pseudolandmarks on the left dm_1 of the
536   Kebara 1 Neanderthal. For orientation: B = buccal, L = lingual, M = mesial, D = distal.

537   Figure 4. Results of the Principal Components Analysis: all samples. The range of variation in
538   recent *H. sapiens* encompasses that of nearly all fossil samples, whereas the fossil samples are
539   more tightly constrained along the first three PCs. Center plot: PC1 against PC2. Upper left: PC1,
540   PC2 and PC3. N, Neanderthal; EHS, Early *Homo sapiens*; RHS, Recent *Homo sapiens*; UPHS,
541   Upper Paleolithic *Homo sapiens*. For orientation: B = buccal, L = lingual, M = mesial, D =
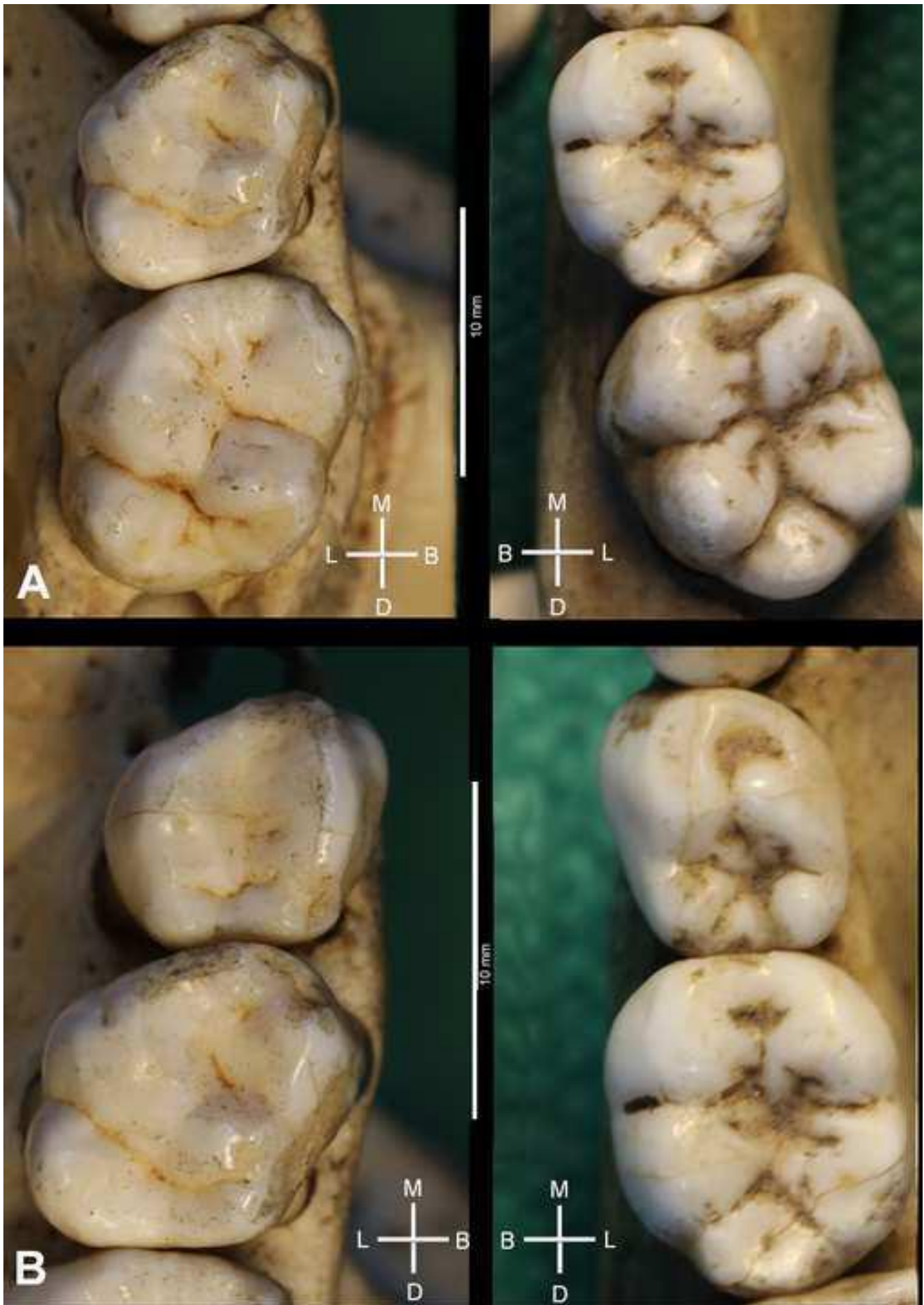542   distal.

543   Figure 5. Results of the Principal Components Analysis of recent *H. sapiens* grouped by
544   geographic origin. With the exception of the South American sample, which has only positive
545   PC2 scores, there appears to be no geographic patterning to ldm1 shape based on the first two
546   principal components. For orientation: B = buccal, L = lingual, M = mesial, D = distal.
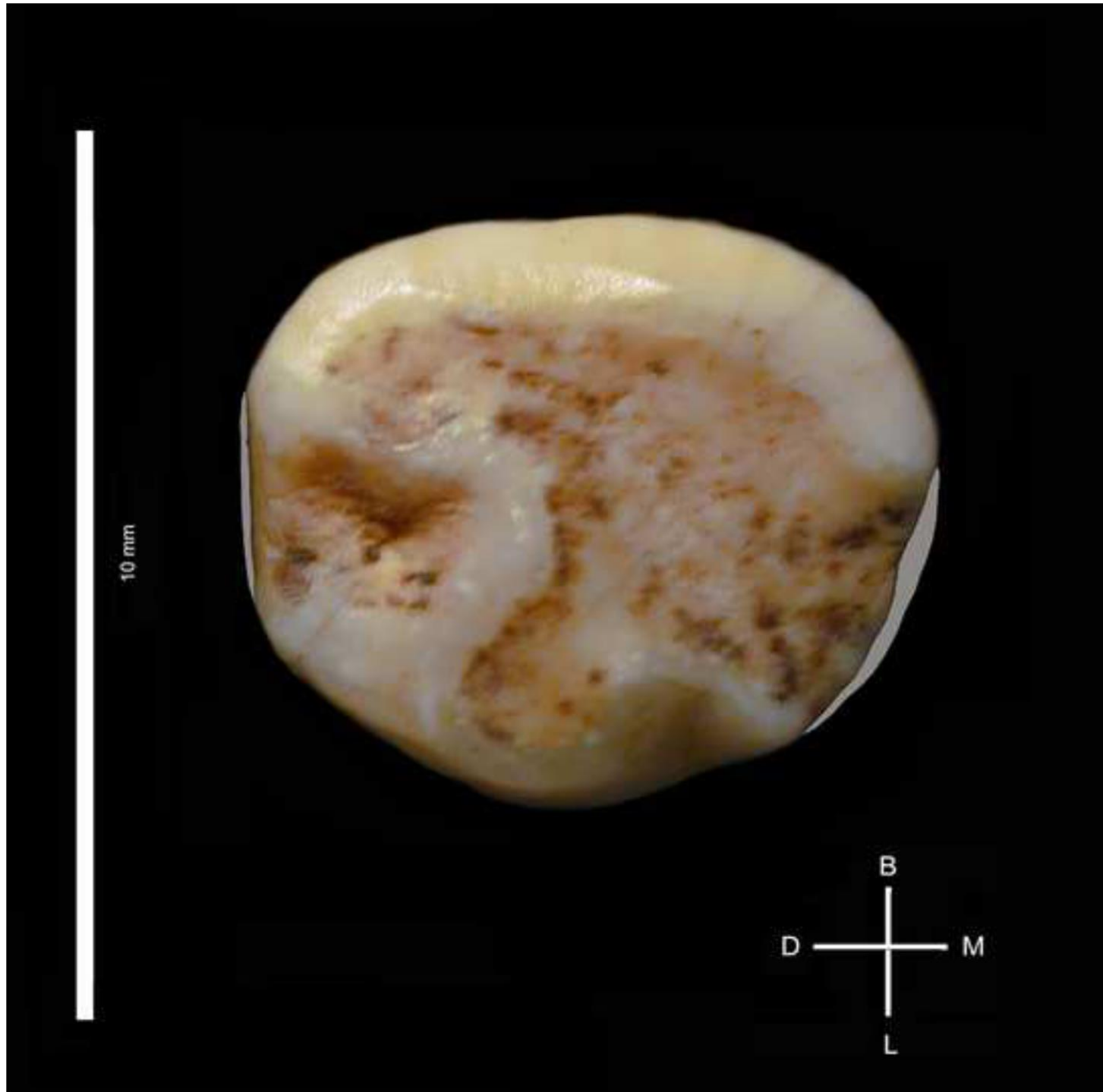
547   Figure 6. Comparison of mean shapes between Neanderthals (left) and Upper Paleolithic *H.*
548   *sapiens* (right). Right arrow indicates mesiobuccal expansion (tuberculum molare) in Upper
549   Paleolithic *H. sapiens*. Left arrow indicates more equal sized buccal and lingual cusps in
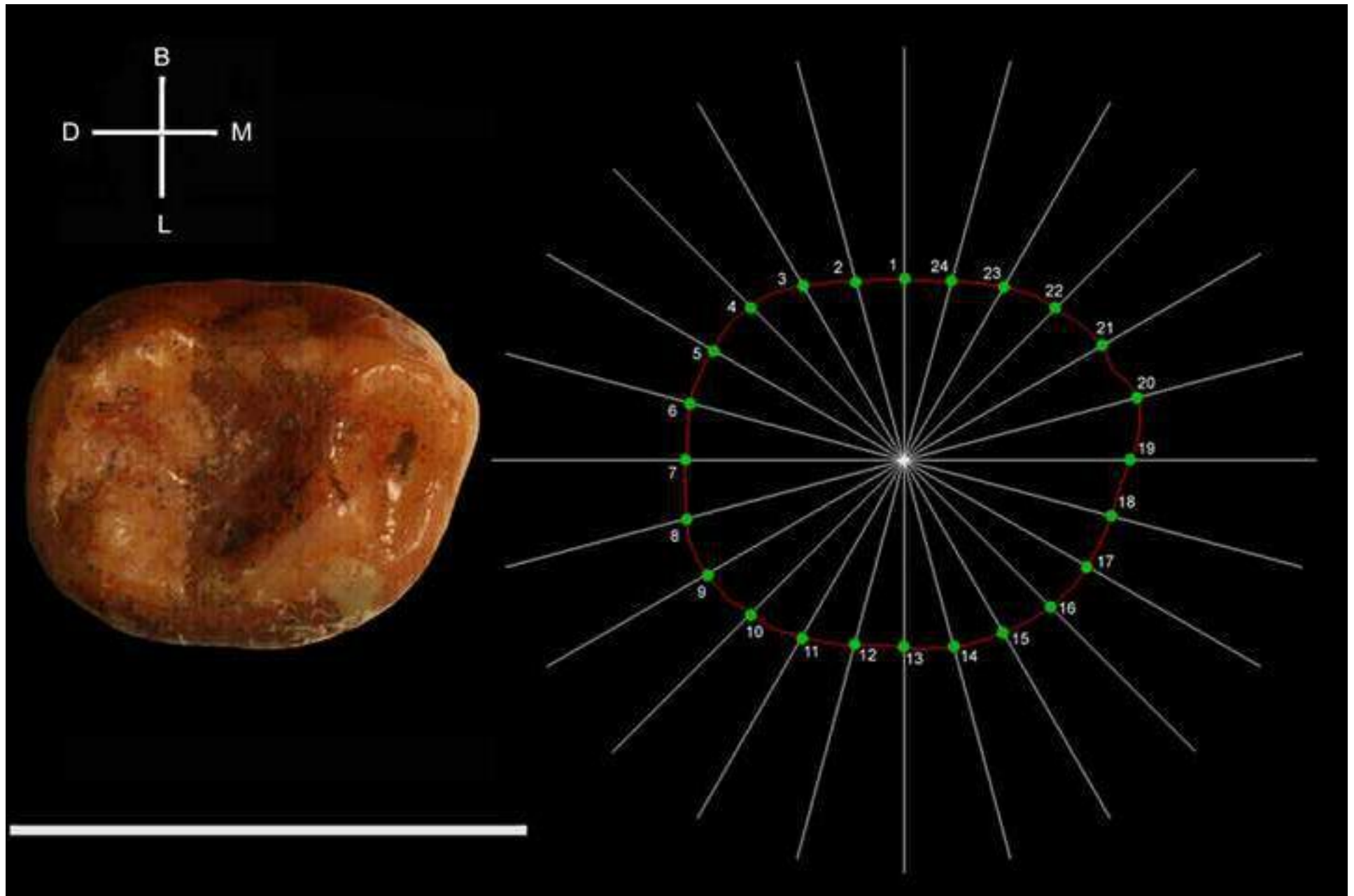550   Neanderthals. For orientation: B = buccal, L = lingual, M = mesial, D = distal.

551   Figure 7. Variation of left dm_1 crown shape within recent *H. sapiens* geographic populations. For
552   orientation: B = buccal, L = lingual, M = mesial, D = distal.
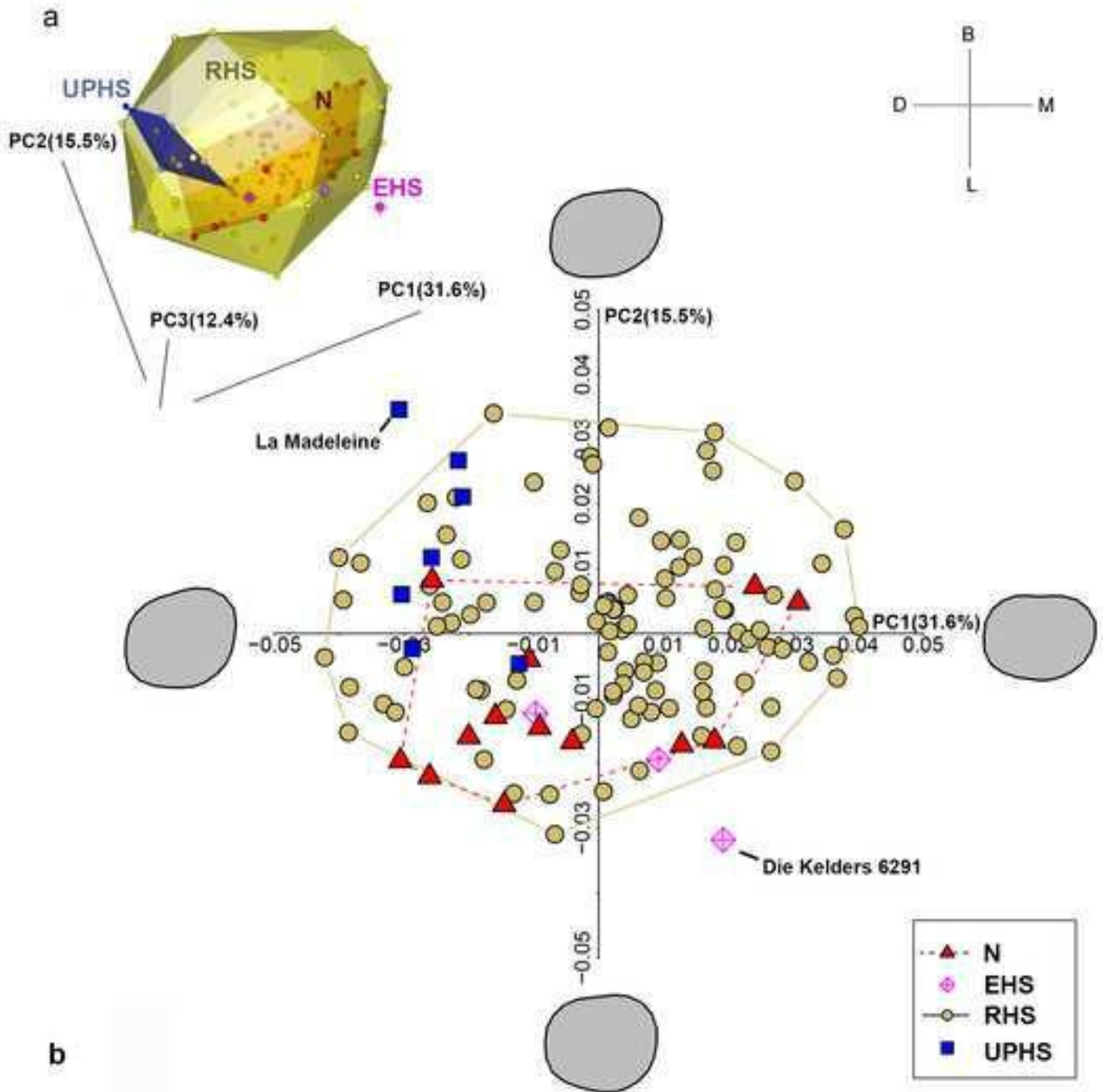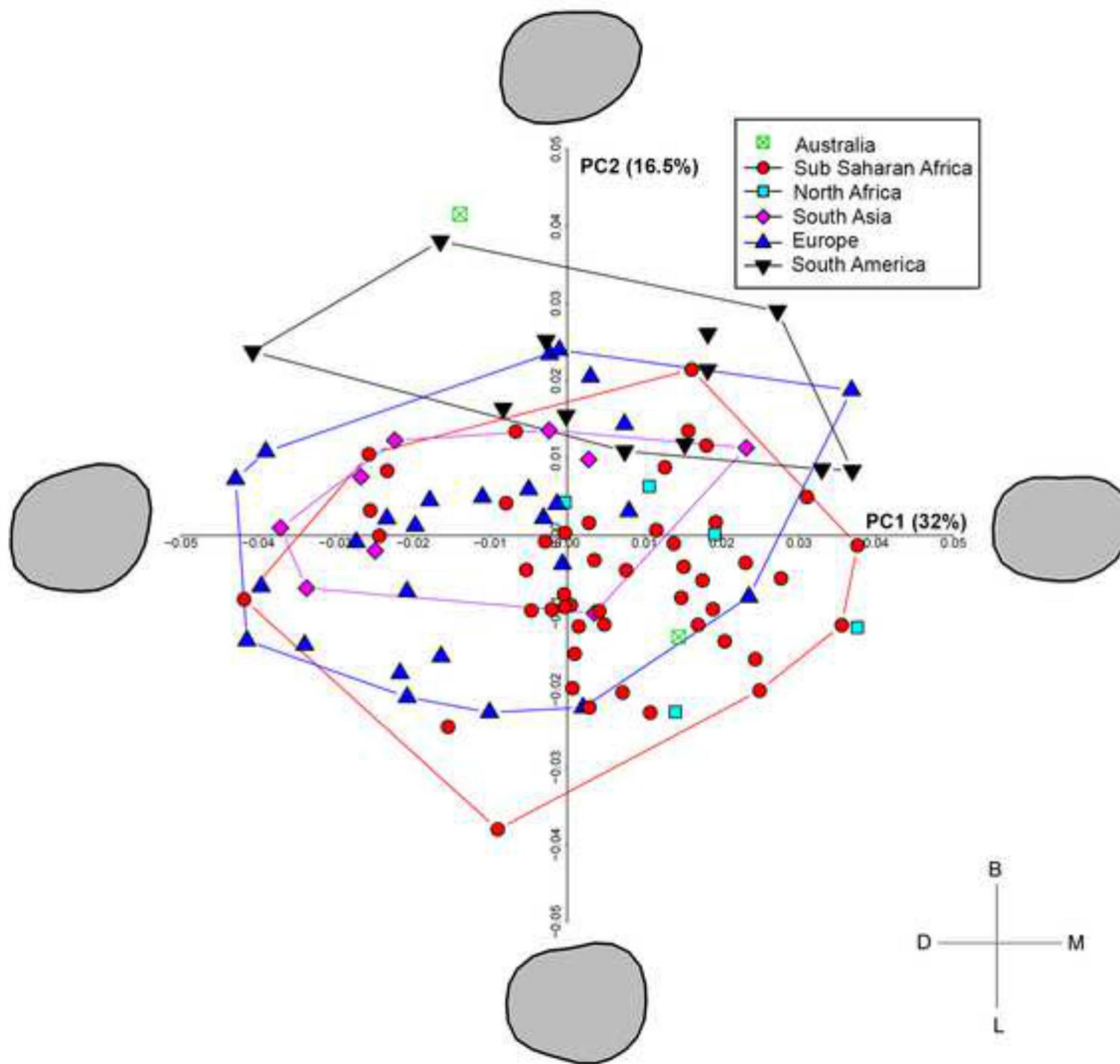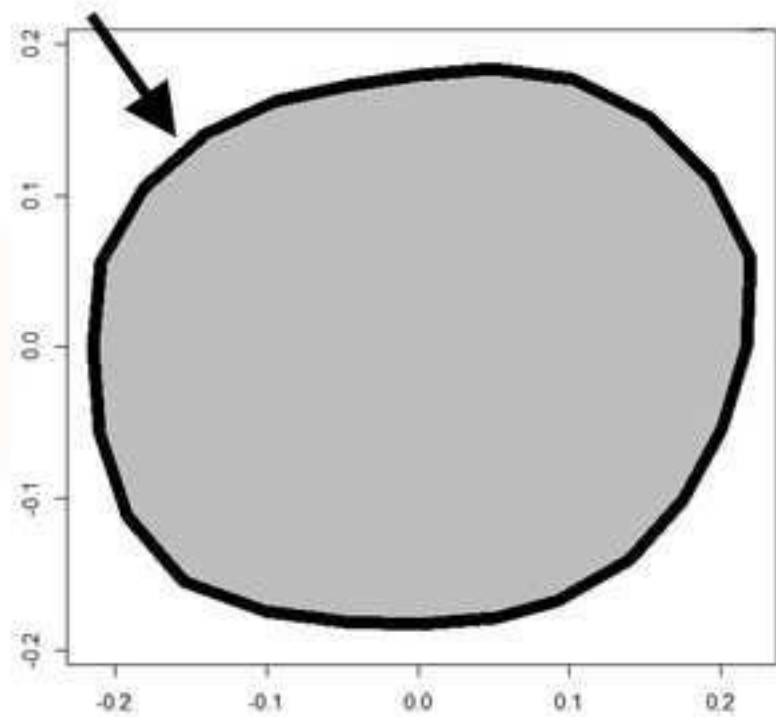
553

554

Figure 1

Figure 3

Figure 4

a

RHS

UPHS

PC2(15.5%)

N

EHS

PC1(31.6%)

PC3(12.4%)

PC2(15.5%)

B

D — M

L

La Madeleine

PC1(31.6%)

Die Kelders 6291

N
EHS
RHS
UPHS

b

NEANDERTHAL MEAN SHAPE
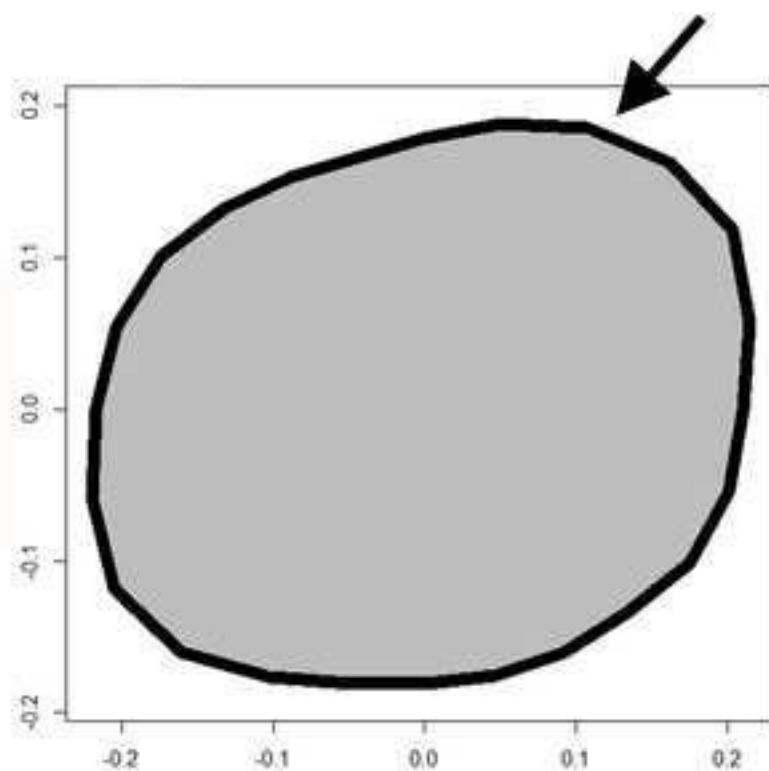
UPPER PALEOLITHIC *H. SAPIENS* MEAN SHAPE

Figure 7
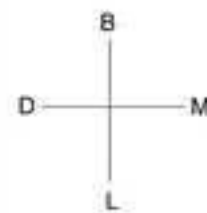
Table 1
Materials used in this study.[a]

	No.	Sites sampled
Early *H. sapiens*	3	Die Kelders, Qafzeh
Upper Paleolithic *H. sapiens*	7	Balla Barlang, Estelas, La Grotte du Figuier, Isturitz, Lagar Velho, Abri de la Madeleine, Roche de Solutré
H. neanderthalensis	13	Archi, Arcy-sur-Cure, Barakai Cave, Bruniquel, Combe Grenal, Engis, Kebara, La Ferrassie, La Chaise, Riparo del Molare, Peche de l'Azé, Roc de Marsal, Mezmaiskaya
Recent *H. sapiens*	103	Africa, Asia, Australia, Europe, South America

[a] See SOM for sources of materials.

Table 2
Permutation tests of differences in crown shape of the dm_1 between fossil and recent human samples. [a]

	Early *H. sapiens*	Neanderthal	Recent *H. sapiens*
H. neanderthalensis (n=13)	0.502		
Recent *H. sapiens* (n=103)	0.182	**0.010**	
Upper Paleolithic *H. sapiens* (n=7)	**0.002**	**0.002**	**0.001**

[a]Significant differences ($p < 0.05$) are in bold.

Table 3
Permutation tests of differences in crown shape of the dm_1 between recent human geographic subsamples.[a-b]

	Europe	North Africa	South America	South Asia
Europe (n=28)				
North Africa (n=5)	**0.025**			
South America (n=12)	**0.001**	0.054		
South Asia (n=9)	0.884	**0.040**	0**.032**	
Sub-Saharan Africa (n=49)	**0.001**	0.884	**0.001**	**0.032**

[a]The Australia sample is excluded in the permutation test because of its small (n=2) sample size'
[b]Significant differences ($p < 0.05$) are in bold.

Table 4
Results of quadratic discriminant functions assignments (fossil and recent *H. sapiens* combined) based on crown shape of the dm_1 by using 8 PCs (accounting for 90.7% of the variation).

	H. neanderthalensis	*H. sapiens*	% correct
H. neanderthalensis (n=13)	8	5	61.5
H. sapiens (n=113)	11	102	90.2

Table 5
Results of quadratic discriminant functions assignments (fossil and recent *H. sapiens* separated) based on crown shape of the dm_1 using 4 PCs (accounting for 70.3% of the variation). Early *H. sapiens* are excluded due to small sample size.

	H. neanderthalensis	*H. sapiens*	Upper Paleolithic *H. sapiens*	% correct
H. neanderthalensis (*n* = 13)	7	4	2	53.8
Recent *H. sapiens* (*n* = 103)	4	79	16	76.7
Upper Paleolithic *H. sapiens* (*n* = 7)	0	4	3	42.9

Table 6
Results of quadratic discriminant functions assignments (Upper Paleolithic *H. sapiens* and *H. neanderthalensis* only) by using 4 PCs (accounting for 70.3% of the variation). Early *H. sapiens* are excluded due to small sample size.

	H. neanderthalensis	Upper Paleolithic *H. sapiens*	% correct
H. neanderthalensis (*n* = 13)	11	2	84.6
Upper Paleolithic *H. sapiens* (*n* = 7)	3	4	57.1