The Impact of Dental Wear on the Analysis of Morphological Affinities based on Dental Non-metric Traits

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ABSTRACT Dental wear is described as a limitation to dental morphological studies, as it obscures important crown trait features, resulting in significant differences on trait frequencies, an essential component for estimating biodistances. However, the actual impact of dental wear on biological distances still requires further characterization. We explore the impact of dental wear on morphological affinities for Brazilian pre-colonial series in the context of worldwide reference series. Twenty crown traits were scored using the Arizona State University Dental Anthropology System, and dental wear was quantified as an ordinal scale between 1 (no wear) and 8 (crown eroded). Eight crown trait frequencies are significantly associated with dental wear (p<0.05), demonstrating its impact on their analysis. To explore this impact on biodistances, data were divided by wear categories (all teeth, low-wear, moderate/severe wear) and morphological affinities among series were compared through Euclidean distances, Mean Measure of Divergence, and Principal Component Analysis. Results show that the impact of wear is only meaningful when a sample contains many wear-biased traits with only moderate/severe wear. We conclude that, despite the impact of wear on individual trait frequencies, its impact on morphological affinities can be mitigated by including other variables or when comparisons focus only on large-scale biological differences.

Dental morphology has a strong genetic component which allows it to be used as a proxy for neutral genetic markers (Hubbard et al., 2015; Irish et al., 2020; Kimura et al., 2009; Rathmann et al., 2017; Rathmann & Reyes-Centeno, 2020). Dental non-metric traits are assumed to lack significant sexual dimorphism, have minimal influence from divergent selection, and have high heritability (Irish et al., 2020; Scott & Irish, 2013; Scott & Turner II, 1997; Turner II et al., 1991). The required methods to analyze and quantify dental morphology are also cost efficient, and since teeth are often found in the archaeological record and highly resilient to taphonomic processes (Hillson, 2005), they are a good alternative to reconstruct population biological affinities and human mobility on individual (Scott, Pilloud, et al., 2018), local (Scott et al., 2013; Turner II & Scott, 1977), regional (Irish et al., 2017; Rathmann et al., 2019; Sutter, 2009; Turner II, 1976), and global scales (Hanihara, 2008; Scott & Irish, 2017; Scott & Turner II, 1997; Sutter, 2005). A prolific example of the latter can be seen in studies discussing the peopling of the Americas (Greenberg et al., 1986; Huffman, 2014; Powell, 1995, 1997; Powell & Neves, 1998; Scott, Schmitz, et al., 2018; Stojanowski et al., 2013; Stojanowski & Johnson, 2015; Sutter, 2005; Turner II & Scott, 2013).

Dental morphology played an important role in discussing the settlement of the Americas since the first half of the 20th century (Dahlberg, 1945; Hrdlička, 1920, 1921). After the development of

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standardized methods for data collection, such as the Arizona State University Dental Anthropology System (ASUDAS), studies of dental non-metric traits increased significantly over the years (Powell, 1995; Scott & Irish, 2017; Scott & Turner II, 1997; Sutter, 2005; Turner II et al., 1991). However, as researchers studied different archaeological series, different conclusions were drawn about how the Americas were first settled by modern humans. Some studies argue that all Native Americans are more strongly related to each other than to any other group outside the Americas, and share a rather homogenous dental morphological pattern, related to Northeast Asians populations that first crossed the Bering Strait (Greenberg et al., 1986; Scott, Schmitz, et al., 2018; Scott & Turner II, 1997; Turner II, 1990; Turner II & Scott, 2013). Others suggest phenotypic variation within the Americas is larger, with some Native American groups biologically related to Southeast Asians, meaning that at least two distinct biological populations crossed the Bering Strait during pre-colonial times (Haydenblit, 1996; Huffman, 2014; Lahr & Haydenblit, 1995; Ortiz, 2013; Powell, 1995, 1997; Powell & Neves, 1998; Powell & Rose, 1999; Sutter, 2005, 2009).

This discrepancy in narratives has often been attributed to issues regarding the replicability of ASUDAS, as observer error is often an anticipated concern (Marado, 2017; Nichol & Turner, 1986; Wu & Turner, 1993). Also, the combination of which morphological traits are used to assess biological affinities may have an important influence on the results (Rathmann & Reyes-Centeno, 2020). Furthermore, dental wear has also been suggested as a noteworthy concern on its own, causing bias in scoring non-metric traits (Burnett et al., 2013; Burnett, 2016; Stojanowski & Johnson, 2015). Dental wear is a physiological phenomenon on which tooth enamel and dentine are gradually worn over time by attrition, abrasion and/or erosion mechanisms (Kaidonis, 2008). Many dental non-metric traits are features located in the tooth crown, so dental wear may gradually erase morphological details and impact scoring decisions (Scott et al., 2016). The effects vary for each particular trait and can result in the under-estimation of trait frequencies (i.e., attributing lower grades or absence to traits that should be scored as higher grades or present), or over-estimation of frequencies (i.e., higher trait expressions are scored regardless of wear, but lower/absent expressions are scored as missing data under the same circumstances) (Burnett et al., 2013; Burnett, 2016). If the error in the estimations of trait frequency is significantly biased between teeth with low and moderate/severe wear, it violates the assumptions that samples have data missing completely at random (MCAR) (Burnett et al., 2013; Stojanowski & Johnson, 2015). Data MCAR is a central tenet in the reconstruction of population parameters based on samples, because it means missing values follow the same distribution as the observed values (Bhaskaran & Smeeth, 2014), and therefore information about the population has not been skewed by the data that was not observable.

Many non-metric dental traits have been shown to be susceptible to wear-related bias: shoveling UI1, cusp number LM2 (Burnett et al., 2013; Stojanowski & Johnson, 2015), distal accessory ridge UC, mesial canine ridge UC, accessory ridges UP, lingual cusp number LP2, hypocone UM2 (Burnett et al., 2013; Burnett, 2016), double shoveling UI1, enamel extensions UM1, deflecting wrinkle LM1 (Stojanowski & Johnson, 2015). However, the clear impact of wear-biased traits on multivariate analysis has not been formally evaluated. It is possible that a certain amount of error is acceptable as long as it does not change the interpretations of the results. In other words, the measured attributes are still valid as long as they are meaningfully reflecting real biological relationships (Houle et al., 2011).

Going back to the example about the peopling of the Americas, the debate around dental wear is particularly relevant. Although there is consilience that Native Americans share a recent common ancestor with Asians, there is no clear agreement about which Asian dental complex they are more related: 1) a specialized pattern which emerged approximately between 20 and 11 thousand years ago (kya) in Northeast Asia, with high frequencies of shoveling UI1, double shoveling UI1, one-rooted UP1, enamel extensions UM1, pegged/reduced/missing UM3, deflecting wrinkle LM1, three-rooted LM1; commonly referred to as the Sinodont pattern (Turner II, 1989, 1990); or 2) a generalized and more simplified pattern which appears between 25 and 40kya in Southeast Asia (Turner II, 2006), with lower trait frequencies of the same above-mentioned traits, and a higher frequency of four-cusped LM2; commonly described as the Sundadont pattern (Scott, Schmitz, et al., 2018; Turner II, 1990).

Some authors suggest that Native Americans have a different derived dental morphological pattern from both Sinodonts and Sundadonts (Scott, Schmitz, et al., 2018; Stojanowski & Johnson, 2015). While keeping ties to Sinodont groups such as Northeast Asians, Native Americans have even higher trait frequencies of some traits (e.g., shoveling UI1, double shoveling UI1), which can be viewed as "super-Sinodont" (Scott, Schmitz, et al., 2018). In other words, it seems that there are considerable differences on the dental morphological patterns between Native American and Asian populations, which is even larger in some traits than the differences observed between Asian Sinodonts and Sundadonts (Scott, Schmitz, et al., 2018).

Since Sundadonty has lower frequencies of several morphological traits, particularly when compared to Native Americans, some scholars argue that the under-estimation of trait frequencies due to dental-wear bias is responsible for the close biological link between Native Americans and Southeast Asians (Scott, Schmitz, et al., 2018; Turner II & Scott, 2013), which has been noted in several independent studies and different archaeological series (Haydenblit, 1996; Huffman, 2014; Lahr & Haydenblit, 1995; Ortiz, 2013; Powell, 1995, 1997; Powell & Neves, 1998; Powell & Rose, 1999; Sutter, 2005, 2009).

To contribute to this discussion, and at the same time to illustrate the impact of dental wear in dental non-metric analyses, we present a case study of a Brazilian coastal series dated to between 10.0 and 1.0 kya. Our study subsets this dataset into different series based on dental wear degrees and compare their morphological affinities within a global reference framework, using a combination of only wear-biased traits, only unbiased traits, and all traits pooled together. These analyses aim to improve our understanding of the impact dental wear has in multivariate statistical analyses, and to explore if at any point dental non-metric traits stop being meaningful markers of biological relationships.

Materials and Methods

To quantify the impact of wear on the morphological affinities of Brazilian coastal populations, we analyzed 431 individuals from the South and Southeast Brazilian coast, dated between ~10.0 and 1.0 kya. Most of our sample comes from a broad archaeological context of shellmound builders, commonly known as *Sambaquis*, which have previously been shown to share a Native American dental morphological pattern (Turner II & Scott, 2013). Our sample includes individuals from the following archaeological sites: Capelinha 1 (n=7), Capelinha 2 (n=1), Itaoca (n=2), Estreito (n=5), Laranjal (n=9), Moraes (n=32), Piaçaguera (n=34), Tenório (n=24), Mar Virado (n=21), Cosipa 4 (n=2), Buracão (n=17), Galheta IV (n=6), Ilha de Espinheiros 2 (n=7), Enseada (n=26), Morro do Ouro (n=70), Itacoara (n=28), Rio Comprido (n=48), Cabeçuda (n=12), Guaraguaçu A & B (n=69), and Matinhos (n=11).

A total of 20 crown traits from ASUDAS were scored (Scott & Irish, 2017; Turner II et al., 1991), and dental occlusal wear was noted according to Smith (1984). To improve sample sizes, we used the total tooth count method to calculate trait frequency: when available, both antimeres were scored for each trait, and sample frequencies were calculated by dividing the total number of positive expressions by the total number of teeth analyzed (Scott, 1980). While this approach may add redundant information to the data, as individuals are often scored twice (Scott, 1980; Scott & Irish, 2017; Turner II & Scott, 1977), previous studies have shown that results based on individual and total counting methods produce very similar results, and thus can be used for comparative purposes (Marado, 2014; Scott, 1980). As the main goal of this study is to explore the impact of wear bias on the estimations of morphological affinities, we opted for the method that would maximize the number of teeth and dental wear information included.

Intra-observer error of dichotomized traits was calculated with a subsample of 128 individuals, analyzed by the first author twice with approximately one month interval between analyses. Only teeth that were scored for dental wear were considered in this analysis, and Cohen's Kappa coefficient of agreement was used to assess the level of agreement between analyses. Kappa's values were classified as follows: 0.00-0.20 (slight agreement); 0.21-0.40 (fair agreement); 0.41-0.60 (moderate agreement); 0.61-0.80 (substantial agreement); 0.81-99 (almost perfect agreement) (Landis & Koch, 1977).

To test the impact of wear on morphological affinities among series, we only included teeth scored for both dental wear and morphological traits, and followed a similar approach to Burnett (2013): three categories of dental wear were established based on the scale of Smith (1984): low wear (Grades 1-3); moderate wear (Grades 4-5); and severe wear (Grades 6-8). As there were very low sample sizes of traits scored on teeth with severe wear, we combined teeth with moderate or severe wear. Afterwards, we compared trait presence and absence between low and moderate/severe wear groups using Fisher's Exact tests.

Finally, we evaluated the morphological affinities among series through multivariate exploratory analyses, comparing our samples with other skeletal series from Southeast Asia, Asia, Circumpolar, North America, Mesoamerica, and South America (Scott & Irish, 2017). All data tables used for comparative purposes are available in Scott and Irish (2017). Furthermore, we split our sample into three series based on dental wear categories: 1) Brazilian coast, which includes all teeth regardless of dental wear; 2) Brazilian coast (low wear), which excludes teeth with moderate/severe dental wear; 3) Brazilian coast (Mod/Sev wear), which uses only teeth with moderate/severe occlusal dental wear. We recognize it is unlikely for a researcher to select only moderate/severe wear traits in any study on dental morphology. However, some archaeological series are very limited, and sometimes only composed by individuals with substantial amounts of dental wear. Thus, we use this series as a way to infer the maximum amount of error that can result from the use of only teeth moderately to severely worn out.

To assess possible trait correlations between groups, and check if correlations varied significantly between combinations of wear-biased and/or unbiased traits, Spearman correlations were calculated over trait frequencies of three different data sets: A) Only wear-biased traits; B) Only unbiased traits; and C) all traits combined. To mitigate the impact of multicollinearity, for each highly correlated pair of variables (r≥0.7), we removed one of those traits from the multivariate analyses.

Next, Euclidean distances and Mean Measure of Divergence without sample size correction were calculated for each of the three datasets, and represented through Kruskal Multidimensional Scaling. Mantel matrix correlation tests were applied to compare distance matrices generated by both methods for each dataset to test the level of similarities between them. The morphological affinities were also explored through Principal Component Analysis, and the first two principal components were extracted from the average trait frequencies for the series and represented in a scatterplot.

Together, these different multivariate analyses allow us to evaluate the impact of wear biases in estimating morphological affinities (and biological relationships) among samples, by illustrating to what degree the inclusion of biased frequencies affect the overall pattern of affinities among series when inserted in a broader comparative framework. Furthermore, as we expect our samples to share a Native American dental complex, as suggested by Turner and Scott (2013), any deviation from this cluster may lead us to assume that dental wear can shift the results significantly, enough to bias our ancestry estimations at a worldwide scale, as suggested by some authors (Scott, Schmitz, et al., 2018; Turner II, 2006; Turner II & Scott, 2013).

All statistical analyses were done in R (R Core Team, 2020), with functions written by two of us (MH and DF), and complemented by the packages ggplot2 (Wickham, 2016), ggfortify (Tang et al., 2016), MASS (Venables & Ripley, 2002), vegan (Oksanen et al., 2013), and irr (Gamer et al., 2012).

Results

Figure 1 shows the intra-observer error for all the analyzed traits in this study. While most traits show substantial agreement or higher, three traits only reached moderate agreement (metaconule UM1, anterior fovea LM1, and groove pattern LM2), and so should be considered with caution. These traits are also traits that show significant bias from dental wear (Table 1), suggesting that dental

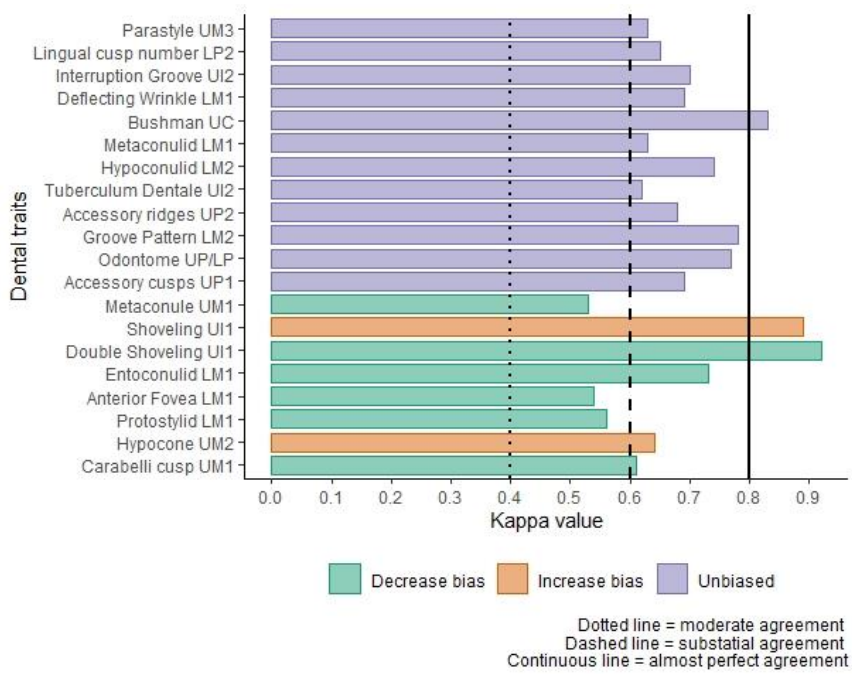

Figure 1. Bar plot with Cohen's Kappa coefficient of agreement for each morphological trait.

wear may play a role in the consistent scoring of these traits. However, it is worth noting that some traits with almost perfect agreement are also wear biased, one by underestimation (double shoveling UI1) and the other by overestimation of trait frequencies (shoveling UI1). Therefore, the role of wear on the replicability of trait analysis depends on the type of trait and should be assessed accordingly.

Figure 2 shows the distribution of dental wear grades for each scored morphological trait. As can be seen, the distribution of trait scores is largely similar between teeth with low and moderate/severe dental wear. Table 1 shows the sample sizes and trait frequencies for the series in this study, as well as the results for the Fisher Exact tests comparing trait frequency by wear degrees. There were 4,191 dental trait scores in total, 2,069 on teeth with low wear and 2,122 on teeth with moderate/severe wear. Half of the dental traits have larger sample sizes on teeth with low wear, and the other half have larger sample sizes on teeth with moderate/severe wear. However, in some traits there is a clear larger sample size for teeth with low wear (e.g., deflecting wrinkle LM1 and accessory ridges UP2). Eight of the 20 traits (40%) show significant dental wear bias (*p*<0.05). The effect of dental wear varies between traits: shoveling UI1 and hypocone UM1 are biased towards increased trait frequencies (25%, 2/8) whereas the remaining trait biases (75%, 6/8) resulted in the underestimation of their frequencies. Therefore, in the Brazilian context, as in other studies, dental wear is more prone to bias traits by underestimating their frequencies (Burnett et al., 2013).

In the analyses comparing the Brazilian series with the reference series, the following traits were excluded because they are not available from Scott and Irish (2017): anterior fovea LM1, accessory ridges UP2, and accessory cusps UP1. This resulted in a dataset of 17 traits, seven of which show significant wear bias. We calculated the absolute mean difference of each trait between all pairs of reference series and compared it with the frequency differences observed between low and moderate/severe wear groups (Table 2), to quantify the magnitude of the wear bias in the context of observed differences among series representing large

Table 1. Sample sizes, frequencies, Fisher's Exact test, and dental wear-bias effect for each dental morphological trait.

Variable	Grade Threshold	Brazilian Coast		Brazilian Coast (low wear)		Brazilian Coast (moderate/severe wear)		Fisher *p*. value	Bias effect
		n	f	n	f	n	f		
Shoveling UI1	3	168	0.857	100	<u>0.81</u>	68	<u>0.926</u>	**0.043**	Increase
Double Shoveling UI1	2	166	0.681	98	<u>0.745</u>	68	<u>0.588</u>	**0.042**	Decrease
Interruption Groove UI2	1	165	0.158	93	0.161	72	0.153	1	-
Tuberculum Dentale UI2	2	163	0.215	90	0.233	73	0.192	0.569	-
Bushman UC	1	175	0.051	84	0.048	91	0.055	1	-
Accessory cusps UP1	1	176	0.091	101	0.129	75	0.04	0.062	-
Accessory ridges UP2	2	84	0.476	77	0.494	7	0.286	0.437	-
Metaconule UM1	1	158	0.089	81	<u>0.136</u>	77	<u>0.039</u>	**0.048**	Decrease
Carabelli cusp UM1	3	229	0.127	78	<u>0.308</u>	151	<u>0.033</u>	**<0.01**	Decrease
Hypocone UM2	3	291	0.832	104	<u>0.644</u>	187	<u>0.936</u>	**<0.01**	Increase
Parastyle UM3	2	228	0.004	148	0.007	80	0	1	-
Lingual cusp number LP2	1	192	0.255	93	0.258	99	0.253	1	-
Deflecting Wrinkle LM1	2	71	0.479	65	0.477	6	0.5	1	-
Anterior Fovea LM1	2	103	0.563	76	<u>0.645</u>	27	<u>0.333</u>	**<0.01**	Decrease
Protostylid LM1	2	227	0.031	73	<u>0.082</u>	154	<u>0.006</u>	**<0.01**	Decrease
Entoconulid LM1	1	167	0.24	79	<u>0.316</u>	88	<u>0.17</u>	**0.031**	Decrease
Metaconulid LM1	1	244	0.111	81	0.099	163	0.117	0.829	-
Groove Pattern LM2	Y	260	0.123	95	0.095	165	0.139	0.332	-
Hypoconulid LM2	1	193	0.777	95	0.789	98	0.765	0.731	-
Odontome UP/LP	1	731	0.01	358	0.014	373	0.005	0.277	-

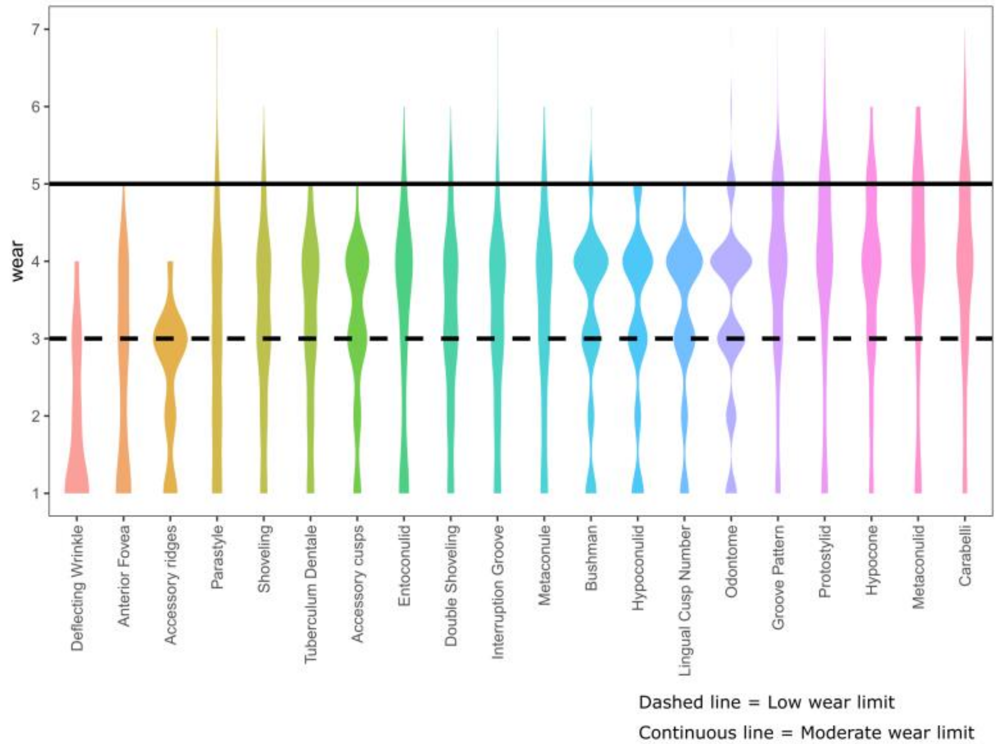

Figure 2. Violin plot showing the frequency of wear degrees for each morphological trait.

Table 2. Maximum trait frequency differences between low and moderate/severe wear subset in the Brazilian Coast groups, comparative information about absolute mean difference in trait frequency among worldwide series, and proportion of differences among reference series that exceed the wear bias observed for Brazilian series.

Traits	Frequency bias between low and moderate/severe wear Brazilian series	Absolute mean frequency differences among reference series	Proportion of differences among comparative series exceeding bias in Brazilian series
Shoveling UI1	0.116	0.263	0.638
Double shoveling UI1	-0.157	0.349	0.65
Interruption grooves UI2	-0.008	0.147	0.96
Tuberculum dentale UI2	-0.041	0.107	0.766
Bushman Canine UC	0.007	0.029	0.672
Metaconule UM1	-0.097	0.123	0.533
Carabelli cusp UM1	**-0.275**	**0.138**	**0.134**
Hypocone UM2	**0.292**	**0.155**	**0.174**
Parastyle UM3	-0.007	0.037	0.826
Lingual cusp number LP2	-0.005	0.25	0.991
Deflecting wrinkle LM1	0.023	0.174	0.929
Protostylid LM1	**-0.076**	**0.047**	**0.217**
Entoconulid LM1	-0.146	0.157	0.484
Metaconulid LM1	0.018	0.045	0.766
Groove pattern LM2	0.044	0.091	0.718
Hypoconulid LM2	-0.024	0.152	0.875
Odontome UP1/LP1	-0.009	0.037	0.835

Bold: Wear-biased traits that show absolute frequency bias larger than the absolute mean frequency differences among reference series.

continental biological profiles. Although seven wear-biased traits show significant wear bias (see Table 1), only three of them (Carabelli cusp, hypocone, and protostylid) show wear bias that exceeds the average difference in the reference series. Therefore, most traits in the Brazilian series show wear biases that are smaller than the majority of differences among the reference series. These three traits should be considered as the most problematic, and may lead to a more significant bias in the patterns of morphological affinities observed in our data.

Before running the multivariate analyses, Spearman correlation tests among the 17 traits were done to check for collinearity of variables. The correlation tests revealed a strong correlation ($r \geq 0.7$) between double shoveling UI1 and groove pattern LM2 (SI2). Therefore, groove pattern LM2 was removed from the analyses using all 17 traits (dataset C). When testing correlations among wear-biased (dataset A) and unbiased (dataset B) traits, no strong correlations were found (SI2), and no traits were removed from the analyses with these datasets.

The results of the multidimensional scaling based on Euclidean distances (Figure 3) and Mean Measures of Divergence (Figure 4) show very similar results, as the two distance measurements show extremely high correlations (Mantel correlation tests: $r=0.950$, $p \leq 0.001$ for biased traits; $r=0.960$, $p \leq 0.001$ for unbiased traits; $r=0.961$, $p \leq 0.001$ for combined traits). Each of the distances matrices produced in this study can be accessed in Supplementary Information 3 (SI3).

The analyses using datasets with biased (Figure 3A and Figure 4A) and with combined traits (Figure 3C and Figure 4C) show a cluster composed by Asian and Southeast Asian groups, a second cluster formed by North American and Circumpolar series, and a third cluster mostly formed by Mesoamerican and South American series. Greater Northwest coast is a constant outlier for North America, since it is within the expected variation for Mesoamerica/South America. Japan is also an outlier of the Asian cluster, standing between them and Mesoamerica/South Americans. Finally, in both Euclidean distances and Mean Measure of Divergence, the Brazilian coast series are within the Mesoamerica/South America cluster, with the wear bias pushing the series slightly away from this cluster.

However, the results using only unbiased traits (Figure 3B and Figure 4B) show important differences from the other analyses. In this case, there are only two clear clusters, one made of Asian and Southeast Asian groups, and another composed by Circumpolar, North American, Mesoamerican and South American series. This reduced number of traits reduces the ability of the analysis to discriminate among most of the geographical regions represented in the reference dataset, which suggests that the inclusion biased traits may be important to infer population structure within the Americas. In other words, this exercise illustrates the fact that removing wear-biased traits may sometimes be more harmful to the study of morphological affinities than their inclusion. Regarding our particular samples, the Brazilian Coast series, although closer to the Native American cluster, is still considerably distant from it, which to some extent may highlight some degree of inter-observer error between the first author of this study and Christy Turner II, who analyzed the worldwide comparative samples (Scott & Irish, 2017). Nevertheless, the Brazilian series appear close to each other, irrespective of the degree of wear considered, which shows that wear bias by itself is not enough to cause the association of series with another geographic region, as suggested before (Turner II, 2006; Turner II & Scott, 2013).

The Principal Component Analyses (Figure 5) show very similar results to Euclidean Distance and Mean Measure of Divergence and helps to identify traits responsible for the population structure within the Americas discussed previously. Shoveling UI1 and double shoveling UI1 are particularly relevant traits to distinguish between Circumpolar/North America and Mesoamerican/South American series, with frequencies being higher on Central and South Native American groups (Figure 5A and Figure 5C). As these traits are missing on the unbiased dataset (Figure 5B), the distinction between Circumpolar, North Americans, Mesoamericans, and South Americans is not evident. Finally, overall, these results reinforce that despite significant differences in frequencies due to dental wear, these differences are not strong enough to change the relative pattern of morphological affinities of the Brazilian series when inserted in a large comparative framework.

Nevertheless, among Brazilian Coast series with different amount of wear, there is a pattern where the subset using only teeth with moderate/severe wear is more separated from other groups (the only method where this pattern is not observed is on the Principal Component Analysis). This suggests that although using only teeth with moderate/severe wear may not change the overall interpretations of the morphological affinities of the series, it is still adding error to the interpretations, especially if the analysis is concerned with patterns of associations within smaller geographic scales.

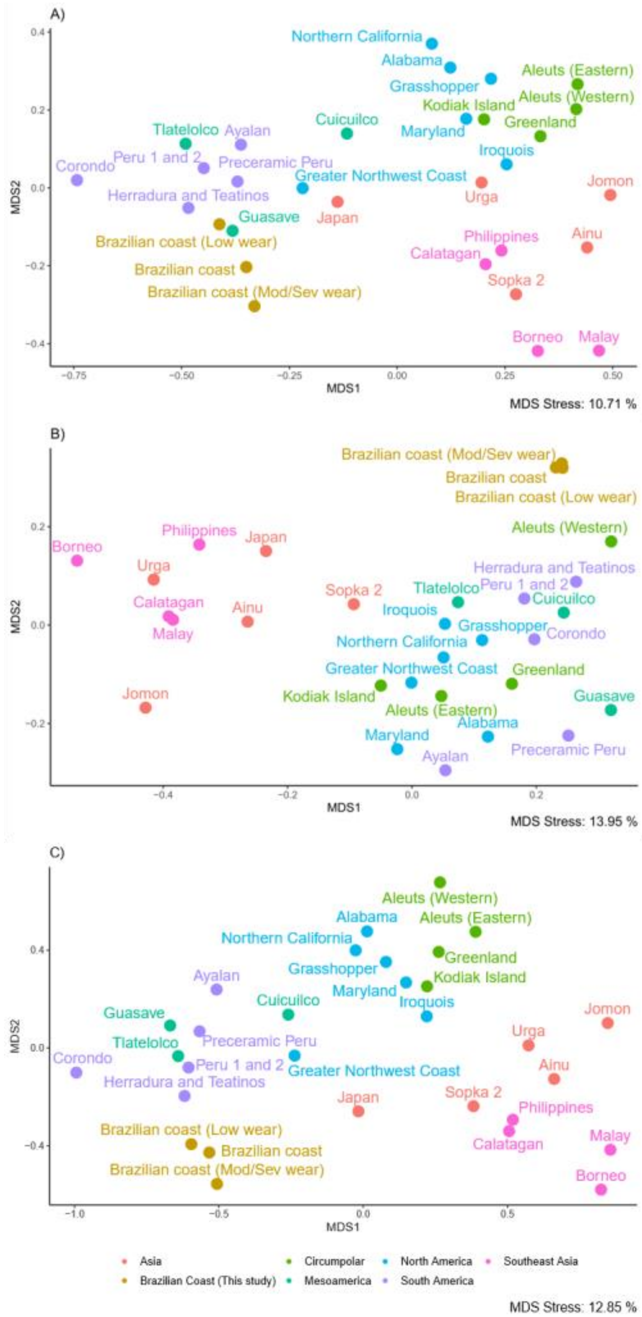

Figure 3. Kruskal non-metric multidimensional scaling of the Euclidean distance using: A) 7 wear-biased ASUDAS traits; B) 10 Unbiased ASUDAS traits; C) 16 ASUDAS traits (9 wear-unbiased and 7 wear-biased traits).

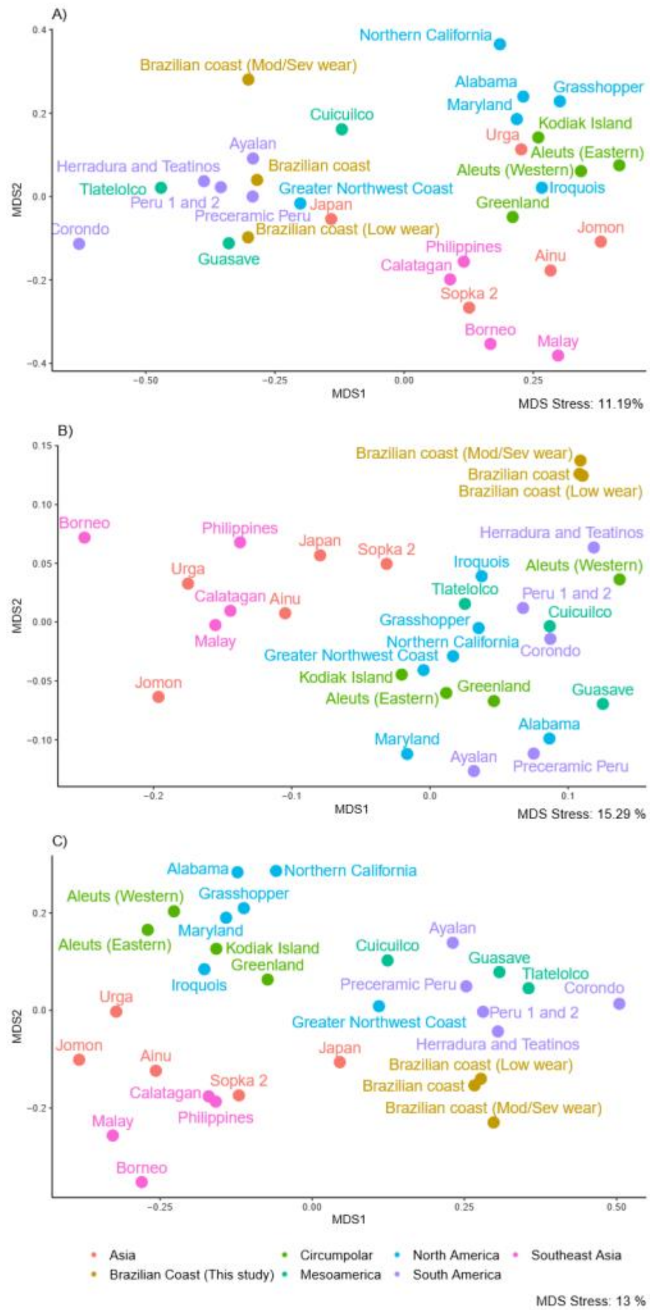

Figure 4. Kruskal non-metric multidimensional scaling of the Mean Measure of Divergence using: A) 7 wear-biased ASUDAS traits; B) 10 Unbiased ASUDAS traits; C) 16 ASUDAS traits (9 wear-unbiased and 7 wear-biased traits).

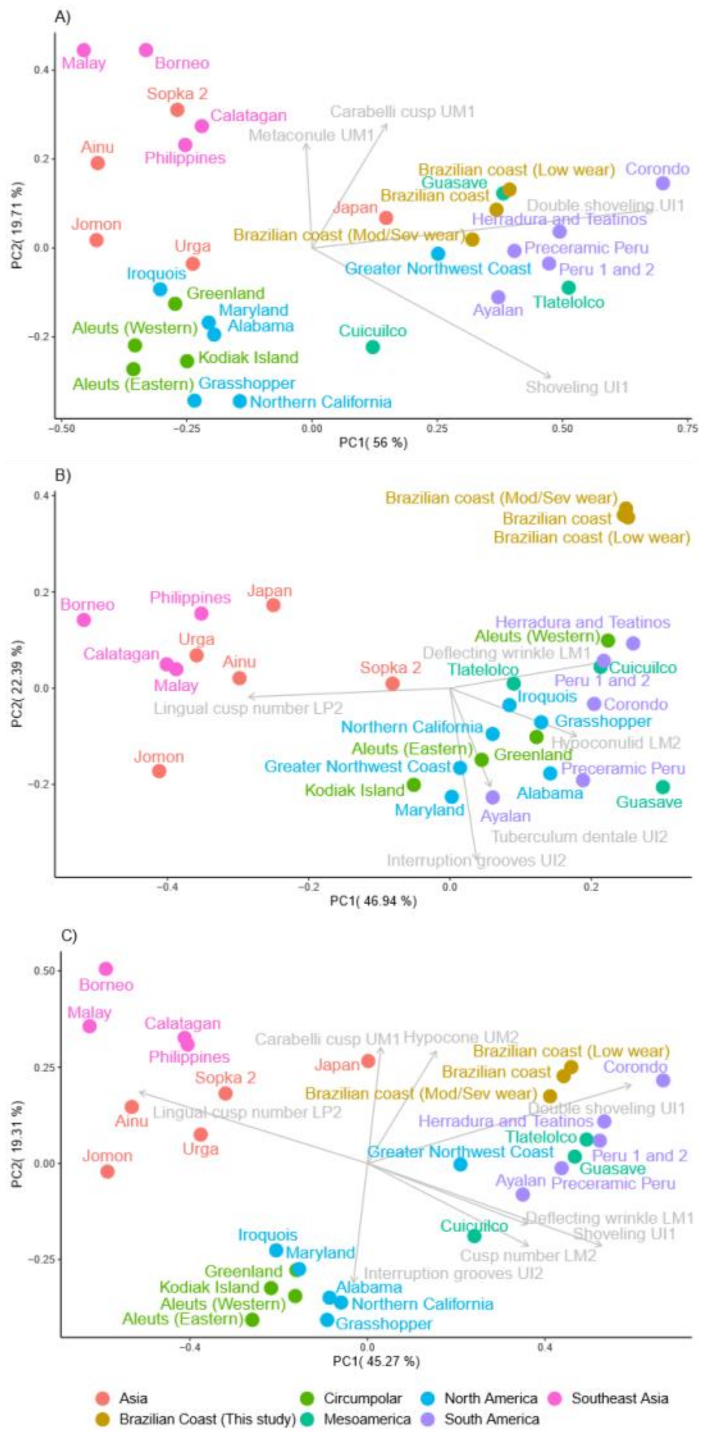

Figure 5. Principal component analysis. Gray arrows show variables most correlated (r>|0.5|) with each axis. A) 7 wear-biased ASUDAS traits; B) 10 Unbiased ASUDAS traits; C) 16 ASUDAS traits (9 wear-unbiased and 7 wear-biased traits).

Discussion

The results show that several traits in the Brazilian series present significantly different frequencies between low and moderate/severe wear groups (shoveling UI1, double shoveling UI1, metaconule UM1, Carabelli cusp UM1, hypocone UM2, anterior fovea LM1, protostylid LM1, entoconulid LM1). These differences can result in variation of up to 31.2% (anterior fovea LM1) of the frequency of the traits. However, when some of these traits are included in multivariate analyses along with other traits, this discrepancy is mitigated, as our series appear closely associated to each other in most analyses, despite the significant trait frequency differences among them. Discrepancies in the multivariate analyses are only relatively important when a series is composed exclusively of teeth with moderate/severe dental wear and when all traits show significant dental-wear bias. But even in these cases, our results do not indicate drastically different patterns of morphological affiliation of the Brazilian series. In reality, we see more important deviations from this pattern when biased traits are removed (see Figures 3B and 4B), suggesting that the removal of biased traits may not be always the ideal solution for studies of dental morphological affinities.

The main reason for these discrepant results, where individual traits show significant differences, but they do not impact the overall pattern of morphological affinities in multivariate space is due to the fact that individual trait frequencies have a small contribution to the overall position of the series in the multivariate space. Even though the frequency of traits can vary as much as 31.2% in some traits, this variation is only a small portion of the final distances between group or have a small contribution to principal component score of that group. Given that the wear bias for most variables is smaller than the average difference seen among the reference series (see Table 2), this small contribution of each trait to the final multivariate results does not significantly impact the pattern of morphological affinities among them. In other words, the wear bias in this case represents a small fraction of the total variance seen among series in the data.

These results support that, even though trait frequency differences should not be overlooked, wear-biased traits should still be considered in studies that are trying to contextualize the morphological affinities of series within larger comparative frameworks (i.e., in situations where it is expected that the wear bias is consistently smaller than the average differences among comparative series; see Table 2). Therefore, our results suggest that it is possible to contextualize better the validity of wear-biased traits in studies of morphological affinities, especially when these traits represent important components of the biological profile of populations. Shoveling UI1 and double shoveling UI1 are two examples of traits that have been noted to be wear biased in different independent studies (Burnett et al., 2013; Stojanowski & Johnson, 2015), including ours. However, they are also very important when characterizing dental variation patterns between Asian and Native American groups (Scott, Schmitz, et al., 2018; Turner II, 1990). When combined with other ASUDAS traits, although biased by dental wear (shoveling UI1, p=0.043, double shoveling UI1, p=0.042) they did not have a significant impact on the pattern of morphological affinities of the Brazilian series in relation to the Mesoamerica/South America cluster. Therefore, in response to the claims that dental wear may be responsible for the dental variation researchers have found within Native American groups (Turner II, 2006; Turner II & Scott, 2013), we argue that it seems rather unlikely, for it would require several traits to have wear-biased frequencies causing differences of the same order of magnitude of what is observed between continents, which is not the case in our analyses.

Our analyses do not show any strong morphological affinities among Native Americans and Southeast Asian groups (Scott & Turner II, 1997; Turner II & Scott, 2013). In this study, as in previous studies, Brazilian coast series are within the dental phenotypic variation of Native Americans (Turner II & Scott, 2013). This occurs in all multivariate analyses, independent of wear-biased traits, or sub-sampled series based on dental wear grades. This is another argument to take into account when excluding teeth or variables based in dental wear alone. In a large scale of analysis, if wear-bias is not very significant, and series are not composed exclusively by teeth with moderate/severe dental wear, removing worn teeth may cause the removal of important diagnostic traits, potentially resulting in more meaningful changes in morphological affinity patterns than if wear-biased traits are kept in the analyses. This is illustrated well by our analyses using only unbiased traits. Furthermore, this also offers some confidence to the interpretation of multivariate morphological affinities of series for which there is no precise information about their dental wear. Although it is often a standard data-collection procedure, not many studies report dental wear grades in dental morphological studies. Our study shows that, although this would be optimal to interpret possible discrepancies between series, it does not imply that such comparisons should not be made when the scale of the variance in the comparison framework is larger than the variance that results from wear bias. Caution must be taken, however, when contextualizing populations within smaller regional contexts, or within populations that share strong morphological affinities, as in these cases the wear-bias can be higher than the differences that define the biological affinities among series. Therefore, the scale of analysis is essential in making the decision of whether to included wear-biased traits, and we recommend that future studies consider the relationship between the variance in the data that can be the result of wear-bias and the variance that is the result of difference between series. As long as the latter is larger than the former, wear-biased traits can be informative of morphological affinities and could be considered in the analyses.

Finally, we agree with previous claims that dental wear is more susceptible to downgrading morphological traits (Turner II, 2006; Turner II & Scott, 2013). Out of the eight identified wear-biased traits, only 25% were biased towards increasing their frequency (2/8), and the remaining 75% (6/8) resulted in the underestimation of the frequencies. As occlusal wear increases, the features of each crown trait become less pronounced, leading the observer to score lower grades, when they should have been scored as not observable. This may occur partially due to the unconscious necessity of an observer to reach substantial sample sizes.

Conclusions

Our study corroborates previous studies showing dental wear bias is a valid concern when analyzing dental non-metric traits, and its assessment should become standard procedure in future studies whenever possible (Burnett et al., 2013). However, while wear-biased traits have an impact on trait frequencies, when combined with other variables, and in a large scale of analysis, its impact may be not meaningful in interpreting the patterns of morphological affinities among series. This impact is directly dependent on the scale of analysis, and regional studies must be more cautious in the inclusion of wear-biased traits, as in contexts with relatively small differences among groups, wear-bias can become meaningful. In other words, the scale of analysis is a key factor when deciding whether to use wear-biased traits.

We hope this study offers a more optimistic perspective about the impact of dental wear in dental morphological studies and gives a better perspective on how meaningful wear-related bias affects the interpretations of morphological affinities among past populations. Our study suggests that eliminating worn teeth by default may not always be the best solution, since it may exclude important discriminatory variables, or invalidate future studies due to a significant reduction on sample sizes.

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REFERENCES

Bhaskaran, K., & Smeeth, L. (2014). What is the difference between missing completely at random and missing at random? *International Journal of Epidemiology*, *43*(4), 1336–1339.

Burnett, S. E. (2016). Crown wear: Identification and categorization. In J. D. Irish & G. R. Scott (Eds.), *A Companion to Dental Anthropology* (First Edit, pp. 415–432). John Wiley & Sons, Inc.

Burnett, S. E., Irish, J. D., & Fong, M. R. (2013). Wear's the problem? Examining the effect of dental wear on studies of crown morphology. In G. R. Scott & J. D. Irish (Eds.), *Anthropological perspectives on tooth morphology: genetics, evolution, variation* (66th ed., pp. 535–554). Cambridge University Press.

Dahlberg, A. A. (1945). The Changing dentition of man. *The Journal of the American Dental Association*, *32*(11), 676–690.

Gamer, M., Lemon, J., & Singh, I. F. P. (2012). irr: Various coefficients of interrater reliability and agreement. R package version 0.84.1. https://cran.r-project.org/web/packages/irr/irr.pdf

Greenberg, J. H., Turner, C. G., Zegura, S. L., Campbell, L., Fox, J. A., Laughlin, W. S., Szathmary, E. J. E., Weiss, K. M., & Woolford, E. (1986). The settlement of the Americas: A comparison of the linguistic, dental, and genetic evidence [and comments and reply]. *Current Anthropology*, *27*(5), 477–497.

Hanihara, T. (2008). Morphological variation of major human populations based on nonmetric dental traits. *American Journal of Physical Anthropology*, *136*(2), 169–182.

Haydenblit, R. (1996). Dental variation among four pre-Hispanic Mexican populations. *American Journal of Physical Anthropology*, *100*(1), 225–246.

Hillson, S. (2005). *Teeth*. Cambridge, Cambridge University Press.

Houle, D., Pélabon, C., Wagner, G. P., & Hansen, T. F. (2011). Measurement and meaning in biology. *The Quarterly Review of Biology*, *86*(1), 3–34.

Hrdlička, A. (1920). Shovel-shaped teeth. *American Journal of Physical Anthropology*, *3*(4), 429–465.

Hrdlička, A. (1921). Further studies of tooth morphology. *American Journal of Physical Anthropology*, *4*(2), 141–176.

Hubbard, A. R., Guatelli-Steinberg, D., & Irish, J. D. (2015). Do nuclear DNA and dental nonmetric data produce similar reconstructions of regional population history? An example from modern coastal Kenya. *American Journal of Physical Anthropology*, *157*(2), 295–304.

Huffman, M. M. (2014). *Biological variation in South America populations using dental non-metric traits: assessment of isolation by time and distance*. The Ohio State University.

Irish, J. D., Lillios, K. T., Waterman, A. J., & Silva, A. M. (2017). "Other" possibilities? Assessing regional and extra-regional dental affinities of populations in the Portuguese Estremadura to explore the roots of Iberia's Late Neolithic-Copper Age. *Journal of Archaeological Science: Reports*.

Irish, J. D., Morez, A., Girdland Flink, L., Phillips, E. L. W., & Scott, G. R. (2020). Do dental nonmetric traits actually work as proxies for neutral genomic data? Some answers from continental- and global-level analyses. *American Journal of Physical Anthropology*.

Kaidonis, J. A. (2008). Tooth wear: the view of the anthropologist. *Clinical Oral Investigations*, *12* (Suppl 1), 21–26.

Kimura, R., Yamaguchi, T., Takeda, M., Kondo, O., Toma, T., Haneji, K., Hanihara, T., Matsukusa, H., Kawamura, S., Maki, K., Osawa, M., Ishida, H., & Oota, H. (2009). A common variation in EDAR is a genetic determinant of shovel-shaped incisors. *American Journal of Human Genetics*, *85*(4), 528–535.

Lahr, M. M., & Haydenblit, R. (1995). Traces of ancestral morphology in Tierra del Fuego and Patagonia. *Ameriacan Journal of Physical Anthropology*, *20*(Suppl), 128.

Landis, J., & Koch, G. (1977). The measurement of observer agreement for categorical data. *Biometrics, 33*(1), 159-174.

Marado, L. M. (2014). *Characterization of the dental morphology of a Portuguese sample from the 19th and 20th centuries*. University of Coimbra.

Marado, L. M. (2017). Dental nonmetric trait intraobserver precision: three observations of a large sample. *Anthropologischer Anzeiger*, *74*(1), 15–23.

Nichol, C. R., & Turner, C. G. (1986). Intra- and interobserver concordance in classifying dental morphology. *American Journal of Physical Anthropology*, *69*(3), 299–315.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P., Stevens, M. H. S., & Wagner, H. (2013). Package 'vegan.' Community Ecology Package, 2(9), 1–295. http://cran.r-project.org/, http://vegan.r-forge.r-project.org/

Ortiz, A. (2013). Dental morphological variation among six Pre-Hispanic South American populations with implications for the peopling of the New World. *Dental Anthropology*, *26*(1–2), 20–32.

Powell, J. F. (1995). *Dental variation and biological affinity among Middle Holocene human populations in North America*. Texas A & M University.

Powell, J. F. (1997). Variação dentária nas Américas: uma visão alternativa. *Revista USP*, *34*, 82–95.

Powell, J. F., & Neves, W. A. (1998). Dental diversity of early New World populations: Taking a bite out of the tripartite model. *American Journal of Physical Anthropology*, *26*, 179–180.

Powell, J. F., & Rose, J. C. (1999). Report on the osteological assessment of the "Kennewick Man" skeleton. In *Report on the Nondestructive Examination, Description and Analysis of the Human Remains from Columbia Park, Kennewick, Washington, 1999*.

R Core Team. (2020). *A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Rathmann, H., & Reyes-Centeno, H. (2020). Testing the utility of dental morphological trait combinations for inferring human neutral genetic variation. *Proceedings of the National Academy of Sciences*, *117*(20), 10769 LP – 10777.

Rathmann, H., Kyle, B., Nikita, E., Harvati, K., & Saltini Semerari, G. (2019). Population history of southern Italy during Greek colonization inferred from dental remains. *American Journal of Physical Anthropology*, *170*(4), 519–534.

Rathmann, H., Reyes-centeno, H., Ghirotto, S., Creanza, N., Harvati, K., Hanihara, T., & Harvati, K. (2017). Reconstructing human population history from dental phenotypes. *Scientific Reports*, *7*(1), 12495.

Scott, G. R. (1980). Population variation of Carabelli's trait. *Human Biology*, *52*(1), 63–78.

Scott, G. R., & Irish, J. D. (2017). *Human tooth crown and root morphology*. Cambridge, Cambridge University Press.

Scott, G. Richard, & Irish, J. D. (Eds.). (2013). *Anthropological perspectives on tooth morphology: Genetics, evolution, variation*. Cambridge, Cambridge University Press.

Scott, G. Richard, & Turner II, C. G. (1997). *The anthropology of modern human teeth: Dental morphology and its variation in recent human populations*. Cambridge, Cambridge University Press.

Scott, G. R., Anta, A., Schomberg, R., & de la Rúa, C. (2013). Basque dental morphology and the "Eurodont" dental pattern. In G.R. Scott & J.D. Irish (Eds.s), *Anthropological perspectives on tooth morphology* (pp. 296-318). Cambridge, Cambridge University Press.

Scott, G. Richard, Maier, C., & Heim, K. (2016). Identifying and recording key morphological (nonmetric) crown and root traits. In J. D. Irish & G. R. Scott (Eds.), *A companion to cental anthropology* (First Edit, pp. 245–264). John Wiley & Sons, Inc.

Scott, G. Richard, Pilloud, M. A., Navega, D., Coelho, J. d'Oliveira, Cunha, E., & Irish, J. D. (2018). rASUDAS: A New Web-Based Application for Estimating Ancestry from Tooth Morphology. *Forensic Anthropology*, *1*(1), 18–31.

Scott, G. Richard, Schmitz, K., Heim, K. N., Paul, K. S., Schomberg, R., & Pilloud, M. A. (2018). Sinodonty, Sundadonty, and the Beringian Standstill model: Issues of timing and migrations into the New World. *Quaternary International*, *466*, 233–246.

Smith, B. H. (1984). Patterns of molar wear in hunger-gatherers and agriculturalists. *American*

Journal of Physical Anthropology, *63*(1), 39–56.
Stojanowski, C. M., & Johnson, K. M. (2015). Observer error, dental wear, and the inference of new world sundadonty. *American Journal of Physical Anthropology*, *156*(3), 349–362.
Stojanowski, C. M., Johnson, K. M., & Duncan, W. N. (2013). Geographic patterns of Early Holocene New World dental morphological variation. *Dental Anthropology*, *26*(3), 7–15.
Sutter, R. C. (2005). The prehistoric peopling of South America as inferred from epigenetic dental traits. *Andean Past*, *7*(1), 183–217.
Sutter, R. C. (2009). Prehistoric population dynamics in the Andes. In J. Marcus & P. R. Williams (Eds.), *Andean civilization: a tribute to Michael E. Moseley* (Monograph, pp. 9–38). UCLA Cotsen Institute of Archaeology Press.
Tang, Y., Horikoshi, M., & Li, W. (2016). ggfortify: unified interface to visualize statistical results of popular R packages. *The R Journal*, *6*(2), 478–489.
Turner II, C. G. (1976). Dental evidence on the origins of the Ainu and Japanese. *Science*, *193* (4256), 911 LP – 913.
Turner II, C. G. (1989). Teeth and prehistory in Asia. *Scientific American*, *260*(2), 88–97.
Turner II, C. G. (1990). Major features of Sundadonty and Sinodonty, including suggestions about East Asian microevolution, population history and Late Pleistocene relationships with Australian aboriginals. *American Journal of Physical Anthropology*, *82*(1), 295–317.
Turner II, C. G. (2006). Dental morphology and the population history of the Pacific rim and basin: commentary on Hirofumi Matsumura and Mark J. Hudson. *American Journal of Physical Anthropology*, *130*(4), 455–461.
Turner II, C. G., & Scott, G. R. (1977). Dentition of Easter Islanders. In A. A. Dahlberg & T. M. Graber (Eds.), *Orofacial growth and development* (pp. 229–249). The Hague: Mouton.
Turner II, C. G., & Scott, G. R. (1977). Dentition of Easter Islanders. In A. A. Dahlberg & T. M. Graber (Eds.), *Orofacial growth and development* (pp. 229–249). The Hague: Mouton.
Turner II, C. G., & Scott, G. R. (2013). The dentition of American Indians: Evolutionary results and demographic implications following colonization from Siberia. In W. Henke & I. Tattersall (Eds.), *Handbook of paleoanthropology* (pp. 1–35). Springer Berlin Heidelberg.
Turner II, C. G., Nichol, C. R., & Scott, G. R. (1991). Scoring procedures for key morphological traits of the permanent dentition: The Arizona State University Dental Anthropology System. In M. A. Kelley & C. S. Larsen (Eds.), *Advances in dental anthropology* (pp. 13–31). Wiley-Liss, Inc.
Venables, W., & Ripley, B. (2002). *Modern applied statistics with S* (4th ed.). Springer.
Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.
Wu, L., & Turner, C. G. (1993). Brief communication: Variation in the frequency and form of the lower permanent molar middle trigonid crest. *American Journal of Physical Anthropology*, *91*(2), 245–248.