

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## An application of zooms to identify archaeological avian fauna from Teotihuacan, Mexico

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1955531> since 2024-02-12T13:39:35Z

*Published version:*

DOI:10.1016/j.jas.2022.105692

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

**AN APPLICATION OF ZOOMS TO IDENTIFY ARCHAEOLOGICAL AVIAN  
FAUNA FROM TEOTIHUACAN, MEXICO**

*To be submitted to Journal of Archaeological Science*

Maria C. Codlin<sup>1</sup>, Katerina Douka<sup>2,3</sup>, Kristine K. Richter<sup>3,4</sup>

<sup>1</sup> Department of Anthropology, Boston University, Boston, MA 02215, USA

<sup>2</sup> Department of Evolutionary Anthropology, Faculty of Life Sciences,  
University of Vienna, Vienna, Austria

<sup>3</sup> Max Planck Institute for the Science of Human History, Department of  
Archaeology, Jena, Germany

<sup>4</sup> Department of Anthropology, Harvard University, Cambridge, MA 02318,  
USA

mcodlin@bu.edu

katerina.douka@univie.ac.at

kkrichter@palaeome.org

**Corresponding author:**

Maria C. Codlin

[mcodlin@bu.edu](mailto:mcodlin@bu.edu)

675 Commonwealth Avenue, Suite 347  
Boston, Massachusetts 02215

26 DO NOT CITE IN ANY CONTEXT WITHOUT PERMISSION OF THE  
27 AUTHOR  
28

## 29    **1. Abstract**

30            The remains of aquatic birds often represent the best surviving  
31    evidence for prehispanic lake exploitation in highland Central Mexico, an  
32    important center of urban development with vast lacustrine resources. Yet  
33    unlike the sustained focus on turkey husbandry in Mesoamerican research,  
34    the economic importance of ducks and other lacustrine birds has received  
35    little attention. The diversity of birds in Central Mexico presents challenges to  
36    species identification from skeletal remains. To overcome these challenges,  
37    we present a new application of ZooMS, a collagen-based identification  
38    technique, to identify archaeological avian fauna from Teotihuacan. We  
39    develop the first database of avian biomarkers to include specimens across  
40    multiple taxonomic groups and apply ZooMS on 295 bone fragments to  
41    identify fragmentary and unidentified avian remains from the Tlajinga district  
42    of Teotihuacan. Our results indicate that ZooMS has good potential to identify

43 avian fauna to at least the family level and that the residents of Tlajinga

44 exploited a range of aquatic birds.

45

46

47 **Keywords:** ZooMS, Teotihuacan, aves, birds, collagen, aquatic

48

## 49 2. Introduction

50 Postclassic (900-1519 CE) communities in Central Mexico heavily  
51 exploited aquatic birds, fish and insects from the expansive lacustrine system  
52 in the Basin of Mexico (Figure 1, De Lucia, 2021; de Sahagún et al., 1963;  
53 Hirth, 2016, p. 28; Parsons, 2010, 2008). However, there is little evidence for  
54 how these lake exploitation economies developed over time, or their role in  
55 supporting the large, densely settled city of Teotihuacan, which dominated  
56 the region during the Classic period (ca. BCE 100 – 550/600 CE) (Sugiyama et  
57 al., 2017; Valadez Azúa, 2013; Widmer and Storey, 2016). Aquatic birds  
58 provide the most direct evidence for the exploitation of lake resources, as the  
59 remains of small lake fish, insects and crustaceans are rarely recovered  
60 archaeologically. Yet traditional zooarchaeological identification of birds in  
61 Central Mexico is challenging, hindered by large groups of winter migratory  
62 birds (Ayala-Pérez et al., 2013; Gamboa et al., 2017; Peterson and Navarro  
63 Sigüenza, 2006) and high species diversity in this center of avian endemism

(Howell and Webb, 1995, p. 15). To address these challenges, we explore the potential of palaeoproteomics, and specifically ZooMS (Zooarchaeology by Mass Spectrometry), to identify archaeological birds from Teotihuacan while also developing new collagen peptide biomarkers for North American avifauna.

ZooMS is a collagen-based method for taxonomic identification of animals based on amino acid substitutions, called single amino acid polymorphisms (SAPs), within Type I collagen—the primary organic component of bone (Buckley et al., 2009; Richter et al., 2022; Welker et al., 2015). Collagen is extracted, digested with the enzyme, trypsin, and analyzed on a matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometer. The resulting spectra are analyzed against lists of reference peptide markers that vary across taxa. In comparison to aDNA analysis, ZooMS is rapid, low-cost, and requires very little bone, allowing for high-throughput analysis and application to extremely small or fragmentary

remains (Buckley et al., 2016; Richter et al., 2011; Speller et al., 2016; Wang et al., 2021). ZooMS, therefore, provides taxonomic identification where traditional zooarchaeological methods could be limited, especially for fragmentary remains and morphologically similar species.

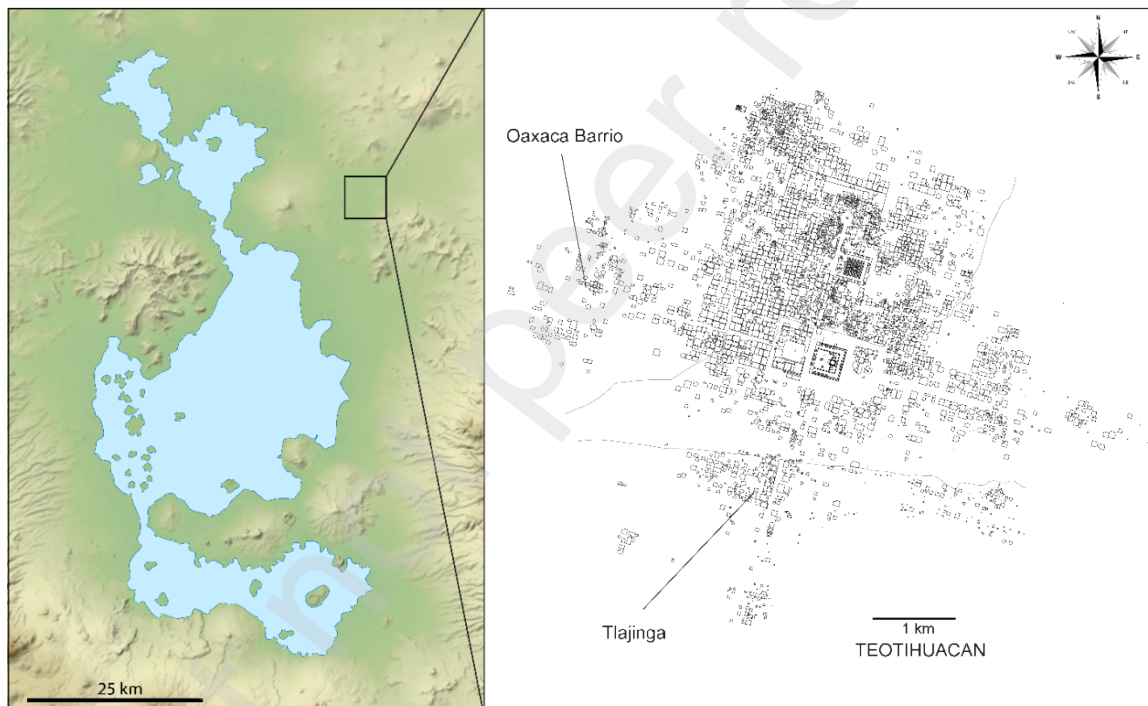


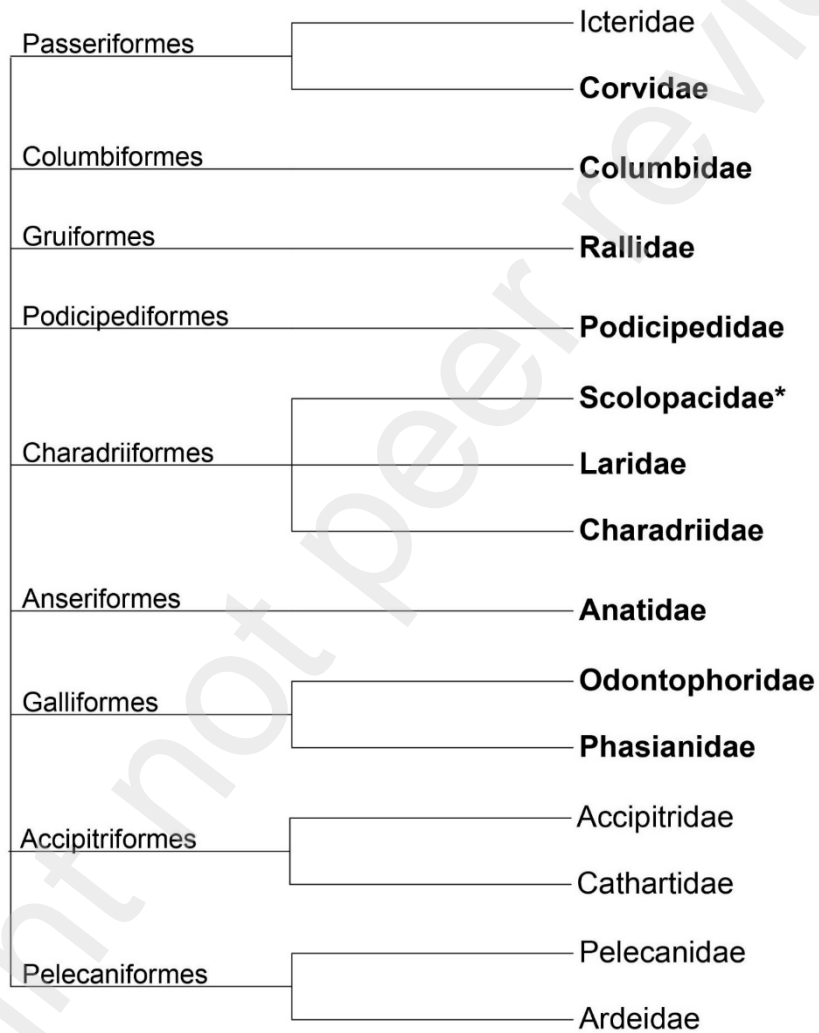
Figure 1. Location of Teotihuacan in the Basin of Mexico. Left: Lake system in Basin of Mexico prior to European contact (after *Lago de Texcoco Posclásico* 2007 by Yavidaxiu, and derivative work by historicaire and Sémhur, CC BY-SA 4.0 via Wikimedia Commons). Right: Map of Teotihuacan (after Millon 1973).



While diagnostic markers have been established to discriminate among many types of mammals, fish and reptiles (Buckley and Kansa, 2011; Harvey et al., 2019b, 2018; Janzen et al., 2021; Peters et al., 2021; Richter et al., 2020; Speller et al., 2016; van der Sluis et al., 2014), the application of ZooMS for avian fauna has been minimal. As Eda et al. (2020) note, this lack of interest stems largely from the slower collagen mutation rate in birds compared to mammals, which limits the number of mutations available to distinguish among taxonomic groupings (Buckley, 2018; Richter et al., 2022). However, despite the slow mutation rate, avian collagen has potential for taxonomic identification at the family (Horn et al., 2019) and sub-family levels (Eda et al., 2020). As of 2022, two peptide markers (COL1 $\alpha$ 2-502 and COL1 $\alpha$ 2-889, nomenclature after Brown et al. 2020) had been published that discriminate four domesticated fowl, including mallard duck (*Anas platyrhynchos*) and three members of the Phasianidae family: chicken (*Gallus gallus*), Japanese quail (*Phasianus coturnix*), and turkey (*Meleagris gallopavo*) (Buckley, 2018;

104 Buckley et al., 2009). Recently, a more detailed study of peptide COL1 $\alpha$ 2-889  
105 noted two variations useful in discriminating archaeological chicken and  
106 indigenous pheasants in Japan (Eda et al., 2020), demonstrating the utility of  
107 ZooMS to address specific archaeological questions. However, two genera of  
108 indigenous pheasants of the Phasianidae family, *Phasianus* and *Syrnaticus*,  
109 were indistinguishable based on this peptide, and the marker used to identify  
110 *Phasianus* is also found in other families of birds (Eda et al., 2020). This  
111 demonstrates that relying on single peptide markers can limit the usefulness  
112 of ZooMS in areas with high avian diversity and highlights the need for further  
113 work on characterizing the collagen sequences of avian fauna.

114 We use these findings as a starting point to examine publicly available  
115 collagen sequences and reference modern samples to develop ZooMS  
116 markers that discriminate among 15 families, representing nine orders of  
117 birds that are frequently found in archaeological sites across the Americas  
118 (Figure 2).



121

122 Figure 2. Phylogenetic tree of avian orders and families represented by modern and archaeological  
 123 specimens (from itol.embl.de, v. 6.5.2, Letunic and Bork, 2021). Individuals from families in bold were  
 124 analyzed by LC-MS/MS. \*Scolopacidae family was identified through comparison of LC-MS/MS  
 125 sequences to collagen sequences for this family.

126

### 127 3. Site Description: Teotihuacan, Mexico

128 Teotihuacan was a large, densely populated urban center, and one of  
129 the few cities in the Americas to have a population of 100,000 or more prior to  
130 European arrival (Figure 1). The city rose around 100 BCE and by its height in  
131 250-550 CE had consolidated a large multiethnic population and presided  
132 over a network of communication corridors that connected the city to natural  
133 resources, trade goods, and other Mesoamerican cultures (Carballo, 2013;  
134 Hirth, 2020; Sanders et al., 1979). Parsons (2010) argued that lake  
135 exploitation in the Basin of Mexico would have intensified during this period  
136 to support growing urban populations and the expansion of settlements into  
137 landscapes that supported maguey (*Agave*) production, but otherwise had  
138 limited agricultural potential. Yet current estimates of aquatic resources at  
139 Tlajinga indicate waterfowl made up no more than 4% of the animals  
140 consumed in the city (Sugiyama et al., 2017, p. 66). On average, aquatic  
141 species make up 33% of the birds identified in residential areas of

142 Teotihuacan, while turkeys alone comprise 45% (Sugiyama et al., 2017, Table  
143 ESM1).

144 Recent excavations at Tlajinga, on the southern periphery of the city,  
145 provides new material to examine avian diversity and the importance of  
146 aquatic birds to urban subsistence (Carballo et al., 2019). The faunal analysis  
147 of these materials is described elsewhere (Codlin, in prep), but in brief, the  
148 assemblage contained 613 fragments of bird bone, only 282 of those were  
149 identified to a taxonomic group.

## 150 **4. Materials and methods**

### 151 *4.1. Modern reference samples*

152 Approximately 10-20 mg of bone was sampled from non-diagnostic  
153 skeletal portions, primarily ribs of 31 modern North American bird specimens  
154 (collections from Boston University and the American Museum of Natural  
155 History [AMNH]). The samples included 17 species of bird from 12 families  
156 commonly identified in bird checklists and archaeological sites in highland

157 Central Mexico: Accipitridae, Anatidae, Ardeidae, Cathartidae, Columbidae,  
158 Corvidae, Icteridae, Laridae, Odontophoridae, Pelecanidae, Podicipedidae,  
159 Rallidae (Table S1). Where possible, multiple individuals from the same  
160 species were sampled.

#### 161 4.2. *Archaeological samples*

162 Two-hundred and ninety-five (n=295) archaeological specimens were  
163 sampled from Tlajinga, and from a nearby neighborhood, the Oaxaca Barrio  
164 (Tlailotlacan). We sampled 259 avian bones from two adjacent apartment  
165 compounds at Tlajinga (17:S3E1 and 18:S3E1), including 192 unidentifiable  
166 specimens and 67 specimens identified morphologically at least to taxonomic  
167 order (e.g. Galliformes). An additional 36 specimens, including five identified  
168 minimally to order, were collected from faunal material from salvage  
169 excavations at the Oaxaca Barrio (Ortega Cabrera, 2012, 2010, 2009).  
170 Approximately 25% of the samples selected from both sites were identified  
171 minimally to the level of family. Material from the Oaxaca Barrio is included in

172 ZooMS analysis, but the archaeological implications are not considered here.  
173 Faunal material from both excavations was recovered in the field using 5 mm  
174 screens, meaning that small birds, including quails, are likely to be under-  
175 represented in the dataset (Tellkamp, 2019). For all archaeological samples,  
176 small fragments of bone, weighing 10-50 mg, were removed from non-  
177 diagnostic portions of bone for analysis.

#### 178 4.3. *Bird collagen database*

179 Avian collagen sequences were downloaded from UniProt and NCBI;  
180 these include data from avian genome sequencing (Feng et al., 2020; Jarvis et  
181 al., 2014; Zhang et al., 2014). The sequences were aligned and compared to  
182 the reference chicken sequences (COL1A1 - P02457, COL1A2 - P02467)  
183 using Jalview (see data at: 10.5281/zenodo.6363113) The sequence data was  
184 theoretically digested with trypsin using the Bacollite R package (v. 1.0,  
185 Hickinbotham et al., 2020) allowing the following post-translational

modifications: oxidation (+15.9949 Da) of proline and deamidation (+0.9840 Da) of asparagine and glutamine.

#### 4.4. Collagen sequencing and peptide mass fingerprinting

Collagen was extracted and digested using established methods (Brown et al., 2020b; Buckley et al., 2009; Welker et al., 2015). Briefly, samples were incubated in 0.6 M hydrochloric acid (HCl) overnight, washed with 50 mM ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) pH 8.0 (AmBic), and incubated briefly in 0.1M of sodium hydroxide (NaOH) before gelatinization in AmBic at 65°C for one hour and digestion with trypsin. Samples were then diluted and spotted 1:1 with  $\alpha$ -cyano-4-hydroxycinnamic acid and analyzed on a Bruker Autoflex Speed LRF MALDI-TOF Mass Spectrometer located at the Max Planck Institute for the Science of Human History, Jena, Germany.

198



199    4.5.    *Marker ID and MS/MS confirmation*

200            A list of candidate marker peaks was generated from visual comparison  
201    of the spectra using mMass (v 5.5.0, Strohalm et al., 2010) and differences in  
202    the theoretical peptide masses generated from Bacollite. One individual from  
203    each pattern of markers identified in the archaeological assemblage (n=14)  
204    was analyzed using LC-MS/MS (Lumos Orbitrap, Mass Spectrometry Facility,  
205    University of Massachusetts Medical School) to confirm the sequences of  
206    candidate markers. Where possible, the matching reference specimens were  
207    also analyzed (n=9).

208            LC-MS/MS data was processed using Byonic (v. 3.4, Bern et al., 2012)  
209    allowing for oxidations of methionine, proline, and lysine, deamidation of  
210    asparagine and glutamine, and acetylation on N-terminal glutamine and  
211    glutamic acid. First, proteins present in the samples were identified using a  
212    database consisting of SwissProt (1/20/2022), whole proteomes from 13  
213    species of bird, plus available avian proteins from UniProt (Table S2).

214 Focused databases for each sample were generated using a protein FDR of  
215 2%. Second, to identify novel collagen peptides, assisted *de novo* sequencing  
216 was conducted using error tolerant searching in Byonic against a database  
217 containing 13 regions of interest from avian COL1 $\alpha$ 2-sequences. Third,  
218 marker confirmation was conducted against a database consisting of the  
219 sequences from the focused databases, excluding COL1 sequences, curated  
220 avian COL1 sequences, and novel collagen peptides from *de novo*  
221 sequencing. Markers were considered confirmed if there were 2 or more  
222 peptide spectral matches with PEP 2D scores below 0.001 with coverage of  
223 the SAP locations (Table S3).

#### 224 4.6. *Identification of archaeological samples and clustering*

225 Clustering and sample identification was conducted using R version  
226 4.1.1 (R Core Team, 2021) and the package MALDI-Quant (v 1.2, Gibb and  
227 Strimmer, 2012) after validation of the parameters used against mMass.  
228 Spectra underwent smoothing, baseline removal, calibration, peak picking,

229 and deisotoping before filtering for number of peaks between 50 and 90, as  
230 peak lists outside of this range correspond to poor quality spectral data.

231 Technical replicates of the resulting spectra were then averaged and the  
232 averaged spectrum for each sample was peak picked and deisotoped. A  
233 binary matrix that recorded the presence or absence of markers (combining  
234 masses of amidated, deamidated, and oxidized versions where possible) was  
235 created from the markers confirmed by LC-MS/MS in the peak lists. The  
236 matrix was used for hierarchical clustering (stats package, R Core Team,  
237 2021) with 20 groups. Spectra in these groups were then visually analyzed for  
238 homogeneity and taxonomic assignment. Low quality spectra which were  
239 previously filtered out were then visually inspected to see if taxonomic  
240 assignment was possible. The complete MALDI-quant workflow is available  
241 at 10.5281/zenodo.6366234. ZooMS data was the integrated with  
242 zooarchaeological data to generate Number of Identified Specimens (NISP)  
243 and Minimum Number of Individuals (MNI) (Lyman, 2008; Table S5).

## 244 **5. Peptide biomarkers for avifauna**

### 245 *5.1. Peptide biomarkers and their taxonomic resolution*

246 We confirmed 59 peptide sequence variations across 12 locations using  
247 LC-MS/MS, corresponding to 71 MALDI marker peaks for the identification of  
248 avian taxa (Table 1 and Table S3). These markers can successfully  
249 discriminate among 15 families based upon data from protein sequences and  
250 modern and archaeological samples (Table 2). Markers in the Anatidae,  
251 Charadriidae, Scolopacidae, Laridae, Podicipedidae, Rallidae, Phasianidae,  
252 Odontophoridae, Corvidae, and Columbidae families were confirmed using  
253 LC-MS/MS. Markers in Accipitridae, Cathartidae, Pelecanidae, Ardeidae, and  
254 Icteridae families are only candidate markers derived from ZooMS spectra  
255 and sequence data.

256 Not all the 71 markers identified are equally useful for identification.

257 As reported in other taxonomic groups, some peptide variants are poorly  
258 visualized in the MALDI either for only modern species or both modern and

archaeological. Other variants overlap with each other, other collagen peptides, or common contaminants. For the marker peaks which overlap with common contaminants ( $m/z$  1193.6,  $m/z$  1566.7, and  $m/z$  2108), LC-MS/MS analysis identified none of the contaminant peptides present in any of our samples. Moreover, no highly diagnostic marker peaks overlap with common contaminants, although care should be taken when interpreting the diagnostic peak for turkey,  $m/z$  1622.6, which is 1 Da removed from peptide derived from the self-digestion of trypsin at  $m/z$  1623.8. In addition, we identified several peptides which are consistently identified as deamidated in the MALDI and LC-MS/MS across all samples (e.g. COL1 $\alpha$ 2-175 and COL1 $\alpha$ 2-520). The most distinct peaks that can be used for identification are highlighted in bold in Table 1.

271 Table 1. Peaks for COL1 $\alpha$ 2 peptide markers identified for birds

Taxonomic ID	Sample #	10-42 <sup>b</sup>		175-192 <sup>b</sup>	292-309 <sup>d</sup>	454-483 <sup>c</sup>	502-519	520-555 <sup>b</sup>	
Anatidae 1	MC148	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
<i>Anas platyrhynchos</i>	MC2	3097.4	3113.4	1603.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	
Anatidae 2	MC123	3097.4	3113.4	1603.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
Anatidae 3	MC182	3097.4	3113.4	1603.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
<i>Oxyura jamaicensis</i>	MC16	3097.4	3113.4	1589.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
Anatidae 4	MC171	3097.4	3113.4	1589.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
<i>Podilymbus podiceps</i>	MC37	<b>3106.4</b>	<b>3122.4</b>	1589.8	1596.8	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
Podicipedidae	MC187	<b>3106.4</b>	<b>3122.4</b>	1589.8	1596.8	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
<i>Gallinula galeata</i>	MC26	3097.4	3113.4	1589.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
<i>Fulica americana</i>	MC30	3097.4	3113.4	1589.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
Rallidae	MC110	3097.4	3113.4	1589.8	1609.7	<b>2804.3</b>	1578.8	<b>3152.4</b>	<b>3168.4</b>
<i>Leucophaeus attricilla</i>	MC28	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	<b>1552.8</b>	<b>3206.5</b>	<b>3222.5</b>
Laridae	MC300	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	<b>1552.8</b>	<b>3206.5</b>	
<i>Charadrius semipalmatus</i>	MC20		<b>3106.4</b>	1589.8	1609.7	<b>2777.3</b>	1578.8	<b>3156.4</b>	<b>3172.4</b>
Scolopacidae <sup>a</sup>	MC232	<b>3071.4</b>	<b>3087.4</b>	1589.8	1609.7	<b>2777.3</b>	<b>1608.8</b>	<b>3166.4</b>	<b>3182.4</b>
<i>Meleagris gallopavo</i>	MC39	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	<b>1622.9</b>	<b>3192.5</b>	<b>3208.5</b>
<i>Gallus gallus</i> <sup>a</sup>	MC114	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	1594.8	<b>3192.5</b>	<b>3208.5</b>
<i>Colinus virginianus</i>	MC32	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	1594.8	<b>3182.4</b>	<b>3198.5</b>
<i>Colinus virginianus</i>	MC207	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	1594.8	<b>3182.4</b>	<b>3198.4</b>
Odontophoridae	MC331	3097.4	3113.4	1603.8	1609.7	<b>2777.3</b>	1594.8	<b>3192.5</b>	
<i>Zenaida macroura</i>	MC23	3097.4	3113.4	1596.8	1579.7	<b>2804.3</b>	1578.8	<b>3150.4</b>	
Columbidae	MC129	3097.4	3113.4	1596.8		<b>2804.3</b>	1578.8	<b>3150.4</b>	<b>3166.4</b>
<i>Corvus corax</i>	MC349	<b>3111.4</b>	<b>3127.4</b>	1603.8	<b>1608.8</b>	<b>3166.5</b>	<b>1552.8</b>	<b>3166.5</b>	<b>3182.4</b>
Unconfirmed markers									
<i>Accipiter cooperii</i>	MC9	3097.4	3113.4	1603.8	1580*	2804.3	1552.8*	3152.4*	3168.4*
<i>Buteo jamaicensis</i>	MC18	3097.4	3113.4	1603.8	1580*	2804.3	1552.8*	3152.4*	3168.4*
<i>Cathartes aura</i>	MC11	3097.4	3113.4	1589.8	1609.7	2804.3			
<i>Ardea alba</i>	MC13	3097.4	3113.4	1589.8	1596.8*	2804.3	1578.8*	3152.4*	3168.4*
<i>Egretta thula</i>	MC25	3097.4	3113.4	1589.8	1596.8*	2804.3	1578.8*	3152.4*	3168.4*

<i>Quiscalus</i>									
<i>mexicanus</i>	MC21	3111.4	3127.4	1603.8	1608.8	2777.3	1578.8	3166.5	
<i>Pelecanus</i>									
<i>erythrorhynchos</i>	MC35	3097.4	3113.4	1603.8	1596.8*	2804.3*	1552.8	3152.4*	3168.4*

272

273 The most diagnostic and least ambiguous peaks are in **bold**. Non-bolded peaks may be  
 274 identical to peaks from other peptide locations and should only be used to support  
 275 identifications based on other markers. The unconfirmed markers are based upon MALDI and  
 276 sequence data, but have not been confirmed by LC-MS/MS. Labelling of peptides follows  
 277 Brown et al. (2020a). These markers are all on the COL1a2 chain.

278

279 \* denotes markers not visible in the MALDI, but expected based on collagen sequences of  
 280 related taxa.

281 <sup>a</sup> Taxonomic identification is based on comparison to publicly available collagen sequences. <sup>b</sup>

282 Peak appears most strongly at deamidated version. <sup>c</sup> Flies poorly in MALDI modern

283 specimens. <sup>d</sup> This marker is not consistently identified in LC-MS/MS analysis. However, this

284 marker does fly in the MALDI and often present at identical masses to other markers. Peak

285 appears most strongly at deamidated version. <sup>e</sup> Appears inconsistently in LC-MS/MS and

286 MALDI when no proline oxidation is present but has identical masses to some other markers.

287 <sup>f</sup> Appears inconsistently in LC-MS/MS, however most birds in available collagen sequences

288 have the peptide sequence responsible for peak at  $m/z$  1221.6.  $m/z$  1225.6 is diagnostic in

289 MALDI and was confirmed in LC-MS/MS.

290 Table 1. *continued*

291

Taxonomic ID	Sample #	604-618 <sup>f</sup>	625-648 <sup>c</sup>	625-653 <sup>c</sup>	658-687	757-789	889-906 <sup>e</sup>	978-990 <sup>b</sup>	
Anatidae 1	MC148	1221.6	2108	2466.2	2511.3	2985.5	1616.8	1632.8	1192.6
<i>Anas platyrhynchos</i>	MC2		2108	2466.2	2511.3	2969.5	1616.8	1632.8	1192.6
Anatidae 2	MC123	1221.6	2108	2466.2	2511.3	2969.5	1616.8	1632.8	1192.6
Anatidae 3	MC182	1221.6	2108	2466.2	2511.3	2984.5		1632.8	1192.6
<i>Oxyura jamaicensis</i>	MC16	1221.6	2108	2466.2	2511.3	2927.5		1660.8	1192.6
Anatidae 4	MC171	1221.6	2108	2466.2	2511.3	2927.5	1644.8	1660.8	1192.6
<i>Podilymbus podiceps</i>	MC37	1221.6	2108	2466.2	2497.2	2927.5	1550.8	1566.8	1220.6
Podicipedidae	MC187	1221.6	2108	2466.2	2497.2	2927.5	1550.8	1566.8	1220.6
<i>Gallinula galeata</i>	MC26	1221.6	2108	2466.2	2497.2	2985.5	1578.8	1594.8	1220.6
<i>Fulica americana</i>	MC30	1221.6	2108	2466.2	2497.2	2985.5	1578.8	1594.8	1220.6
Rallidae	MC110	1221.6	2108	2466.2	2497.2	2985.5	1578.8	1594.8	1220.6
<i>Leucophaeus attricilla</i>	MC28	1221.6	2108	2466.2	2531.2	2927.5	1550.8	1566.8	1220.6
Laridae	MC300	1221.6	2108		2531.2	2927.5	1550.8	1566.8	1220.6
<i>Charadrius semipalmatus</i>	MC20	1221.6	2108	2466.2	2511.3	2955.5	1550.8	1566.8	1220.6
Scolopacidae <sup>a</sup>	MC232	1221.6	2108	2466.2	2559.2	2985.5	1550.8	1566.8	1220.6
<i>Meleagris gallopavo</i>	MC39	1221.6	2108	2466.2	2539.3	2927.5	1578.8	1594.8	1220.6
<i>Gallus gallus</i> <sup>a</sup>	MC114	1221.6	2108		2539.3	2927.5	1604.8	1620.8	1220.6
<i>Colinus virginianus</i>	MC32	1221.6	2108	2466.2	2539.3	2927.5	1550.8	1566.8	1220.6
<i>Colinus virginianus</i>	MC207	1221.6	2108	2466.2	2539.3	2927.5	1550.8	1566.8	1220.6
Odontophoridae	MC331	1221.6	2108	2466.2	2539.3	2927.5	1550.8	1566.8	1220.6
<i>Zenaida macroura</i>	MC23		2108	2466.2	2497.2	2881.5	1592.8	1608.8	1192.6
Columbidae	MC129	1225.6	2108	2466.2	2497.2	2881.5		1608.8	1192.6
<i>Corvus corax</i>	MC349	1221.6	2135	2493.2	2525.3	2927.5		1580.8	1220.6
Unconfirmed markers									
<i>Accipiter cooperii</i>	MC9	1221.6	2108*	2466.2*	2511.3	2913	1578.8	1594.8	1220.6
<i>Buteo jamaicensis</i>	MC18	1221.6	2108*	2466.2*	2511.3	2913	1578.8	1594.8	1220.6
<i>Cathartes aura</i>	MC11	1221.6			2497.2	2913	1578.8	1594.8	1220.6



<i>Ardea alba</i>	MC13	1221.6	2108*	2466.2*	2511.3	2939	1550.8	1566.8	1220.6
<i>Egretta thula</i>	MC25	1221.6	2108*	2466.2*	2511.3	2939	1550.8	1566.8	1220.6
<i>Quiscalus</i>	MC21	1221.6			2511.3	2927.5			1220.6
<i>mexicanus</i>									
<i>Pelecanus</i>	MC35	1221.6	2108*	2466.2*	2497.2	2927.5	1578.8	1594.8	1220.6
<i>erythrorhynchos</i>									

292

293 *See notes on previous page*

294

295 Table 2. Summary of taxa analyzed and comparison of morphological and ZooMS  
 296 identifications

Order	Family	ID type	Morphological identification	ZooMS identification
Anseriformes	Anatidae	Modern	<i>Anas platyrhynchos</i>	Anatidae 2 <i>Oxyura</i> sp.
Anseriformes	Anatidae	Modern	<i>Oxyura jamaicensis</i>	(Anatidae 4)
Anseriformes	Anatidae	Archaeological	Anatidae	Anatidae 1
Anseriformes	Anatidae	Archaeological	Anatidae	Anatidae 3
Anseriformes	Anatidae	Archaeological	Anatidae	Anatidae 5*
Anseriformes	Anatidae	Archaeological	Anatidae	Anatidae 6*
Columbiformes	Columbidae	Modern	<i>Zenaida macroura</i>	Columbidae
Columbiformes	Columbidae	Modern	<i>Columbina talpacoti</i>	Columbidae <sup>+</sup>
Columbiformes	Columbidae	Archaeological	Columbidae	Columbidae
Charadriiformes	Scolopacidae	Archaeological	Charadriiform	Scolopacidae*
Charadriiformes	Charadriidae	Modern	<i>Charadrius semipalmatus</i>	Charadriidae
Charadriiformes	Laridae	Modern	<i>Leucophaeus attricilla</i>	Laridae
Charadriiformes	Laridae	Archaeological	Laridae	Laridae
Passeriformes	Corvidae	Archaeological	<i>Corvus corax</i>	Corvidae
Passeriformes	Icteridae	Modern	<i>Quiscalus mexicanus</i>	Icteridae <sup>2</sup>
Podicipediformes	Podicipedidae	Modern	<i>Podilymbus podiceps</i>	Podicipedidae
Podicipediformes	Podicipedidae	Archaeological	<i>Podiceps nigricollis</i>	Podicipedidae
Galliformes	Odontophoridae	Modern	<i>Colinus virginianus</i>	<i>Colinus virginianus</i>
Galliformes	Odontophoridae	Archaeological	Odontophoridae	Odontophoridae
Gruiformes	Rallidae	Modern	<i>Fulica americana</i>	Rallidae
Gruiformes	Rallidae	Modern	<i>Gallinula galeata</i>	Rallidae
Galliformes	Phasianidae	Archaeological	Phasianidae	<i>Gallus gallus</i> * <i>Meleagris</i>
Galliformes	Phasianidae	Archaeological	<i>Meleagris gallopavo</i>	<i>gallopavo</i>
Accipitriformes	Accipitridae	Modern	<i>Accipiter cooperii</i>	Accipitridae <sup>+</sup>
Accipitriformes	Accipitridae	Modern	<i>Buteo jamaicensis</i>	Accipitridae <sup>+</sup>
Accipitriformes	Cathartidae	Modern	<i>Cathartes aura</i> <i>Pelecanus</i>	Cathartidae <sup>+</sup>
Pelecaniformes	Pelecanidae	Modern	<i>erythrorhynchos</i>	Pelecanidae <sup>+</sup>
Pelecaniformes	Ardeidae	Modern	<i>Egretta thula</i>	Ardeidae <sup>+</sup>

Pelecaniformes Ardeidae Modern *Ardea alba* Ardeidae+

297

298 \* Taxonomic identification is based only on sequence data and similarities to other

299 analyzed taxa, not MALDI reference data. + ZooMS identification is estimate as

300 markers are not confirmed by LC-MS/MS

301

### 302 5.1.1. Galliformes

303 Galliformes includes two families important for urban subsistence at  
304 Teotihuacan, Phasianidae and Odontophoridae. Reference samples were  
305 collected from *Colinus virginianus* (northern bobwhite) while *Meleagris*  
306 *gallopavo* (turkey) and *Gallus gallus* (chicken) are available in collagen  
307 sequence data. While no peptides can clearly discriminate the two families,  
308 COL1 $\alpha$ 2-889, COL1 $\alpha$ 2-520, and COL1 $\alpha$ 2-502 allow us to discriminate among  
309 *Gallus*, *Meleagris*, and *Colinus*, as well as other quails of the Odontophoridae  
310 family that are present in archaeological data. COL1 $\alpha$ 2-757 may have the  
311 potential to further discriminate among other quail genera present in Central  
312 Mexico (*Callipepla*, *Dendrortyx* and *Cyrtonyx*), but we have not been able to  
313 unambiguously confirm the marker without further reference material.

### 314 5.1.2. Charadriiformes

315 Charadriiformes is a diverse order of aquatic birds, and many species  
316 are found within the five families of this order common in Central Mexico:

Laridae, Scolopacidae, Charadriidae, Recurvirostridae and Jacanidae (Peterson and Navarro Sigüenza, 2006). Reference samples were collected for species from Laridae and Charadriidae, while collagen sequences are available for species in Charadriidae and Scolopacidae. The samples from these three families are distinguished by variations across six peptide locations: COL1 $\alpha$ 2-10, COL1 $\alpha$ 2-175, COL1 $\alpha$ 2-502, COL1 $\alpha$ 2-658, COL1 $\alpha$ 2-757, and COL1 $\alpha$ 2-520. Given that only one species from each group was sampled, these peptide markers may be specific to genus or species within these families, and there does not appear to be any peptide marker specific to this order of birds.

### 5.1.3. *Anseriformes*

Anatidae (duck, geese and swans) are the only family from Anseriformes present in Central Mexico (Howell and Webb, 1995) and we identified the greatest diversity of markers among this family. The most common genera in our study region are *Anas*, *Oxyura*, *Spatula*, *Aythya*, and

332 *Mareca*, while *Anser*, *Mergus*, and *Bucephala* may have been more common  
333 in the past (Ayala-Pérez et al., 2013; de Sahagún et al., 1963, pp. 26, 34–38,  
334 57; Howell and Webb, 1995). Reference samples were acquired from *Anas*  
335 and *Oxyura* and sequence data are available for *Anas*, *Oxyura*, and *Anser*.  
336 Four main marker locations are important for Anatidae: COL1 $\alpha$ 2-658,  
337 COL1 $\alpha$ 2-889, COL1 $\alpha$ 2-454, and COL1 $\alpha$ 2-757. We identify six archaeological  
338 duck groups that are distinguishable based on various combinations of these  
339 four peptides (Figure 3). *Anas* and *Oxyura* are distinguished by differences in  
340 COL1 $\alpha$ 2-889 and COL1 $\alpha$ 2-757. *Anser* and *Anas* are distinguished at peptides  
341 COL1 $\alpha$ 2-658 and COL1 $\alpha$ 2-889.

342       The peptides identified in Anatidae 2 closely match *Anas*  
343 *platyrhynchos*. Archaeological specimens in this group likely include the  
344 closely related *Anas diazi* (Mexican duck), which was common in the region  
345 (Ayala-Pérez et al., 2013), and may also include other taxa in the *Anas* genus  
346 or dabbling ducks of the Anatini tribe, such as *Spatula* and *Mareca*. Markers

347 for Anatidae 4 are identical to *Oxyura jamaicensis* (ruddy duck) which is the  
348 only species of its tribe found in Central Mexico (Howell and Webb, 1995, pp.  
349 172–3). It is likely, then, that all archaeological specimens identified as  
350 Anatidae 4 belong to *Oxyura jamaicensis*. Further reference samples are  
351 required to identify Anatidae groups 1, 3, 5, and 6, although the presence of  
352 peak  $m/z$  2497.5 for COL1 $\alpha$ 2-658 in Anatidae 5 and 6 suggests they could be  
353 types of geese, based on sequence data for *Anser*.

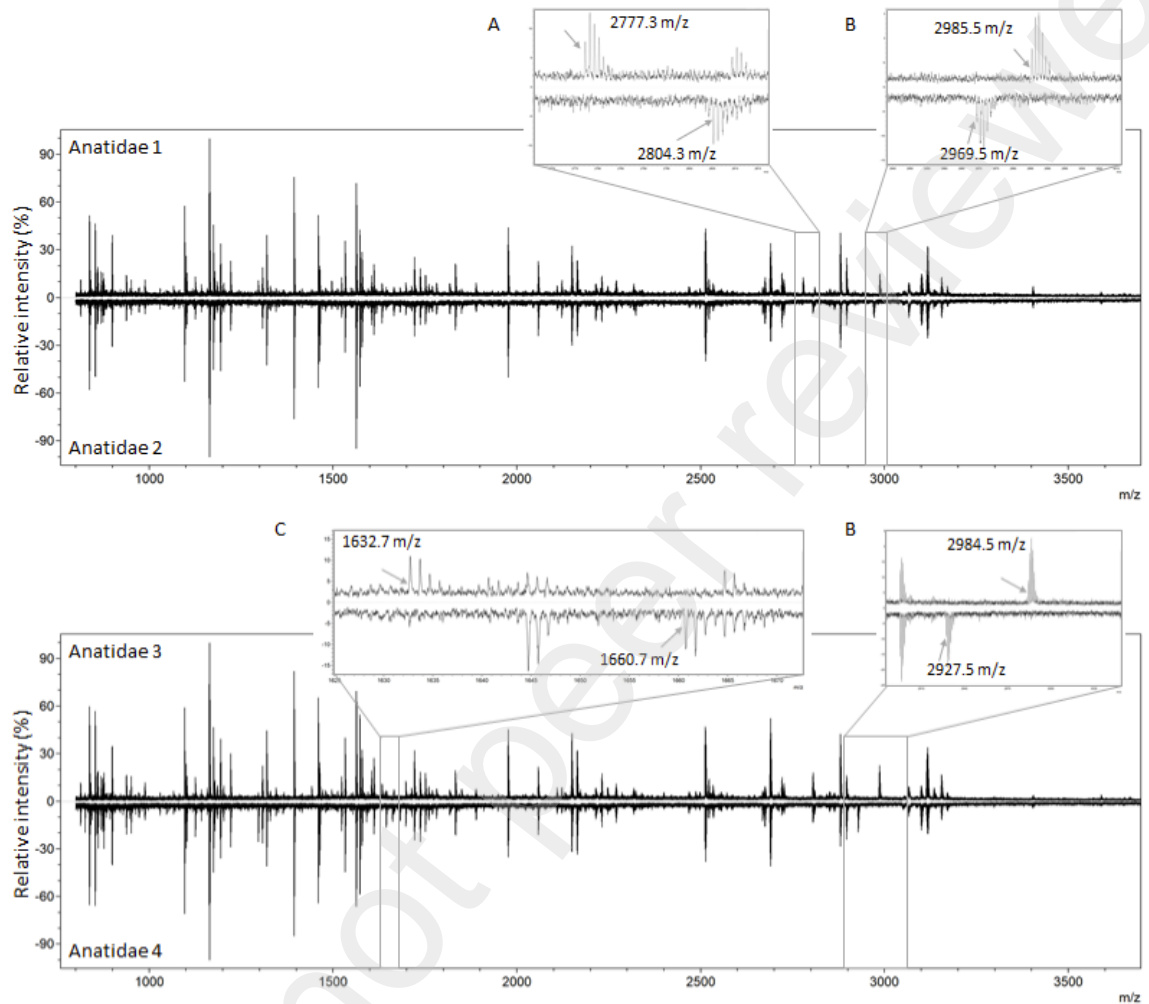


Figure 3. Examples of peptide markers that distinguish four groups of Anatidae. Anatidae 1 and 3 are not identified to taxonomic group, while Anatidae 2 is identical to *Anas platyrhynchos* and Anatidae 4 is identical to *Oxyura jamaicensis*. A) the difference between the COL1  $\alpha$  2-454 marker at  $m/z$  2777.3 for Anatidae 1, and  $m/z$  2804.3 found in the other Anatidae groups. B) unique peaks for each Anatidae group among the COL1  $\alpha$  2-757 marker—Anatidae 1:  $m/z$  2985.5, Anatidae 2:  $m/z$  2969.5, Anatidae 3:  $m/z$  2984.5, and Anatidae 4:  $m/z$  2927.5. C) the difference between the COL1  $\alpha$  2-889 marker at  $m/z$  1660.7 in Anatidae 4 and  $m/z$  1632.7 that is present in other ducks of the Anatidae family.



365 5.1.4. *Gruiformes, Podicipediformes, Columbiformes, and Passeriformes*

366 Gruiformes, Podicipediformes and Columbiformes are each  
367 represented by two species from one family: Rallidae, Podicipedidae, and  
368 Columbidae respectively (Table 2). While Podicipedidae and Columbidae are  
369 the only families from these orders present in the Basin of Mexico, Rallidae is  
370 the most diverse family of three Gruiformes (Peterson and Navarro Sigüenza,  
371 2006). Although specimens in each order are distinguished from other birds  
372 based on multiple markers, species within each family are indistinguishable  
373 from each other. Based on the large numbers of markers that distinguish  
374 families within other orders studied here, the samples from Rallidae are likely  
375 to be distinct from other families in the order Gruiformes.

376 Passeriformes are the largest order of birds with upwards of 140  
377 families. An archaeological specimen for *Corvus corax* (raven) of the Corvidae  
378 family is similar to *Quiscalus mexicanus* (Mexican grackle), except for the  
379 marker at COL1α2-658. Compared to other taxa in the study, however, *Corvus*

380 and *Quiscalus* have the highest number of unique markers and many more  
381 variations among passerine families are visible in the available collagen  
382 sequences.

#### 383 5.1.5. *Pelecaniformes and Accipitriformes*

384 Pelecaniformes and Accipitriformes are represented by multiple  
385 reference samples but were not submitted for LC-MS/MS analysis as the  
386 peptide marker patterns were not observed in any archaeological samples.  
387 MALDI peaks observed in these samples are presented in Table 1, but these  
388 unconfirmed peaks are not included in the biomarker list for identification of  
389 taxa. Accipitridae is the largest of three families of Accipitriformes in Central  
390 Mexico, including Cathartidae and Pandionidae (Peterson and Navarro  
391 Sigüenza, 2006). While *Buteo* and *Accipiter* from Accipitridae cannot be  
392 separated by MALDI marker, Accipitridae and Cathartidae are distinguished  
393 from each other by markers at COL1 $\alpha$ 2-175 and COL1 $\alpha$ 2-658. Similarly,  
394 among the Pelecaniformes, Ardeidae is the largest of three families common

in Central Mexico, also including Pelecanidae and Threskiornithidae (Peterson and Navarro Sigüenza, 2006). *Egretta* and *Ardea* in the Ardeidae family are indistinguishable, while Ardeidae are distinct from Pelecanidae at multiple markers.

## 5.2. Comparison to established peptide markers

The overall mutation rate of avian collagen is lower than mammals. However, several peptide regions appear to have particularly high variability, meaning avian taxonomic groups can be identified more effectively than predicted by the slower mutation rate. Peptides COL1 $\alpha$ 2-520 and COL1 $\alpha$ 2-757, for example, both have at least five locations of SAPs in their respective 36- and 33-number amino acid sequences. These combinations of variants correspond to seven unique peptide sequences for each marker location that was confirmed with LC-MS/MS. While COL1 $\alpha$ 2-889 is a shorter peptide with only 18 amino acids, SAPs occur in three locations and correspond to eight unique peptide sequences. Overall, the taxonomic resolution achievable in

birds appears to be only slightly less than that of mammals. For example, five markers have been identified that distinguish taxa within the family Bovidae (Janzen et al., 2021), while we identified four peptide markers that distinguish taxa within the family Anatidae.

COL1 $\alpha$ 1 508-519 has been previously noted a useful marker to discriminate broad groups of mammals (Buckley et al., 2014). This peptide appears conserved across all birds in this study, present at  $m/z$  1162. However recent research suggests this marker and amino acid sequence is also shared with Australian marsupials and some reptiles (Harvey et al., 2019a; Peters et al., 2021).

As in mammals, most of the diagnostic bird biomarkers are identified from COL1  $\alpha$  2. Some potential markers were identified on COL1  $\alpha$  1, but these are not presented as they could not be consistently confirmed by LC-MS/MS. Of the avian markers identified here, all but two are homologous to locations where biomarkers are identified in the collagen sequences of mammals or

425 fish: COL1 $\alpha$ 2 454, COL1 $\alpha$ 2 502, COL1 $\alpha$ 2 757, and COL1 $\alpha$ 2 978 (Pep E, C, G  
426 and A, Buckley et al., 2009); COL1 $\alpha$ 2 292 (P2, Buckley et al., 2014), COL1 $\alpha$ 2  
427 10 (Pep 9, Buckley et al., 2016), COL1 $\alpha$ 2 889 (Janzen et al., 2021, although  
428 first described as a bird marker by Buckley, 2018 and Eda et al. 2020),  
429 COL1 $\alpha$ 2-604 (Harvey et al., 2019a), COL1 $\alpha$ 2-625 (Harvey et al., 2018),  
430 COL1 $\alpha$ 2-658 (Richter et al., 2020). These locations seem to have good  
431 discriminant ability across animal types. While Wang et al. (2021) noted  
432 COL1 $\alpha$ 2 175 as a non-diagnostic marker shared by mammals, this marker  
433 does discriminate among birds, although it overlaps with identical masses  
434 from other markers. One novel marker presented here, COL1 $\alpha$ 2-520-555, is  
435 highly diagnostic for avian identification, but should be confirmed through  
436 visual inspection of spectra. This marker is often missing in modern  
437 specimens and as the peak is broad with a large isotope distribution, the  
438 correct peak in the distribution is often not properly identified during

automatic peak picking and deisotoping, resulting in being incorrectly identified 1-2 Da off.

## 6. Analysis of archaeological samples

### 6.1. *Taxonomic discrimination via clustering*

All 31 modern reference specimens produced high quality spectra and the peaks picked were consistent across replicates and individuals. Modern and archaeological samples were assigned to one of 20 groups based on hierarchical clustering of marker peaks (Figure 4, see also Figure S1 and Table S1). Two-hundred eighty-five of the 295 archaeological samples clustered into groups, while 10 samples were removed by the screening process. In contrast, only 5 of the 31 reference specimens passed initial screening due to higher rates of noise in spectra for modern samples. However, these parameters were kept due to the excellent discrimination of archaeological samples in the clusters. Four of the five reference specimens clustered where expected, while one sample clustered separately from archaeological

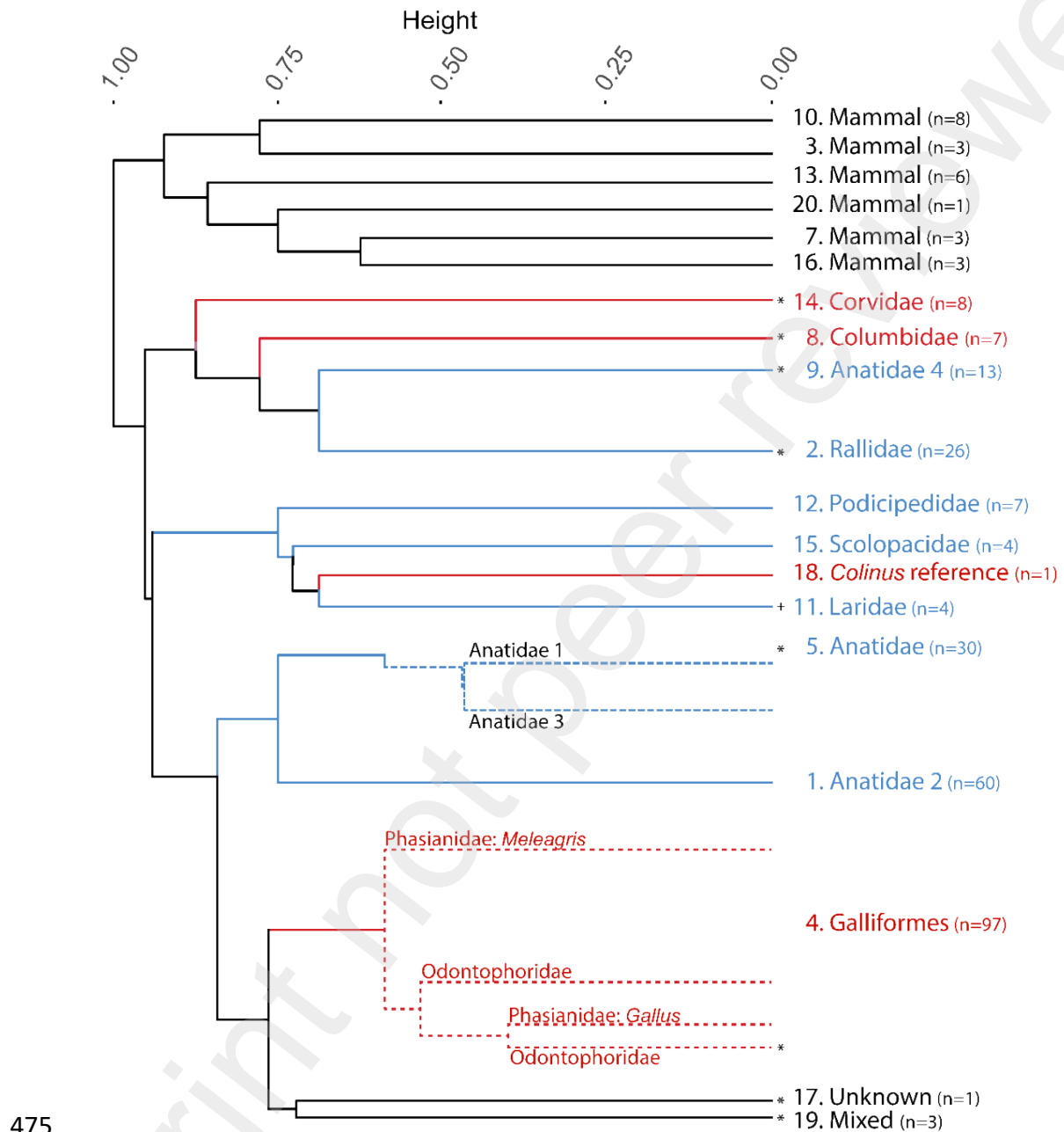
454 samples. Identification of samples within each group was independently  
455 verified by visual inspection of marker peaks and further identification of taxa  
456 within clusters was possible.

457 Six of the 20 clusters contained a total of 30 archaeological samples  
458 matching established mammal biomarkers, while Corvidae, Columbidae,  
459 Rallidae, Podicipedidae, and Scolopacidae each clustered separately. Laridae  
460 clustered apart, with the only erroneous clustering of a non-related sample  
461 (MC306, identified as passeriform). Galliformes clustered together with one  
462 distinct sub-grouping of *Meleagris*, and another distinct group comprising  
463 both Odontophoridae and *Gallus*. Three clusters contained Anatidae. Cluster  
464 1 largely contained specimens from Anatidae 1, while Cluster 9 grouped all  
465 Anatidae 4 samples. Cluster 5 contained two sub-groups, which broadly  
466 separated Anatidae 1 and 3.

467 Eleven samples produced high-quality spectra but were not identifiable  
468 to a taxonomic group by visual inspection. Each of these samples presented

469 the peak at  $m/z$  1162, common to all birds and additional avian biomarkers.  
470 However, these 11 samples represent ten unique peak lists and group across  
471 multiple taxonomic clusters (Figure 4). This suggests there could be ten  
472 distinct taxa yet to be identified in this assemblage. Of the ten archaeological  
473 samples that were excluded prior to clustering, one was identified as  
474 *Meleagris*, and nine were too poor to identify.





475

476 Figure 4. Schematic of hierarchical clustering results for archaeological samples.  
 477 Solid lines represent assigned clusters while dashed lines indicate where additional  
 478 groupings were observed within assigned clusters. Aquatic birds are shown in blue  
 479 and terrestrial birds in red. \* denotes presence of unknown taxa, while + denotes  
 480 incorrect classification at order or family level. See detailed clustering diagram in  
 481 Figure S1.

482

483        This clustering achieved the highest accuracy when limiting variation  
484    among spectra. Averaging replicates analyzed at multiple dilutions and  
485    removing poor spectra prior to clustering greatly improves assignment of  
486    samples to the correct taxonomic group. Moreover, both accuracy and  
487    precision is improved by matching to carefully curated peak lists and  
488    combining the presence of markers with deamidated peaks and varying  
489    number of proline oxidations. Our clustering demonstrates the utility of this  
490    workflow to rapidly assign a large number of archaeological samples, each  
491    with multiple replicates, to broad taxonomic groups.

492    *6.2.    Identifications and collagen preservation at Tlajinga*

493        Some differences were observed between the morphological and  
494    collagen-based identifications (see Table S1). Thirty specimens were  
495    identified as mammals and an additional nine had avian identifications that  
496    differed from their morphological identification. Given the difficulties in

identifying small mammals and birds from fragmented specimens, these are certainly errors in morphological analysis (Driver, 2011; Wolverton, 2013). Nevertheless, the archaeological sample was not selected randomly, and the high number of erroneous morphological identifications is partly due to sampling bias towards less confidently identified fragments. Moreover, these discrepancies highlight, as others have argued, the importance of checking the quality of zooarchaeological identifications using molecular methods, particularly for difficult to identify taxa (Driver, 2011; Horsburgh et al., 2016; Speller et al., 2016).

Nine carbonized bone specimens produced low quality spectra, likely because collagen begins to break down around 70°C. However, at least 24 other samples with evidence of burning or partial carbonization produced identifiable spectra, suggesting that heat exposure does not completely rule out ZooMS analysis because of differential temperatures reached at different parts of the bone during burning. As ZooMS requires intact peptides, but less

512 overall collagen than isotopes or radiocarbon analyses (Harvey et al., 2016;  
513 Wang et al., 2021), more research is needed to establish the level of heat  
514 exposure tolerance. That all non-carbonized samples (n=262) produced  
515 spectra suitable for taxonomic identification suggests that the collagen  
516 preservation at Tlajinga is very good and that archaeological avian bones can  
517 readily produce successful spectra using ZooMS.

518 *6.3. The archaeology of birds at Tlajinga*

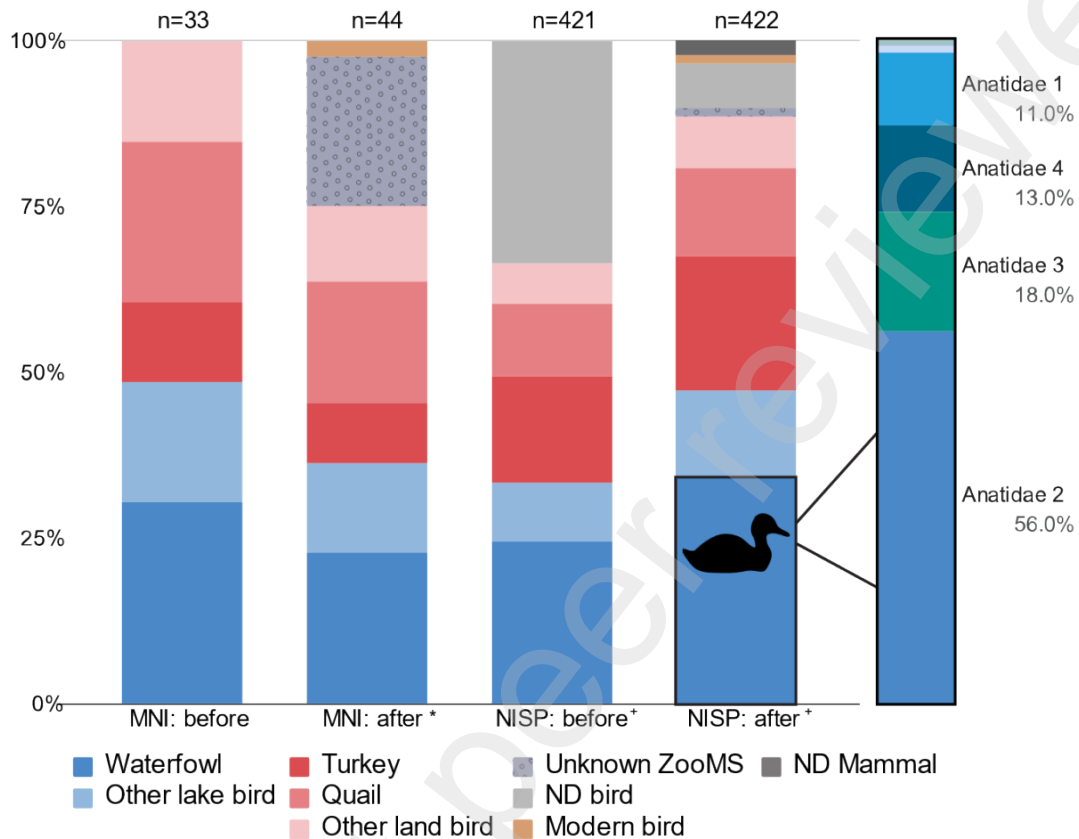
519 ZooMS identifications of elements were incorporated into the existing  
520 zooarchaeological data from Tlajinga to calculate site NISP and MNI for bird  
521 taxa (Figure 5, see also Table S5). Overall, 18 avian taxa were identified in the  
522 Tlajinga assemblage, while an additional 10 taxa remain unidentified. The  
523 total number of identified bird elements at Tlajinga increased from 280 to  
524 384. While the MNI increased from 33 to 44, 10 of these additional birds  
525 represent the 10 unidentified taxonomic groups. Therefore, the MNI pre and

526 post ZooMS analysis changed very little, highlighting that the new MNI is  
527 more representative of taxonomic diversity rather than taxonomic abundance.

528 The integrated results from ZooMS and morphological identifications  
529 (Figure 5) confirm that waterfowl are the most common type of bird identified  
530 at Tlajinga and, together with other aquatic birds, make up almost 50% of the  
531 assemblage by NISP and 36% by MNI. Overall, these results are consistent  
532 with elsewhere at Teotihuacan, where aquatic birds make up roughly a third of  
533 the avian assemblage by MNI (Sugiyama et al., 2017). Turkeys, however make  
534 up a much smaller proportion of the avian assemblage at Tlajinga compared  
535 to the Teotihuacan average. Together, this suggests that aquatic taxa may  
536 have been more important to urban subsistence at Tlajinga than other areas  
537 of the city, which may have placed greater reliance on terrestrial resources  
538 like turkeys.

539

540



541

542 Figure 5. Relative abundance of all avian taxa identified at Tlajinga before and after  
 543 ZooMS analysis. Call out presents relative proportions of the six Anatidae  
 544 distinguished by ZooMS. \* Includes taxa identified from shaft fragments, + includes  
 545 only bones identified to element.

### 546 6.3.1. Taxonomic diversity at Tlajinga: aquatic taxa

547 At Tlajinga, eleven aquatic bird taxa were identified by ZooMS and

548 morphological analyses, including six members of the Anatidae family, two

549 members of the Rallidae family, *Fulica americana* (American coot) and

550 *Gallinula galatea* (common gallinule), as well as examples of Laridae (gulls),

551 Scolopacidae (sandpipers), and Podicipedidae (grebes). Four Anatidae groups  
552 are represented by multiple specimens (Figure 5). Anatidae 2, which includes  
553 taxa in the *Anas* genus, is the most abundant of the ducks identified by  
554 ZooMS at Tlajinga (56% of Anatidae by NISP), followed by Anatidae 3 (18%),  
555 Anatidae 4, identified as *Oxyura jamaicensis* (13%), and Anatidae 1 (11%).  
556 Anatidae 1 and 3 make up 11% and 18% respectively of the ducks identified  
557 with ZooMS. Anatidae 5 and 6 are rare, each represented by a single  
558 specimen.

559 As Anatidae are typically not identified beyond family level at  
560 Teotihuacan (Sugiyama et al., 2017, Table ESM1), the identification of at  
561 least six Anatidae taxa provides additional information into exploitation of  
562 lake resources at the city. Understanding taxonomic diversity of aquatic birds  
563 in urban and rural settlements provides an opportunity to examine changes in  
564 specialization or diversification of the production and distribution of lake  
565 resources throughout Central Mexico. Our results demonstrate that the

566 residents of Tlajinga were exploiting a wide range of aquatic birds, including  
567 multiple types of ducks whose habitats and behaviors vary across species.  
568 This suggests that the production and distribution systems that contributed  
569 to the faunal assemblage at Tlajinga may have employed a diversified  
570 strategy, exploiting a wide range of animals, rather than specializing in the  
571 acquisition of particular taxa. This also supports the argument that other lake  
572 resources, such as small fish and insects, may also have played an important  
573 role in urban subsistence during the Classic period (e.g., Parsons, 2010;  
574 Widmer and Storey, 2016).

575       Moreover, many aquatic birds are migratory, while others, including  
576 *Oxyura jamaicensis* and *Anas diazi*, breed in Central Mexico year-round.  
577 Careful examination of the relative abundance of Anatidae species via ZooMS  
578 alongside other paleo-proteomic techniques such as peptide-based  
579 identification of eggshells (Demarchi et al., 2020; Presslee et al., 2017;  
580 Stewart et al., 2014), provides new opportunities to examine the seasonality



581 of lake exploitation and the implication of intensive lake exploitation on avian  
582 populations in the past.

583 *6.3.2. Taxonomic diversity at Tlajinga: terrestrial taxa*

584 Seven types of non-aquatic birds were identified at Tlajinga from the  
585 combined ZooMS and morphological analysis, including Columbidae (pigeons  
586 and doves), Odontophoridae (New World quails), *Colinus virginianus*,  
587 *Meleagris gallopavo*, *Corvus corax*, *Gallus gallus*, and *Accipiter cooperii*  
588 (Cooper's hawk). Compared to morphological identifications, ZooMS provided  
589 additional discrimination potential for Phasianidae and Odontophoridae  
590 families. In addition to *Meleagris gallopavo*, we identified specimens that  
591 closely match theoretical peaks for *Gallus gallus*. Domestic chickens were  
592 introduced to Central Mexico after European arrival and these specimens  
593 derive from an area of the site with early colonial features. The discovery of  
594 this taxon in deposits below these features provides additional information to  
595 assess the location and extent of colonial period deposits at Tlajinga.

ZooMS also provides the ability to discriminate between *Colinus virginianus* and other quails of the Odontophoridae family. While this result was unexpected and deserves further analysis, the ability to discriminate between these taxa presents an opportunity to investigate the exploitation of quails in Central Mexico. Some Central Mexican quails prefer more open woodland or grassland environments (*Colinus* and *Cyrtonyx*), while others prefer denser forested environments (*Dendrortyx*) (Howell and Webb, 1995, pp. 226, 229, 231). Greater taxonomic precision through ZooMS could be employed to understand environmental and agricultural shifts within the valley, while integration with proteomic techniques for eggshell identification would provide a means to examine the hypothesis that *Colinus virginianus* were raised for urban consumption at Teotihuacan (Widmer and Storey, 2016).

Finally, the ten unidentified taxa highlight that rare avian taxa are underrepresented in traditional zooarchaeological analysis. Rare taxa may not

611 have major implications for understanding urban subsistence but can provide  
612 additional insight into birds acquired for other reasons, such as their colorful  
613 plumage, bird song, or symbolic meanings especially when combined with  
614 pictographic or historical references such as the murals recently excavated at  
615 civic-ceremonial structures in Tlajinga (Carballo et al., 2021).

616 To address these new avenues for research, the integration of  
617 zooarchaeological and ZooMS data needs to be considered carefully in  
618 project planning. Initially, we did not expect to discriminate among Anatidae,  
619 and so specimens that were identified confidently to Anatidae were not  
620 selected for ZooMS analysis. Consequently, the relative abundances of  
621 Anatidae groups shown in Figure 5 are estimates of abundance, as  
622 morphological identifications in the non-ZooMS assemblage may be biased  
623 towards one taxon or another. For larger projects that require confident  
624 assessments of relative abundance, it may be productive to choose fewer  
625 skeletal elements (i.e., the most robust limb bones) and to analyze all

626 identified and unidentified specimens in the collection. With further research  
627 on reference materials, we expect that our ability to discriminate among birds  
628 with peptide mass fingerprinting will increase and some of the less specific  
629 identifications of taxa in this study may be refined.

## 630 **7. Conclusion**

631 With the confirmation of a 71-MALDI-marker panel across 12 peptides,  
632 we have demonstrated that ZooMS is suitable for identification of avian taxa  
633 from archaeological contexts to at least the level of family. The slower  
634 mutation rate of avian collagen appears to be offset by high variability in  
635 particular regions, meaning that ZooMS can also identify birds to sub-family  
636 levels. While Buckley (2018) and Eda et al. (2020) previously noted  
637 differences that discriminate among members of the Phasianidae family, we  
638 demonstrate that sub-family differences are also possible among  
639 Odontophoridae and Anatidae.

640           Using ZooMS we revealed higher avian taxonomic diversity from the  
641   faunal assemblage at Tlajinga, Teotihuacan during the Classic period  
642   including the exploitation of a range of aquatic birds. Combining ZooMS with  
643   morphology allowed for an increased recovery of rare taxa which are often  
644   underrepresented in reference collections and thus less frequently identified  
645   compared to commonly exploited taxa. To fully take advantage of ZooMS, the  
646   ability to integrate ZooMS into traditional zooarchaeological analyses such as  
647   NSIP and MNI should be considered. Going forward, the enhanced ability to  
648   identify avian remains to the family and subfamily level has great potential to  
649   elucidate a wide range of societal topics, from urban provisioning systems,  
650   seasonality of settlements or hunting activities, and past environmental  
651   changes.

652

## 653 **8. Data accessibility**

654 MALDI raw data and MS2s for confirmed biomarker sequences is  
655 available through Zendo at <https://doi.org/10.5281/zenodo.6363114>. MS/MS  
656 data is available through ProteomeXchange (PXD034547) through MassIVE  
657 (MSV000089660) at DOI: <https://doi.org/10.25345/C5N29PB27>. R code for  
658 MALDI analysis and clustering is available through Zenodo at  
659 <https://doi.org/10.5281/zenodo.6366234>. All other data are included in the  
660 manuscript and/or supplemental materials.

## 661 **9. Acknowledgements**

662 Material in this article was based on work supported by the U.S.  
663 National Science Foundation (NSF) under Dissertation Improvement Grant  
664 (BCS-1916358), NSF Grant (BCS-13212447), the Max Planck Group, and  
665 Harvard University. Permission for destructive sampling of archaeological  
666 material was provided by the Instituto Nacional de Antropología e Historia in  
667 Mexico City. We thank Raúl Valadez Azúa, Verónica Ortega Cabrera, David

668 Carballo, Catherine West and the American Museum of Natural History for  
669 access to modern and archaeological faunal material from Tlajinga and  
670 Tlailotlacan, Sandra Hebestreit for lab support and running of the MALDI at  
671 the Max Planck Institute for the Science of Human History; Scott Shaffer and  
672 Khaja Muneeruddin at University of Massachusetts Medical School for  
673 processing the MS/MS samples; Dusan Boric, Alana Masciana, and Kevin  
674 Uno at Columbia University for providing US based lab space; Christina  
675 Warinner, The Proteomics Core Facility, and FAS Research Computing at  
676 Harvard University for supporting infrastructure for data analysis. Many  
677 thanks to Catherine West and John M. Marston for their support in  
678 conceptualization of this project and feedback on early drafts of the  
679 manuscript.

680 **Author contributions:**

681 M.C.: Conceptualization, Writing - Original Draft, Formal Analysis, Visualization,  
682 Investigation, Funding Acquisition

683 K.D.: Writing, Writing - Review & Editing, Funding Acquisition

684 K.R.: Conceptualization, Writing - Original Draft, Writing - Review & Editing,  
685 Visualization, Investigation

686

687 **Competing Interest Statement:** The authors declare that they have no  
688 competing interests.



## 689 10. Bibliography

- 690 Ayala-Pérez, V., Arce, N., Carmona, R., 2013. Distribución espacio-temporal de aves  
691 acuáticas invernantes en la ciénega de Tláhuac, planicie lacustre de Chalco,  
692 México. *Revista Mexicana de Biodiversidad* 84, 327–337.  
693 <https://doi.org/10.7550/rmb.28632>
- 694 Bern, M., Kil, Y.J., Becker, C., 2012. Byonic: advanced peptide and protein identification  
695 software. *Curr Protoc Bioinformatics* Chapter 13, Unit13.20.  
696 <https://doi.org/10.1002/0471250953.bi1320s40>
- 697 Brown, S., Douka, K., Collins, M., Richter, K.K., 2020a. On the standardization of  
698 ZooMS nomenclature. *Journal of Proteomics* 104041.  
699 <https://doi.org/10.1016/j.jprot.2020.104041>
- 700 Brown, S., Hebestreit, S., Wang, N., Boivin, N., Douka, K., Richter, K.K., 2020b.  
701 Zooarchaeology by Mass Spectrometry (ZooMS) for bone material - Acid  
702 insoluble protocol. *protocols.io*.  
703 <https://doi.org/dx.doi.org/10.17504/protocols.io.bf43jqyn>
- 704 Buckley, M., 2018. Zooarchaeology by Mass Spectrometry (ZooMS) Collagen  
705 Fingerprinting for the Species Identification of Archaeological Bone Fragments,  
706 in: Giovas, C.M., LeFebvre, M.J. (Eds.), *Zooarchaeology in Practice*. Springer  
707 International Publishing, pp. 227–247. [https://doi.org/10.1007/978-3-319-64763-](https://doi.org/10.1007/978-3-319-64763-0_12)  
708 [0\\_12](https://doi.org/10.1007/978-3-319-64763-0_12)
- 709 Buckley, M., Collins, M., Thomas-Oates, J., Wilson, J.C., 2009. Species Identification by  
710 Analysis of Bone Collagen Using Matrix-Assisted Laser Desorption/Ionisation  
711 Time-of-Flight Mass Spectrometry. *Rapid Communications in Mass Spectrometry*  
712 23, 3843–3854. <https://doi.org/10.1002/rcm.4316>
- 713 Buckley, M., Fraser, S., Herman, J., Melton, N.D., Mulville, J., Pálsdóttir, A.H., 2014.  
714 Species identification of archaeological marine mammals using collagen fi  
715 ngerprinting 41. <https://doi.org/10.1016/j.jas.2013.08.021>
- 716 Buckley, M., Gu, M., Shameer, S., Patel, S., Chamberlain, A.T., 2016. High-throughput  
717 collagen fingerprinting of intact microfaunal remains; A low-cost method for  
718 distinguishing between murine rodent bones. *Rapid Communications in Mass*  
719 *Spectrometry* 30, 805–812. <https://doi.org/10.1002/rcm.7483>
- 720 Buckley, M., Kansa, S.W., 2011. Collagen fingerprinting of archaeological bone and  
721 teeth remains from Domuztepe, South Eastern Turkey. *Archaeological and*  
722 *Anthropological Sciences* 3, 271–280. <https://doi.org/10.1007/s12520-011-0066-z>
- 723 Carballo, D.M., 2013. The Social Organization of Craft Production and Interregional  
724 Exchange at Teotihuacan, in: Hirth, K.G., Pillsbury, J. (Eds.), *Merchants, Trade,*  
725 *and Exchange in the Pre-Columbian World*. *Dumbarton Oaks and Trustees for*  
726 *Harvard University*, Washington, D.C., pp. 113–140.
- 727 Carballo, D.M., Barba, L., Ortiz, A., Blancas, J., Sariñana, D.H., Codlin, M., Saucedo,  
728 A., Rodríguez, G.D.T., 2021. Excavations at the Southern Neighborhood Center  
729 of the Tlajinga District, Teotihuacan, Mexico. *Latin American Antiquity* 32, 557–  
730 576.

- Carballo, D.M., Hirth, K.G., Hernández Sariñana, D., Buckley, G.M., Mejía Ramón, A.G., Kennett, D.J., 2019. New Research at Teotihuacan's Tlajinga District, 2012–2015. *Ancient Mesoamerica* 30, 95–113. <https://doi.org/10.1017/S0956536118000159>
- Codlin, M.C., in prep. Household Subsistence and Animal Acquisition at Tlajinga, Teotihuacan, Mexico. To be submitted to *Ancient Mesoamerica*.
- De Lucia, K., 2021. Household lake exploitation and aquatic lifeways in postclassic Xaltocan, Mexico. *Journal of Anthropological Archaeology* 62, 101273. <https://doi.org/10.1016/j.jaa.2021.101273>
- de Sahagún, B., Anderson, A.J.O., Dibble, C.E., 1963. Book 11: Earthly Things, Florentine Codex. School of American Research.
- Demarchi, B., Presslee, S., Sakalauskaite, J., Fischer, R., Best, J., 2020. The role of birds at Çatalhöyük revealed by the analysis of eggshell. *Quaternary International* 543, 50–60. <https://doi.org/10.1016/j.quaint.2020.02.009>
- Driver, J.C., 2011. Identification, classification and zooarchaeology. *Ethnobiology Letters* 2, 19–39. <https://doi.org/10.14237/eb1.2.2011.19-39>
- Eda, M., Morimoto, M., Mizuta, T., Inou, T., 2020. ZooMS for birds: Discrimination of Japanese archaeological chickens and indigenous pheasants using collagen peptide fingerprinting. *Journal of Archaeological Science : Reports* 34, 102635. <https://doi.org/10.1016/j.jasrep.2020.102635>
- Feng, S., Stiller, J., Deng, Y., Armstrong, J., Fang, Q., Reeve, A.H., Xie, D., Chen, G., Guo, C., Faircloth, B.C., Petersen, B., Wang, Z., Zhou, Q., Diekhans, M., Chen, W., Andreu-Sánchez, S., Margaryan, A., Howard, J.T., Parent, C., Pacheco, G., Sinding, M.-H.S., Puetz, L., Cavill, E., Ribeiro, Â.M., Eckhart, L., Fjeldsø, J., Hosner, P.A., Brumfield, R.T., Christidis, L., Bertelsen, M.F., Sicheritz-Ponten, T., Tietze, D.T., Robertson, B.C., Song, G., Borgia, G., Claramunt, S., Lovette, I.J., Cowen, S.J., Njoroge, P., Dumbacher, J.P., Ryder, O.A., Fuchs, J., Bunce, M., Burt, D.W., Cracraft, J., Meng, G., Hackett, S.J., Ryan, P.G., Jönsson, K.A., Jamieson, I.G., da Fonseca, R.R., Braun, E.L., Houde, P., Mirarab, S., Suh, A., Hansson, B., Ponnikas, S., Sigeman, H., Stervander, M., Frandsen, P.B., van der Zwan, H., van der Sluis, R., Visser, C., Balakrishnan, C.N., Clark, A.G., Fitzpatrick, J.W., Bowman, R., Chen, N., Cloutier, A., Sackton, T.B., Edwards, S.V., Foote, D.J., Shakya, S.B., Sheldon, F.H., Vignal, A., Soares, A.E.R., Shapiro, B., González-Solís, J., Ferrer-Obiol, J., Rozas, J., Riutort, M., Tigano, A., Friesen, V., Dalén, L., Urrutia, A.O., Székely, T., Liu, Y., Campana, M.G., Corvelo, A., Fleischer, R.C., Rutherford, K.M., Gemmell, N.J., Dussex, N., Mouritsen, H., Thiele, N., Delmore, K., Liedvogel, M., Franke, A., Hoepfner, M.P., Krone, O., Fudickar, A.M., Milá, B., Ketterson, E.D., Fidler, A.E., Friis, G., Parody-Merino, Á.M., Battley, P.F., Cox, M.P., Lima, N.C.B., Prosdociimi, F., Parchman, T.L., Schlinger, B.A., Loiselle, B.A., Blake, J.G., Lim, H.C., Day, L.B., Fuxjager, M.J., Baldwin, M.W., Braun, M.J., Wirthlin, M., Dikow, R.B., Ryder, T.B., Camenisch, G., Keller, L.F., DaCosta, J.M., Hauber, M.E., Louder, M.I.M., Witt, C.C., McGuire, J.A., Mudge, J., Megna, L.C., Carling, M.D., Wang, B., Taylor, S.A., Del-Rio, G., Aleixo, A., Vasconcelos, A.T.R., Mello, C.V.,

- 775 Weir, J.T., Haussler, D., Li, Q., Yang, H., Wang, J., Lei, F., Rahbek, C., Gilbert,  
776 M.T.P., Graves, G.R., Jarvis, E.D., Paten, B., Zhang, G., 2020. Dense sampling of  
777 bird diversity increases power of comparative genomics. *Nature* 587, 252–257.  
778 <https://doi.org/10.1038/s41586-020-2873-9>
- 779 Gamboa, A.G.M., Dreja, E.A.S., Armillas, M.O.F., Piña, I.A.P., Herrada, A.M., 2017.  
780 Lista de aves en el humedal de Tláhuac.  
781 <https://doi.org/10.13140/RG.2.2.30915.50724>
- 782 Gibb, S., Strimmer, K., 2012. MALDIquant: a versatile R package for the analysis of  
783 mass spectrometry data. *Bioinformatics* 28, 2270–2271.  
784 <https://doi.org/10.1093/bioinformatics/bts447>
- 785 Harvey, V.L., Daugnora, L., Buckley, M., 2018. Species identification of ancient  
786 Lithuanian fish remains using collagen fingerprinting. *Journal of Archaeological*  
787 *Science* 98, 102–111. <https://doi.org/10.1016/j.jas.2018.07.006>
- 788 Harvey, V.L., Egerton, V.M., Chamberlain, A.T., Manning, P.L., Buckley, M., 2016.  
789 Collagen Fingerprinting: A new screening technique for radiocarbon dating  
790 ancient bone. *PLoS ONE* 11, e0150650.  
791 <https://doi.org/10.1371/journal.pone.0150650>
- 792 Harvey, V.L., Egerton, V.M., Chamberlain, A.T., Manning, P.L., Sellers, W.I., Buckley,  
793 M., 2019a. Interpreting the historical terrestrial vertebrate biodiversity of Cayman  
794 Brac (Greater Antilles, Caribbean) through collagen fingerprinting. *Holocene* 29,  
795 531–542. <https://doi.org/10.1177/0959683618824793>
- 796 Harvey, V.L., LeFebvre, M.J., DeFrance, S.D., Toftgaard, C., Drosou, K., Kitchener,  
797 A.C., Buckley, M., 2019b. Preserved collagen reveals species identity in  
798 archaeological marine turtle bones from Caribbean and Florida sites. *Royal*  
799 *Society Open Science* 6, 191137. <https://doi.org/10.1098/rsos.191137>
- 800 Hickinbotham, S., Fiddymont, S., Stinson, T.L., Collins, M.J., 2020. How to get your  
801 goat: automated identification of species from MALDI-ToF spectra.  
802 *Bioinformatics* 36, 3719–3725. <https://doi.org/10.1093/bioinformatics/btaa181>
- 803 Hirth, Kenneth.G., 2020. Teotihuacan Economy from the Inside Out, in: Hirth, K., G.,  
804 Carballo, D., M., Arroyo, B. (Eds.), *Teotihuacan: The World beyond the City*.  
805 *Dumbarton Oaks, Washington D.C.*, pp. 97–138.
- 806 Hirth, K.G., 2016. *The Aztec Economic World*. Cambridge University Press, Cambridge.
- 807 Horn, I.R., Kenens, Y., Palmblad, N.M., Van Der Plas-Duivesteijn, S.J., Langeveld,  
808 B.W., Meijer, H.J.M., Dalebout, H., Marissen, R.J., Fischer, A., Florens, F.B.V.,  
809 Niemann, J., Rijdsdijk, K.F., Schulp, A.S., Laros, J.F.J., Gravendeel, B., 2019.  
810 Palaeoproteomics of bird bones for taxonomic classification. © Zoological  
811 *Journal of the Linnean Society* XX, 1–16.  
812 <https://doi.org/10.1093/zoolinlean/zlz012/5470657>
- 813 Horsburgh, K Ann, Orton, Jayson, Klein, Richard G, Horsburgh, K A, Orton, J, Klein, R  
814 G, 2016. Beware the Springbok in Sheep's Clothing: How Secure Are the Faunal  
815 Identifications upon Which We Build Our Models? *African Archaeological*  
816 *Review* 33, 353–361. <https://doi.org/10.1007/s10437-016-9231-1>
- 817 Howell, S.N.G., Webb, S., 1995. *A guide to the birds of Mexico and northern Central*  
818 *America*. Oxford University Press, Oxford.

- 819 Janzen, A., Richter, K.K., Mwebi, O., Id, S.B., Onduso, V., Gatwiri, F., Ndiema, E.,  
 820 Katongo, M., Goldstein, T., Douka, K., Boivin, N., 2021. Distinguishing African  
 821 bovids using Zooarchaeology by Mass Spectrometry ( ZooMS ): New peptide  
 822 markers and insights into Iron Age economies in Zambia. *Plos One* 16, e0251061.  
 823 <https://doi.org/10.1371/journal.pone.0251061>
- 824 Jarvis, E.D., Mirarab, S., Aberer, A.J., Li, B., Houde, P., Li, C., Ho, S.Y.W., Faircloth,  
 825 B.C., Nabholz, B., Howard, J.T., Suh, A., Weber, C.C., Fonseca, R.R. da, Li, J.,  
 826 Zhang, F., Li, H., Zhou, L., Narula, N., Liu, L., Ganapathy, G., Boussau, B.,  
 827 Bayzid, Md.S., Zavidovych, V., Subramanian, S., Gabaldón, T., Capella-  
 828 Gutiérrez, S., Huerta-Cepas, J., Rekepalli, B., Munch, K., Schierup, M., Lindow,  
 829 B., Warren, W.C., Ray, D., Green, R.E., Bruford, M.W., Zhan, X., Dixon, A., Li,  
 830 S., Li, N., Huang, Y., Derryberry, E.P., Bertelsen, M.F., Sheldon, F.H.,  
 831 Brumfield, R.T., Mello, C.V., Lovell, P.V., Wirthlin, M., Schneider, M.P.C.,  
 832 Prosdocimi, F., Samaniego, J.A., Velazquez, A.M.V., Alfaro-Núñez, A., Campos,  
 833 P.F., Petersen, B., Sicheritz-Ponten, T., Pas, A., Bailey, T., Scofield, P., Bunce,  
 834 M., Lambert, D.M., Zhou, Q., Perelman, P., Driskell, A.C., Shapiro, B., Xiong,  
 835 Z., Zeng, Y., Liu, S., Li, Z., Liu, B., Wu, K., Xiao, J., Yinqi, X., Zheng, Q.,  
 836 Zhang, Y., Yang, H., Wang, J., Smeds, L., Rheindt, F.E., Braun, M., Fjeldsa, J.,  
 837 Orlando, L., Barker, F.K., Jönsson, K.A., Johnson, W., Koepfli, K.-P., O'Brien,  
 838 S., Haussler, D., Ryder, O.A., Rahbek, C., Willerslev, E., Graves, G.R., Glenn,  
 839 T.C., McCormack, J., Burt, D., Ellegren, H., Alström, P., Edwards, S.V.,  
 840 Stamatakis, A., Mindell, D.P., Cracraft, J., Braun, E.L., Warnow, T., Jun, W.,  
 841 Gilbert, M.T.P., Zhang, G., 2014. Whole-genome analyses resolve early branches  
 842 in the tree of life of modern birds. *Science* 346, 1320–1331.  
 843 <https://doi.org/10.1126/SCIENCE.1253451>
- 844 Letunic, I., Bork, P., 2021. Interactive Tree Of Life (iTOL) v5: an online tool for  
 845 phylogenetic tree display and annotation. *Nucleic Acids Research* 49, W293–  
 846 W296. <https://doi.org/10.1093/nar/gkab301>
- 847 Lyman, R.L., 2008. *Quantitative Paleozoology*. Cambridge University Press, Cambridge.
- 848 Ortega Cabrera, V., 2012. Proyecto de Investigación Arqueológica Barrio Oaxaqueño,  
 849 Tlailotlacan, Teotihuacán: Informe Técnico Temporada 2012. Instituto Nacional  
 850 de Anthropología e Historia.
- 851 Ortega Cabrera, V., 2010. Proyecto de Investigación Arqueológica Barrio Oaxaqueño,  
 852 Tlailotlacan, Teotihuacan, Temporada 2010: Informe Técnico de Excavacion y  
 853 Análisis de Materiales Arqueológicos. Instituto Nacional de Anthropología e  
 854 Historia.
- 855 Ortega Cabrera, V., 2009. Proyecto de Investigación Arqueológica Barrio Oaxaqueño,  
 856 Tlailotlacan, Teotihuacan, Temporada 2009: Informe Técnico de Excavaciones  
 857 Arqueológicas y Análisis de Materiales. Instituto Nacional de Anthropología e  
 858 Historia.
- 859 Parsons, J.R., 2010. The Pastoral Niche in Pre-Hispanic Mesoamerica, in: *Pre-Columbian*  
 860 *Foodways: Interdisciplinary Approaches to Food, Culture, and Markets in*  
 861 *Ancient Mesoamerica*. Springer, New York, pp. 109–136.  
 862 [https://doi.org/10.1007/978-1-4419-0471-3\\_4](https://doi.org/10.1007/978-1-4419-0471-3_4)

- Parsons, J.R., 2008. Beyond Santley and Rose (1979): The Role of Aquatic Resources in the Prehispanic Economy of the Basin of Mexico. *Journal of Anthropological Research* 64, 351–366.
- Peters, C., Richter, K.K., Manne, T., Dortch, J., Paterson, A., Travouillon, K., Louys, J., Price, G.J., Petraglia, M., Crowther, A., Boivin, N., 2021. Species identification of Australian marsupials using collagen fingerprinting. *Royal Society Open Science* 8, 211229. <https://doi.org/10.1098/rsos.211229>
- Peterson, A.T., Navarro Sigüenza, A.G., 2006. Hundred-year changes in the avifauna of the Valley of Mexico, Distrito Federal, Mexico. *Huitzil* 7, 4–14.
- Presslee, S., Wilson, J., Woolley, J., Best, J., Russell, D., Radini, A., Fischer, R., Kessler, B., Boano, R., Collins, M., Demarchi, B., 2017. The identification of archaeological eggshell using peptide markers. *Science and Technology of Archaeological Research* 1424300. <https://doi.org/10.1080/20548923.2018.1424300>
- R Core Team, 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria. URL <https://www.R-project.org/>.
- Richter, K., K., McGrath, K., Masson-MacLean, E., Hickinbotham, S., Tedder, A., Britton, K., Bottomley, Z., Dobney, K., Hulme-Beaman, A., Zona, M., Fischer, R., Collins, M.J., Speller, C.F., 2020. What's the catch? Archaeological application of rapid collagen-based species identification for Pacific Salmon. *Journal of Archaeological Science* 116, 105116. <https://doi.org/10.1016/j.jas.2020.105116>
- Richter, K.K., Codlin, M.C., Seabrook, M., Warinner, C., 2022. A primer for ZooMS applications in archaeology. *Proceedings of the National Academy of Sciences* 119, e2109323119. <https://doi.org/10.1073/pnas.2109323119>
- Richter, K.K., Wilson, J., Jones, A.K.G., Buckley, M., van Doorn, N., Collins, M.J., 2011. Fish 'n chips: ZooMS peptide mass fingerprinting in a 96 well plate format to identify fish bone fragments. *Journal of Archaeological Science* 38, 1502–1510. <https://doi.org/10.1016/j.jas.2011.02.014>
- Sanders, W.T., Parsons, J.R., Santley, R.S., 1979. *The Basin of Mexico: Ecological Processes in the Evolution of a Civilization*. Academic Press, New York.
- Speller, C., van den Hurk, Y., Charpentier, A., Rodrigues, A., Gardeisen, A., Wilkens, B., McGrath, K., Rowsell, K., Spindler, L., Collins, M., Hofreiter, M., 2016. Barcoding the Largest Animals on Earth: Ongoing Challenges and Molecular Solutions in the Taxonomic Identification of Ancient Cetaceans. *Philosophical Transactions of the Royal Society* 371, 20150332. <https://doi.org/10.1098/rstb.2015.0332>
- Stewart, J.R.M., Allen, R.B., Jones, A.K.G., Kendall, T., Penkman, K.E.H., Demarchi, B., O'Connor, T., Collins, M.J., 2014. Walking on Eggshells: A Study of Egg Use in Anglo-Scandinavian York Based on Eggshell Identification Using ZooMS. *International Journal of Osteoarchaeology* 24, 247–255. <https://doi.org/10.1002/oa.2362>

- 905 Strohalm, M., Kavan, D., Novák, P., Volný, M., Havlíček, V., 2010. mMass 3: A Cross-  
 906 Platform Software Environment for Precise Analysis of Mass Spectrometric Data.  
 907 Anal. Chem. 82, 4648–4651. <https://doi.org/10.1021/ac100818g>
- 908 Sugiyama, N., Valadez Azúa, R., Rodríguez Galicia, B., 2017. Faunal Acquisition,  
 909 Maintenance, and Consumption: How the Teotihuacanos Got Their Meat.  
 910 Archaeological and Anthropological Sciences 9, 61–81.  
 911 <https://doi.org/10.1007/s12520-016-0387-z>
- 912 Tellkamp, M.P., 2019. A story told from a small-mesh screen: the importance of  
 913 songbirds and ground doves to the Guangala people at the El Azúcar  
 914 archeological site in coastal Ecuador. Archaeological and Anthropological  
 915 Sciences 11, 6411–6421. <https://doi.org/10.1007/s12520-018-00772-6>
- 916 Valadez Azúa, R., 2013. Una Ciudad Prehispánica Vista a través de la Fauna, in: Götz,  
 917 C.M., Rivas, J., Cárdenas, J., Hernández, H., Zimmermann, M., Ramos, C. (Eds.),  
 918 Culturas Americanas y Su Ambiente: Perspectivas Desde La Zooarqueología,  
 919 Paleoetnobotánica y Etnobiología. Götz C, Rivas J, Cárdenas J, Hernández H,  
 920 Zimmermann y M, Ramos C., pp. 219–237.
- 921 van der Sluis, L.G., Hollund, H.I., Buckley, M., De Louw, P.G.B., Rijdsdijk, K.F., Kars,  
 922 H., 2014. Combining histology, stable isotope analysis and ZooMS collagen  
 923 fingerprinting to investigate the taphonomic history and dietary behaviour of  
 924 extinct giant tortoises from the Mare aux Songes deposit on Mauritius.  
 925 Palaeogeography, Palaeoclimatology, Palaeoecology 416, 80–91.  
 926 <https://doi.org/10.1016/j.palaeo.2014.06.003>
- 927 Wang, N., Samantha, B., Peter, D., Sandra, H., Maxim, K., Sindy, L., Oshan, W.,  
 928 Stefano, G., Michael, C., Kolska, H., Matthew, S., Glenn, S., Michael, S.,  
 929 Korzow, R., Katerina, D., 2021. Testing the efficacy and comparability of ZooMS  
 930 protocols on archaeological bone. Journal of Proteomics 233, 104078.  
 931 <https://doi.org/10.1016/j.jprot.2020.104078>
- 932 Welker, F., Soressi, M., Rendu, W., Hublin, J.J., Collins, M., 2015. Using ZooMS to  
 933 identify fragmentary bone from the Late Middle/Early Upper Palaeolithic  
 934 sequence of Les Cottés, France. Journal of Archaeological Science 54, 279–286.  
 935 <https://doi.org/10.1016/j.jas.2014.12.010>
- 936 Widmer, R.J., Storey, R., 2016. Skeletal Health and Patterns of Animal Food  
 937 Consumption at S3W1:33 (Tlajinga 33), Teotihuacan. Archaeological and  
 938 Anthropological Sciences 9, 51–60. <https://doi.org/10.1007/s12520-016-0417-x>
- 939 Wolverton, S., 2013. Data Quality in Zooarchaeological Faunal Identification. Journal of  
 940 Archaeological Method and Theory 20, 381–396. [https://doi.org/10.1007/s10816-](https://doi.org/10.1007/s10816-012-9161-4)  
 941 [012-9161-4](https://doi.org/10.1007/s10816-012-9161-4)
- 942 Zhang, G., Li, C., Li, Q., Li, B., Larkin, D.M., Lee, C., Storz, J.F., Antunes, A.,  
 943 Greenwold, M.J., Meredith, R.W., Ödeen, A., Cui, J., Zhou, Q., Xu, L., Pan, H.,  
 944 Wang, Z., Jin, L., Zhang, P., Hu, H., Yang, W., Hu, J., Xiao, J., Yang, Z., Liu, Y.,  
 945 Xie, Q., Yu, H., Lian, J., Wen, P., Zhang, F., Li, H., Zeng, Y., Xiong, Z., Liu, S.,  
 946 Zhou, L., Huang, Z., An, N., Wang, Jie, Zheng, Q., Xiong, Y., Wang, G., Wang,  
 947 B., Wang, Jingjing, Fan, Y., da Fonseca, R.R., Alfaro-Núñez, A., Schubert, M.,  
 948 Orlando, L., Mourier, T., Howard, J.T., Ganapathy, G., Pfenning, A., Whitney,

949 O., Rivas, M.V., Hara, E., Smith, Julia, Farré, M., Narayan, J., Slavov, G.,  
 950 Romanov, M.N., Borges, R., Machado, J.P., Khan, I., Springer, M.S., Gatesy, J.,  
 951 Hoffmann, F.G., Opazo, J.C., Håstad, O., Sawyer, R.H., Kim, H., Kim, K.-W.,  
 952 Kim, H.J., Cho, S., Li, N., Huang, Y., Bruford, M.W., Zhan, X., Dixon, A.,  
 953 Bertelsen, M.F., Derryberry, E., Warren, W., Wilson, R.K., Li, S., Ray, D.A.,  
 954 Green, R.E., O'Brien, S.J., Griffin, D., Johnson, W.E., Haussler, D., Ryder, O.A.,  
 955 Willerslev, E., Graves, G.R., Alström, P., Fjeldså, J., Mindell, D.P., Edwards,  
 956 S.V., Braun, E.L., Rahbek, C., Burt, D.W., Houde, P., Zhang, Y., Yang, H.,  
 957 Wang, Jian, Avian Genome Consortium, Jarvis, E.D., Gilbert, M.T.P., Wang, Jun,  
 958 Ye, C., Liang, S., Yan, Z., Zepeda, M.L., Campos, P.F., Velazquez, A.M.V.,  
 959 Samaniego, J.A., Avila-Arcos, M., Martin, M.D., Barnett, R., Ribeiro, A.M.,  
 960 Mello, C.V., Lovell, P.V., Almeida, D., Maldonado, E., Pereira, J., Sunagar, K.,  
 961 Philip, S., Dominguez-Bello, M.G., Bunce, M., Lambert, D., Brumfield, R.T.,  
 962 Sheldon, F.H., Holmes, E.C., Gardner, P.P., Steeves, T.E., Stadler, P.F., Burge,  
 963 S.W., Lyons, E., Smith, Jacqueline, McCarthy, F., Pitel, F., Rhoads, D., Froman,  
 964 D.P., 2014. Comparative genomics reveals insights into avian genome evolution  
 965 and adaptation. *Science* 346, 1311–1320. <https://doi.org/10.1126/science.1251385>  
 966