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The role of birds at Çatalhöyük revealed by the analysis of eggshell

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Abstract

The exceptional eggshell assemblage from Çatalhöyük was studied using an integrated approach combining morphology (by optical and scanning electron microscopy) and palaeoproteomics (by mass spectrometry). We provide taxonomic classification for 90 fragments, of which only 11 remain undetermined. The striking predominance of Anseriformes (probably including greylag geese, as well as ducks and swans) in all types of deposits examined, including middens and burial fills, suggests that these eggs were exploited as food and, at the same time, had a special significance for the inhabitants of the site. We detected the presence of crane eggshell in contexts associated with both the world of the living and the world of the dead (consistent with the well-known importance of this bird at Çatalhöyük), as well as the possible but infrequent occurrence of bustards and herons. Overall, we suggest that eggshell analysis, together with osteological data and the archaeological context, can provide the basis for a nuanced understanding of the relationship between humans and birds in the past.

Keywords

Eggshell; taxonomy; birds; morphology; microscopy; ancient proteins; mass spectrometry; Çatalhöyük; Neolithic.

1. Introduction

The role of birds in the lives of human beings can vary widely: from food resource including meat and eggs (but not necessarily both at the same time and in all cultures), to source of ornamental "goods" (feathers, talons (Romandini et al., 2014; Blasco et al., 2019; Finlayson et al., 2019)) or even objects of cult (Russell and McGowan, 2003; Russell, 2018a). Birds also have a parallel role beyond death: ritual food offerings, symbols of rebirth, active subjects in funerary rituals (for

example, defleshing by vultures (Pilloud et al., 2016)). In some instances, birds may be thought of as the link between the world of the living and that of the dead (e.g. Best and Mulville, 2017). Active behaviours of humans towards birds include observing, fearing, worshipping, catching, managing in captivity, taming, mimicking, domesticating, feeding, killing, butchering, selecting any desired anatomical parts, and disposing of the remains (Serjeantson, 2009). The relationship between birds and their human counterparts can vary dramatically, not only by species, but also by the lifestage of the bird.

The study of archaeological avian bone assemblages is crucial in order to unpick these different strands. For example, the bird bones at Çatalhöyük suggest that cranes had ritual significance, but also were a food item (Best et al in prep; Russell 2018a; 2018b). Complementary information on bird-human relationships can also be obtained through the analysis of eggshell assemblages. Fragments of avian eggshell can be studied and identified morphologically (Keepax, 1981; Sidell, 1993) or using mass-spectrometry-based proteomic analyses (Stewart et al., 2013/4; Presslee et al., 2017). The extent of resorption of the mammillae can potentially provide information on incubation stage and hatching (Chien et al., 2009), although taphonomy plays an important and yet poorly understood role (Sichert et al., 2019). For example, the ritual role of (possibly fertilised) chicken eggshell has been recently highlighted in conversion period (12^{th}–13^{th} century AD) graves from Kukruse in Estonia (Jonuks et al., 2016), as well as Late Roman burials from Ober-Olm (Sichert et al., 2019). Lack of interactions between humans and avifauna can also be inferred from the eggshell record; e.g. the excellent preservation of proteins in Accipitridae eggshell at the Palaeolithic cave of El Mirón (northern Spain) allowed us to infer that bearded vultures and humans probably did not occupy the site at the same time (Demarchi et al., 2019).

The largest-known Neolithic proto-city of Çatalhöyük (Figure 1) in modern-day Turkey (7100–6000 cal BC), is an exceptional site in its own right, but also because the (relatively small (Mulville, 2014)) assemblage of bird bones has been thoroughly studied and recently published (Russell and McGowan, 2003; Russell, 2018a; 2018b). Russell's work highlights a striking pattern whereby birds at Çatalhöyük were obviously valued for their feathers, clearly had a symbolic role (notably cranes, vultures, crows, and spoonbills), but were not necessarily an important food resource. This is in contrast to other sites in the same region (Baird et al., 2018), despite the extensive presence of marshes and wetlands around the site (Charles et al., 2000).

Extensive recovery by flotation and hand-collection during Ian Hodder's excavations at the site has resulted in an assemblage of unparalleled size and preservation; over 940 units produced eggshell, equivalent to at least 5kg of material (Sidell and Scudder, 2004; Mulville, 2014). Preliminary analysis on a subsample of eggshells (n fragments = 40) in 2015 showed that the assemblage was dominated by Anseriformes species, probably more than one on the basis of the surface morphologies (Best et al., 2015). Here we analyse a further sample (n fragments = 50) of eggshells, selected on the basis of their morphology and archaeological context, in order to have a representative sample from midden layers, burial infills, floors and activity areas. We also reconsider the 2015 results in response to updated analysis techniques. By combining the results from the 2015 and 2019 studies we aim to:

1) Identify and characterise the 90 fragments of eggshells from the site, using a combination of morphological observations (by optical and scanning electron microscopy) and protein analyses (MALDI-MS and LC-MS/MS)
2) Highlight any patterns in avian eggshell representation in living areas (floors, middens) vs areas pertaining to the world of the dead (burial fills)
3) Compare the information obtained by analysing eggshell with that obtained by osteological studies of the Çatalhöyük material.

Approximate location of Figure 1

2. Materials and methods

2.1 Samples analysed

Permission was obtained to export eggshell for analysis in 2014 and in 2018. The material was selected to encompass the temporal breadth of the site, and to represent different areas of the settlement. A first batch of 40 samples was selected randomly and analysed in 2015 (hereafter "2015 batch"), in order to assess which birds dominated the eggshell assemblage, and characterise the protein preservation. A further 50 samples were deliberately selected in order to obtain identification of morphologically distinct eggshell fragments (hereafter "2019 batch"). Table 1 shows the details of the 90 samples analysed, including the archaeological unit, the Hodder level, the deposit categories inferred from the Çatalhöyük project database (midden, floors, burial fill, fill, activity and midden activity). We also report the identification obtained by morphological observation and protein analysis, the inference derived from the combination of the two methods, any signal relative to developmental stage and/or taphonomy, and the eggshell's thickness.

2.2. Microscopy

All specimens were examined by optical microscopy and Keyence Digital Microscope (VHX 5000 series). Where detailed morphological features of the mammillae were present (and were not too damaged by taphonomy or chick development) SEM analysis was employed. For all specimens measurements were taken of the mean number of pores and mammillae per mm^2, the ratio of the mammillae to palisade layer, and eggshell thickness (following Sidell (1993) and Keepax (1981)). The eggshell thickness is averaged from 10 measurements for each specimen and considered a useful but rough indicator for establishing size groupings, since Maurer (Maurer et al., 2012) has demonstrated that thickness can vary significantly within an individual egg. Where SEM analysis took place the internal surface of each sample was then examined and described following set criteria to aid taxonomic identification, including:

1. The regularity, size, shape and spacing of the mammillae;
2. The depth of fissuring and the sutures form and depth;
3. Fiber trails and struts;

Examination of the internal surface was conducted at 300x, 800x and 1000x magnification to allow further comparison with reference materials.

Changes to the surface of the mammillae were also recorded for all specimens (Table 1). This can be caused by both chick development (resorption caused by the chick mobilising calcium from the eggshell), or by taphonomic processes (such as acidic corrosion) - these markers can be very difficult to separate morphologically and terminology for recording this is often used interchangeably. However, no evidence for damage indicates that the egg was freshly laid or infertile. Meanwhile, changes in the mammillae surface may either indicate stage of chick development/hatching (Bradley Beacham and Durand, 2007) or inform on taphonomic processes in the burial environment (Sichert et al., 2019). In this paper we used “no resorption” to indicate a lack of developmental activity, and we use “corrosion” where developmental and taphonomic signals cannot be conclusively separated. Corrosion is described by its regularity/irregularity, and intensity of mammillae destruction (minimal, moderate, high, very high). Where corrosion is very regular, it is more likely to result from chick development, whereas patchy, irregular corrosion is often taphonomic in nature (Morel 1990; Sichert et al., 2019).

Approximate location of Table 1

2.3 Palaeoproteomics

Samples for ancient protein analyses were prepared according to published protocols (Presslee et al., 2017). In brief, eggshell fragments were weighed (5-40 mg), powdered using clean micro-pestles directly in eppendorf tubes, exposed to bleach (NaOCl, 12% w/v for 72 hours) in order to isolate the intracrystalline fraction (Stewart et al., 2013/4; Crisp et al., 2013; Demarchi et al., 2016; Presslee et al., 2017). Bleached powders were demineralised in cold 0.6M hydrochloridric acid (HCl), the solutions neutralised, lyophilised and resuspended in ammonium bicarbonate (50 mM). Alkylation / reduction of disulphide bonds was carried out using dithiothreitol (65°C, 60 min; Sigma Aldrich) and iodoacetamide (room temperature, 45 min; Sigma Aldrich). After overnight digestion at 37°C with trypsin (0.5 µg/µL), samples were acidified and purified using C18 solid-phase extraction (Pierce zip-tip) according to the manufacturer’s instructions. Eluted peptides were spotted directly on a MALDI plate (see below) and the remaining volume evaporated to dryness using a centrifugal evaporator before LC-MS/MS analyses.

All samples were analysed in triplicate by MALDI-MS (Bruker Ultraflex III MALDI-ToF mass spectrometer). 1 µl of sample was spotted onto an MTP384 Bruker ground steel MALDI target plate and 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution (1% in 50% Acetonitrile/0.1% Trifluoroacetic acid (v/v/v)) was added and mixed. Samples were analysed using the following parameter settings: ion source, 25 kV; ion source, 21.4 kV; lens voltage, 9 kV, laser intensity 40–55% and mass range 800–4000 Da. Peptide masses below 650 Da were suppressed. Each sample was externally calibrated against an adjacent spot containing a mixture of six peptides (des-Arg1 Bradykin $m/z = 904.681$, Angiotensin I $m/z = 1295.685$, Glu1-Fibrinopeptide B $m/z = 1750.677$, ACTH (1–17 clip) $m/z = 2093.086$, ACTH (18–39 clip) $m/z = 2465.198$ and ACTH (7–38 clip) $m/z = 3657.929$). Data analysis was performed using the open-source software mMass (Strohalm et al., 2010): three replicates were averaged for each sample, then peaks with signal-to-noise ratios > 6 and height > 62% were considered and matched to a list of taxon-specific *m/z* values (Presslee et al., 2017). All spectra are reported in Supplementary Information 2.

Five samples (CH15_7, CH15_12, CH15_34, CH19_21, CH19_38) were also analysed by LC-MS/MS as described in Fischer and Kessler (2015) and following the guidelines for palaeoproteomics detailed in Demarchi et al. (2016) and Hendy et al. (2018). Briefly, peptides were separated on a PepMAP C18 column (75 µm × 500 mm, 2 µm particle size, Thermo) using a Dionex Ultimate 3000 UPLC at 250 nL/min and acetonitrile gradient from 2% to 35% in 5% dimethyl sulfoxide/0.1% formic acid. Blanks were analysed between each sample. Peptides were detected with a Q-Exactive mass spectrometer (Thermo) at a resolution of 70,000 @ 200 *m/z*. Up to 15 precursors were selected for High-energy Collision Dissociation (HCD) fragmentation. Resulting product ion spectra were searched against a protein database obtained from NCBI (restricting the taxonomy to "birds", downloaded 22/02/2019) and containing common contaminants (cRAP: https://www.thegpm.org/crap/) using the software PEAKS (version 8.5). For PEAKS, FDR rate was set at 0.5%, with protein identifications accepted with −10lgp scores ≥ 40 and ALC (%) ≥ 80.

3. Results

3.1 Representativity of the sample studied

The eggshell fragments analysed were not evenly distributed among the different deposit categories (Figure 2): the majority of samples came from middens (n=51), followed by burial fills (n=18), floors (n=11), midden activity (n=4), activity (n=3), fill (n=3). As a consequence, the representativity of the sample is limited for activity areas, non burial fills and floors, and the discussion will therefore mainly focus on midden deposits and burial fills, thus comparing and contrasting the world of the living and the world of the dead.

3.1 Anseriformes

The identification of Anseriformes eggshell was obtained on the basis of the taxon-specific markers of Presslee et al. (2017) detected in the MALDI-ToF spectra (Figure 2, Table 2) and of eggshell morphology. In order to obtain protein sequence data, which could further confirm the attribution of the spectra to eggshells of Anseriformes birds, we performed LC-MS/MS analyses of two samples (CH15_7 and CH15_12). In both instances, the majority of the protein sequences identified (ovocleidin-116, serum albumin, ovalbumin, lactadherin, serotransferrin, BPI-fold-containing family B member 4-like; Supplementary Information 1) yielded a match to sequences from *Anas* and *Anser* (as shown by the pie charts in Figure 2).

It is currently not possible to discriminate between different species of Anseriformes, neither using MALDI-MS markers nor sequence data, for two main reasons:

i) The majority of the MALDI-MS markers (Table 2) belong to ansocalcin, a C-type lectin originally sequenced from goose eggshell (Lakshminarayanan et al., 2003) but, as discussed elsewhere (Presslee et al., 2017) detected in both goose and duck eggshells by palaeoproteomics.

ii) Similarly, the sequences we identified by LC-MS/MS belonged to both *Anser* and *Anas* (other bird taxa also yielded a match, albeit less frequently, as seen in the pie charts reported in Figure 2).

In general, the agreement between the morphological and molecular analyses was very strong (Table 1), i.e. where diagnostic features were available all eggshell identified as Anseriformes or Anseriformes-like by mass spectrometry was also independently identified as certain or potential goose, duck (and possibly swan) by microscopy. Additionally, six eggshell fragments were classified as goose (or cf. goose) by microscopy, but produced low-quality spectra; three of these were either burnt or heat-stained. A single fragment (CH19_6) identified morphologically as cf. goose yielded a spectrum that could not be determined as Anseriformes. Relatively low protein coverages in the archaeological samples (around 50-60% for the top proteins, see Table 3) and lack of high-coverage genomes of various Anseriformes species prevent complete refinement of the taxonomic determination at this stage. However, microscopy indicated that geese were more common in the eggshell assemblage than ducks, and suggested that a range of species are represented in both groups. From the shell thickness and morphology it is likely that the majority of the goose eggs belong to greylag geese (Figure 3A and Table 1). This is the only goose species that today breeds in the area, and as such the potential identification of multiple geese species is interesting (Russell (2018a). It is possible that the reference materials currently available do not cover all variations within greylag goose eggshell, or for large duck species such as shelduck (*Tadorna tadorna*). Alternatively, this eggshell may include other species commonly identified in the bone assemblage by Best (in prep) and by Russell and McGowan (2005), such as the white-fronted goose (*Anser albifrons*) and the lesser white-fronted goose (*Anser erythropus*). Although neither of these breeds in the area today, interestingly the lesser white-fronted goose does breed in Eastern Turkey, Syria, and Greece, which may suggest different breeding distributions in the past (https://www.iucnredlist.org/, accessed 03/12/2019). At present, other related birds such as black geese [*Branta*] cannot be eliminated using mass spectrometry or microscopy.

A small number of the eggshells are above the thickness range usually assigned to geese (Keepax, 1981; Sidell, 1993; Maurer et al., 2012), and as such swan eggs may also be present in the assemblage (although none could currently be confidently identified by morphology; Figure 7). Overall, it is clear that the inhabitants of Çatalhöyük consistently exploited the eggs of various Anseriformes species (especially geese). Anseriformes represented more than 50% of the whole assemblage (54 out of 90 fragments confidently identified as Anseriformes, and 61 of 90 fragments when probable Anseriformes are included). Interestingly, Anseriformes represented over 75% of the fragments from the randomly-selected 2015 batch (30 out of 40 fragments), indicating that their true prevalence is probably underestimated in this small-scale analysis.

Just over half of the Anseriformes and probable Anseriformes (31 of 61 samples) exhibited no resorption, indicating that the majority of eggs were taken when recently laid (Figure 3B). A further 6 specimens had very patchy corrosion which is likely to be taphonomic damage. The remaining 24 fragments had mostly uniform corrosion which could represent either chick development, taphonomic damage, or a combination of the two. Of these, 11 fragments had high levels of corrosion which may indicate live young hatching on site, or eggshell material collected at point

of hatch (although taphonomy cannot be eliminated here). The majority of these highly corroded fragments came from the midden, but with one from a burial fill and another from a house floor.

Approximate location of Figure 2

Approximate location of Table 2

Approximate location of Table 3

Approximate location of Figure 3

3.2 Non-Anseriformes

Initial microscopic identification indicated that a wide range of species were present in the assemblage, including non-anseriformes. For the CH19 batch a diverse range of morphologically distinct samples were deliberately selected by one of us (JB), in the hope of achieving accurate identification of these other taxa.

The non-Anseriformes eggshell appears to be diverse, and in several instances identification is not possible. Within this, three MALDI-ToF spectral “fingerprints” could be identified, which were then associated to three broad size-categories of eggshell (not necessarily reflecting the size of the birds that they came from): small-sized (n=4; representative spectrum shown in Figure 5A), medium-sized (n=3; representative spectrum shown in Figure 5B) and large-sized (n=9; representative spectrum shown in Figure 5C). The spectra are clearly different, but we were unable to univocally identify these taxa on the basis of the published MALDI-MS markers of Presslee et al. (2017): each spectrum showed the presence of markers pertaining to different species, contrary to what was observed for the Anseriformes samples (see Figure 2). Therefore, LC-MS/MS analyses were conducted on three samples (all data are included in Supplementary Information 1): CH19_38 (small-size), CH19_21 (medium-size) and CH15_34 (large size). Bioinformatic searches of the product ion spectra against the NCBI birds proteomes did not yield straightforward identification of the three taxa: indeed, the pie charts in Figure 5 are strikingly different from those obtained for Anseriformes (Figure 2).

Approximate location of Figure 4

The large-size bird (eggshell) could be tentatively identified as a Gruiformes on the basis of the higher proportion of spectra that could be assigned to this order (Figure 5F), and via microscopy, which indicated that several measurements (including mammillae density, characteristics and eggshell thickness) were appropriate for crane. However, species not represented in the microscopic reference material could not be unequivocally ruled out. Of the nine fragments in this large group, six had no resorption of the shell, two had patchy, very minimal corrosion and one had minimal uniform corrosion (potentially indicating some initial chick development).

In contrast, the medium-size sample yielded a mixed signal, with roughly an equal number of peptide sequences assigned to Gruiformes, Galliformes and Passeriformes (Figure 3E). Interestingly, the top-scoring protein for this sample was ovalbumin from *Chlamydotis macqueenii,* a bustard. Bustards are present at the site (Russell, 2018a), and the shell thickness measurements do fall within the range of another bustard species, *Otis tarda* (although comparative material for detailed morphological analysis was not available at the time of analysis). Therefore, it is possible that sample CH19_21 is indeed a fragment of eggshell pertaining to this taxon. However, more reference material for both proteomics and microscopy would be needed to rule out other possibilities; this will be a priority of our future work. Two fragments in this category had no resorption and one was too abraded to determine damage to the mammillae.

Sample CH19_38, representative of the small-size category, could not be clearly determined. Microscopy suggested that a large heron such as *Ardea cinerea* is a potential candidate, and palaeoproteomic analyses gave a mixed signal, as identified peptides/spectra were assigned to proteins from a variety of avian orders (Figure 5D), which however do include Pelecaniformes. Herons are a very common bird at Çatalhöyük and, as such, their presence in the eggshell assemblage would not be surprising, particularly given the presence of juvenile heron at the site (Best et al. in prep; Russell 2018a). We will be sourcing additional reference material and conducting a more in-depth assessment in the future, based on the results of this preliminary identification. One of the fragments in this group showed no resorption, another had minimal patchy corrosion (probably taphonomic), one moderate uniform corrosion, and one very extensive uniform corrosion. It is probable that the latter two represent eggs with developing chicks inside.

Four samples were undetermined by MALDI-MS and were not subjected to further in-depth palaeoproteomic analyses. CH15_25 was not identifiable by proteomics or microscopy, but CH15_23 was suggestive of a possible vulture egg morphologically, and two Accipitriformes markers (Demarchi et al., 2019) were observed in the MALDI mass spectrum. CH19_6 and CH19_13 can be assigned very tentatively to goose and bustard respectively based on morphology.

Approximate location of Figure 5

3.3 Distribution of eggshell taxa

Figure 6 shows the distribution of eggshell taxa (identification obtained by combining proteomics and morphology, see Table 1) in different deposit categories. Anseriformes dominate the eggshell assemblages recovered from both middens and burial fills, demonstrating the importance of these birds, and particularly geese, at the site. Both geese and ducks are found in all three of these deposit categories. The overall profile between the middens and the burial fills are very similar. This may also suggest a lack of separation between the world of the dead and the world of the living, a recurrent theme at Çatalhöyük, with birds that had significance in life also playing a role in death. It appears that the eggshell fragments were intentionally incorporated into several of the grave environments, although it cannot be ruled out that some inadvertently entered the fill. Of the 18 samples from burial fills, five (28%) produced no usable proteomic data, compared to 10%

of the samples from middens and associated activities. Given the generally excellent preservation of the proteins encountered in this study, we attribute this pattern to burning, which is supported by some of the fragments yielding low-quality spectra being from scorched layers. Several of the eggshell fragments from burial fills exhibit physical evidence of burning (for example, see Figure 6) but heat exposure is known to affect protein preservation even without visible alteration of the biomineral (e.g. Crisp, 2013). The data could therefore be interpreted as evidence of ritual burning of the remains interred with the dead, or the selection of burnt material for incorporation in the burial activities. It may also simply represent waste disposal or food discard, as there are also large quantities of burnt bone found at the site. The redeposition of material certainly needs to be considered as a factor influencing distribution of eggshell and its interpretation, especially at a complex site such as Çatalhöyük. As such, although we have identified some differences between midden and burial fills material (e.g. level of burning), we stress that this cannot be automatically assigned to intentional incorporation.

Approximate location of Figure 6

The large quantity of eggshell recovered from midden deposits suggests that many of the eggs were food or activity waste, although their presence may also have played some role in the closing of buildings. The large number of samples with no resorption or minimal/patchy corrosion suggests that many eggs would have been used as food or in other activities where undeveloped eggs are needed, e.g. as paint binders. This hypothesis is interesting and could be potentially tested by analysing paint remains using proteomics. Although quantifying eggshell is very challenging, the quantity recovered from the site suggests that bird eggs were a more common food item than bird meat, since the avian bone assemblage is relatively small (see Best et al. in prep. and Russell, 2018a). Interestingly, 45% of the fragments from floor deposits (5 of 11 specimens) produced no usable proteomic data, indicating that these eggs may have suffered more taphonomic damage.

Non-Anseriformes are represented in small numbers in the three largest deposit categories (floors, midden and burial), and it is probable that additional non-Anseriformes fragments are present in the unidentified material.

Approximate location of Figure 7

4. Discussion and conclusions

This study focussed on the multi-disciplinary analysis of 90 eggshell fragments from Çatalhöyük. Each fragment was characterised using microscopy-based morphological observations and mass spectrometry-based protein analysis. The combination of the two approaches was successful in identifying the fragments, generally at order level, and with a high level of agreement between morphological and molecular data (Table 1). By combining the methods we were able to provide higher resolution for several fragments than would have been possible using either technique in isolation.

Most fragments (68%) were determined to be Anseriformes or very probable Anseriformes, highlighting the striking prevalence of geese and ducks at the site. The highest proportion of geese and ducks were recovered from midden deposits (world of the living), but they were also well represented in burial fills (world of the dead). Anseriformes eggs are a nutritious and seasonally-abundant resource, clearly linked to the local environment, and the presence of wetlands. Therefore, while the use of bird meat as a food resource is not frequent at Çatalhöyük, as highlighted by a number of studies, including the most recent reassessment by Russell (2018a; 2018b) and Best (in prep.), the exploitation of eggs seemed to be a common feature. Food itself can be special, and egg consumption/use might have had symbolic resonance due to its link with the seasons' cycle, or through association with the living birds that inhabited the landscape around Çatalhöyük and played many different roles in living at, and experiencing, the site. Seasonality and connotations of new-life may have made eggs an appropriate material for deliberate placement in graves.

When combined with the proteomic analyses, the microscopy indicates that many of the Anserifomes are large geese, and the greylag goose in particular is a likely candidate for much of the eggshell material. Greylag geese still breed around the site today (Russell 2018a). These birds often gather to breed in colonies and could have radically changed the appearance and audiality of the landscape surrounding the site during their summer breeding season. It appears that Anseriformes egg collection was a key part of avian exploitation, potentially explaining why the adult birds do not seem to have been killed in large numbers, as this would have then deprived the fowlers of the valuable egg resources. It seems that life at Çatalhöyük involved an intimate knowledge of birds both in their use as food items, in their movement around the landscape and for their symbolic lives.

Among non-Anseriformes, the presence of crane egg in particular should be noted. The crane seems to have been consumed as food but also played a ritual role at the side (Best et al. in prep; Russell, 2018a, 2018b). It is possible that like the birds themselves, their eggs may have also been used in both mundane and special ways. Whilst the wetland environments around the site would have provided suitable nesting environments for Anseriformes, Gruiformes and some Pelecaniformes, it is possible that some egg resources may have been brought from further afield or traded either as food or as special items.

Overall, we confirmed the importance of an integrated zooarchaeological and molecular approach for the study of ancient eggshell. We also highlighted the excellent preservation of eggshell proteins at this 8000-year-old site, despite the hot environment. Fragments which did not yield proteins are therefore likely to having been burnt, possibly during ritual activities, as a high proportion of burnt eggshell was found in burial deposits. The high presence of burning in floors, where the highest proportion of undetermined eggshell was recovered, may result from cooking activities.

We have also highlighted that while deep-branching Anseriformes (Galloanserae) can be readily identified via proteomics as their protein make-up is very distinctive (Figure 2), deciphering the eggshell proteome composition of Neoaves is more challenging (Figure 5). This is consistent with

the later and “sudden” evolutionary radiation of Neoaves at around 50 million years ago. Phylogenetic analyses based on the reconstructed sequences of specific proteins will be the focus of future work, in the hope that these will improve taxonomic resolution for this and other sites. We hope to assess the rest of the Çatalhöyük assemblage as soon as resources allow. In the meanwhile, we will continue producing integrated focused datasets, which can provide a baseline for obtaining more nuanced interpretations of the role of birds in the lives of humans in the past.

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Data availability

All the mass spectrometry proteomics (LC-MS/MS) data have been deposited in the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository (Vizcaíno et al., 2013) with the data set identifier PXD014558.

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FIGURE CAPTIONS

Figure 1: Location of Çatalhöyük in present-day Turkey.

Figure 2: MALDI-TOF spectra for Anseriformes samples CH15_07 (A) and CH15_12 (B), showing the main Anseriformes markers. Pie charts represent the taxonomic composition of proteomes, calculated as the proportion of spectra assigned to different avian orders by bioinformatic analysis of LC-MS/MS data obtained on samples CH15_07 (C) and CH15_12 (D). For each protein group, only the top-scoring protein identifiers were considered. Note that Anseriformes spectra clearly dominate the composition, i.e. the majority of the product ion spectra could be unequivocally assigned to Anseriformes.

Figure 3: Goose eggshell fragments. A: CH15_5 Anseriformes - goose eggshell fragment, likely *Anser anser,* with an average thickness of 0.6 mm. B: CH15_19 Anseriformes - goose eggshell fragment showing no resorption, indicating that minimal chick development had taken place, or that the egg was infertile.

Figure 4: Eggshell thickness for three specimens from the three MALDI-ToF "fingerprints" determined by MS analysis and also recognised by microscopy. A: CH19_38 (small), B: CH19_21 (medium) and C: CH15_34 (large).

Figure 5: MALDI-TOF spectra for non-Anseriformes samples CH15_38 (A), CH19_21 (B) and CH15_34 (C), showing the mixed-taxa markers. Pie charts represent the taxonomic composition of proteomes, calculated as the proportion of spectra assigned to different avian orders by bioinformatic analysis of LC-MS/MS data obtained on samples CH19_21 (D), CH19_38 (E) and CH15_34 (F). For each protein group, only the top-scoring protein identifiers were considered. Note that the taxonomic signal is mixed in all instances.

Figure 6: Relative abundance of avian types identified in each deposit category. Geese silhouettes highlight the dominance of this taxon in midden and burial fill deposits, while floor sediments contained a higher proportion of undetermined fragments; low sample numbers imply that this information would not be meaningful for activity, midden activity and fill deposits.

Figure 7. A highly charred eggshell fragment: CH19_9 from unit 22676 - a burial fill. This fragment is damaged but is most likely to be swan. A: external surface of eggshell. B: thickness and ratio of the mammillae to palisade layer.

Figure 1

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Figure 2

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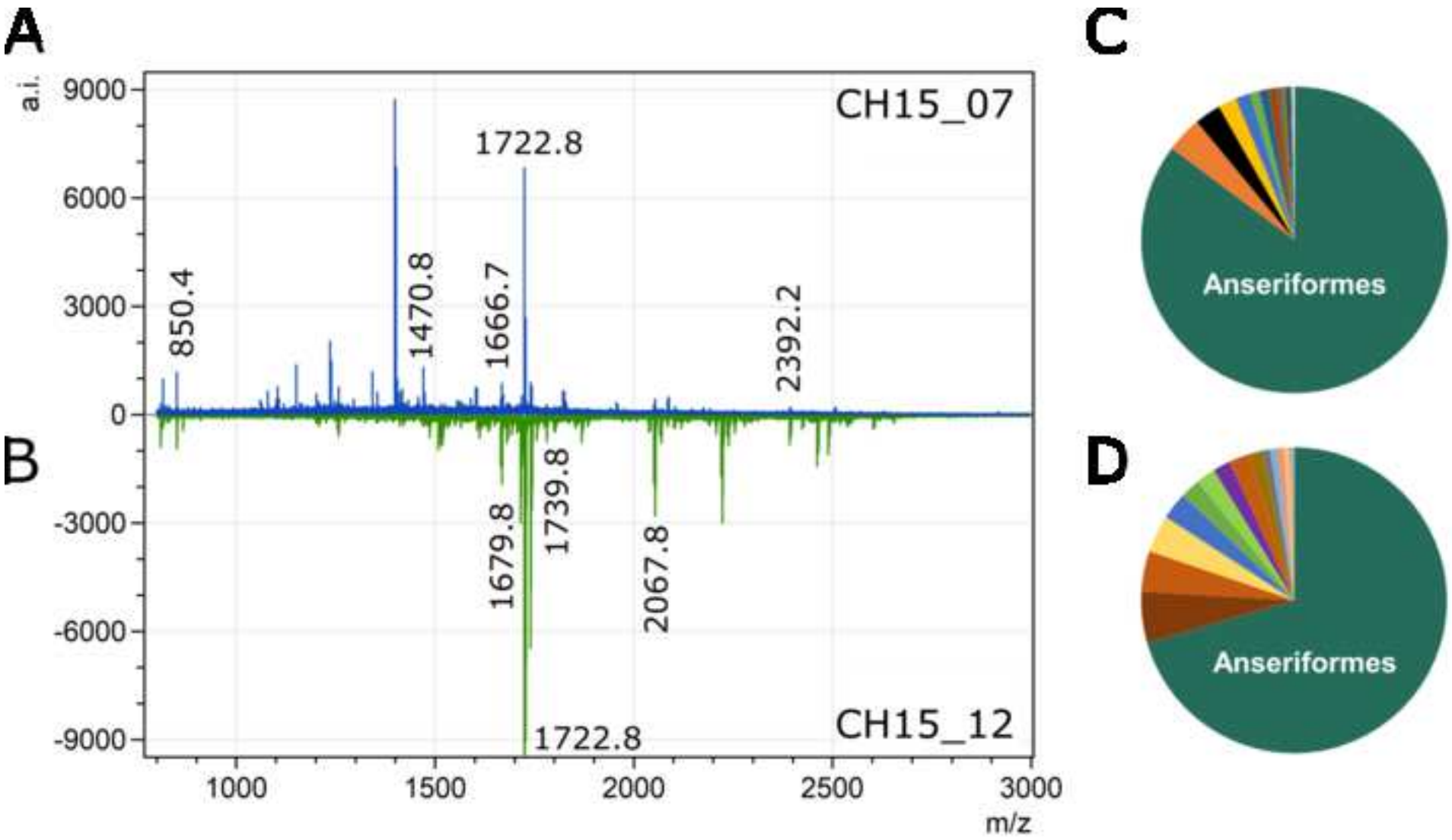

Figure 3

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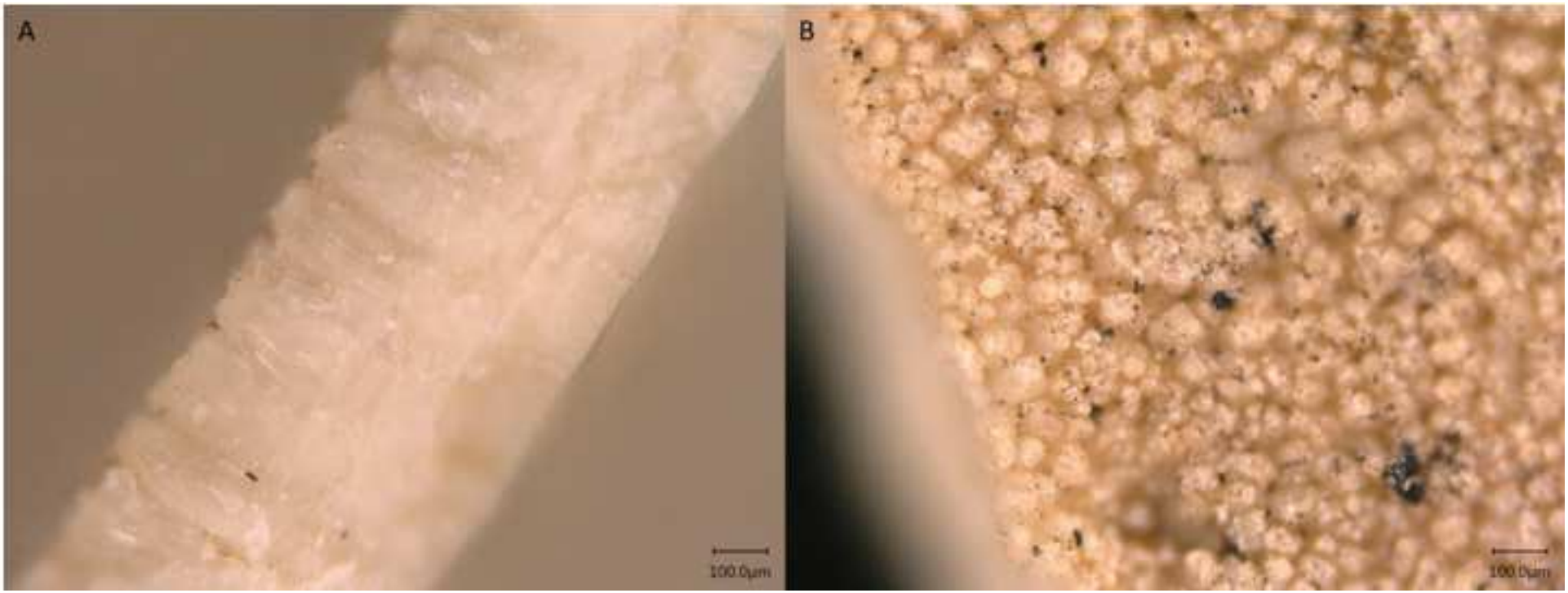

Figure 4

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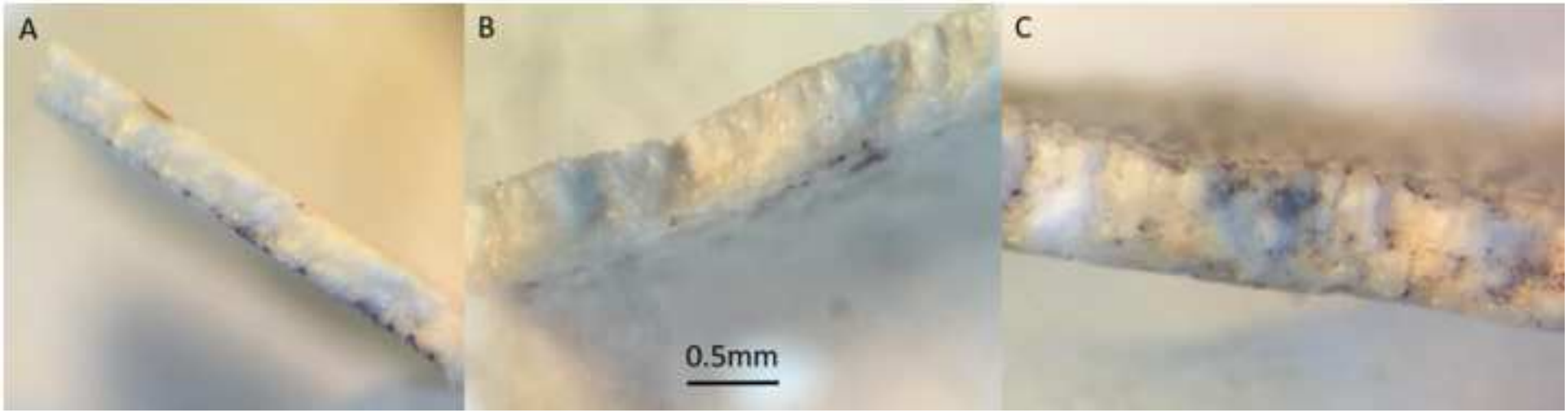

Figure 5

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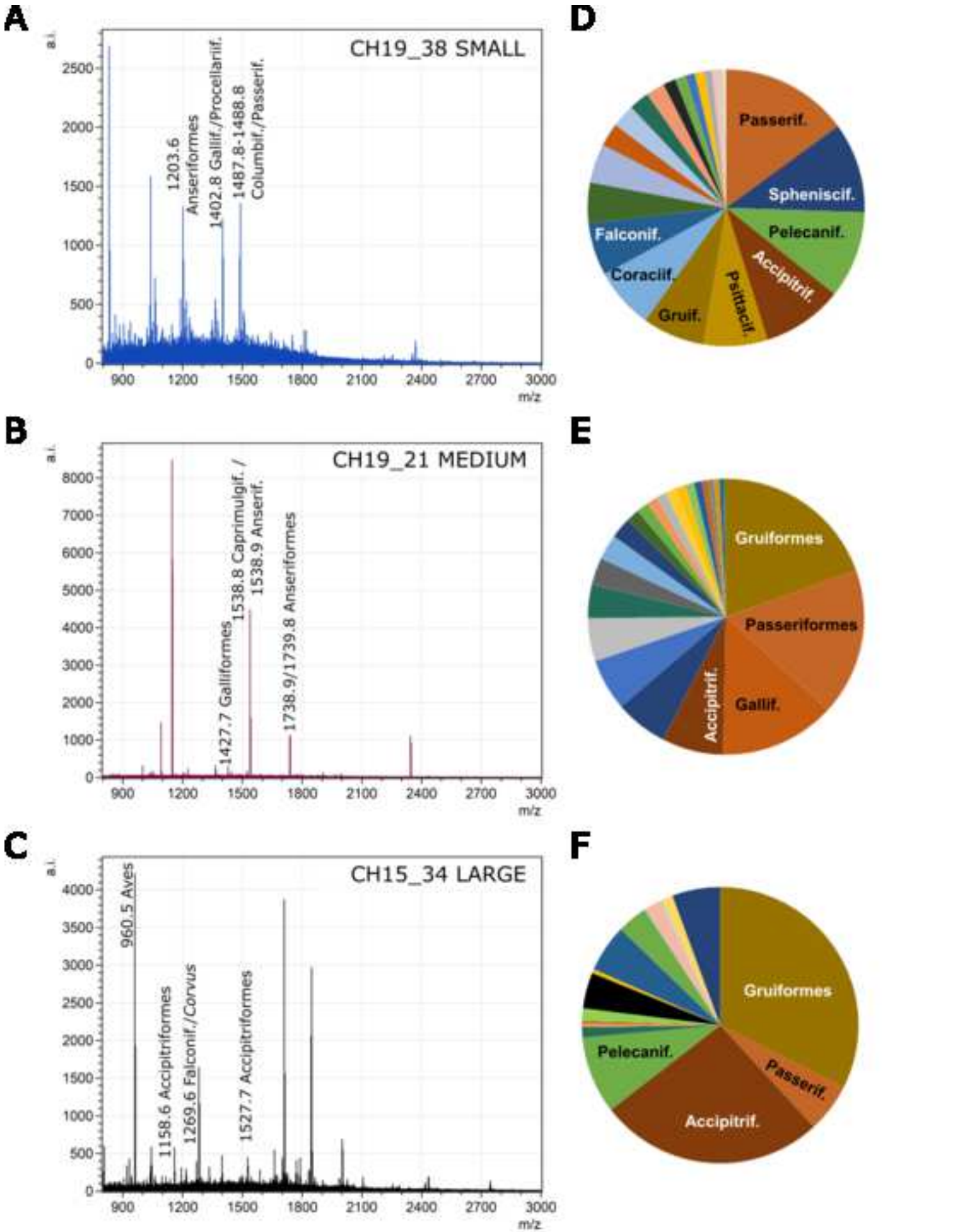

Figure 6

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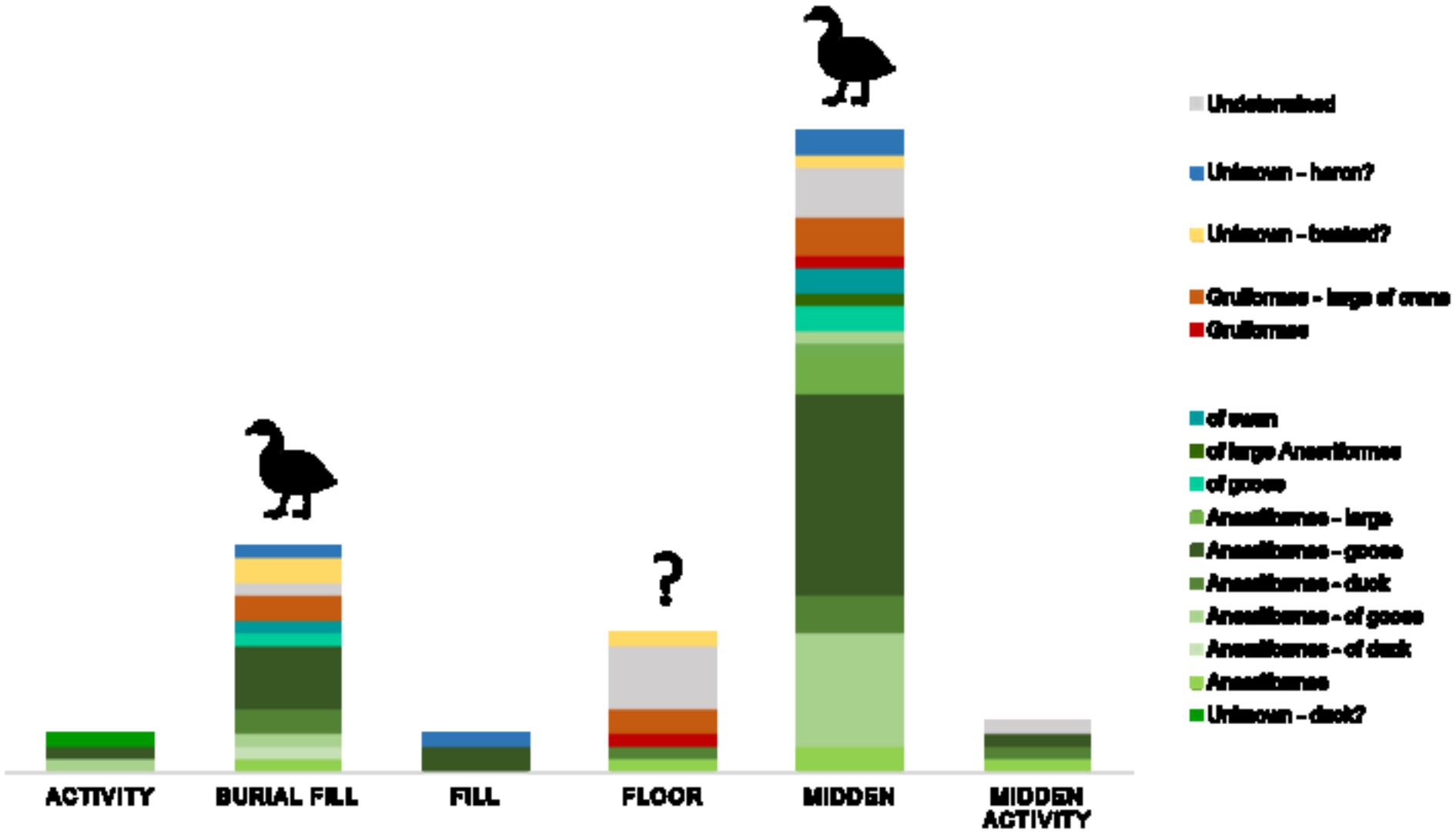
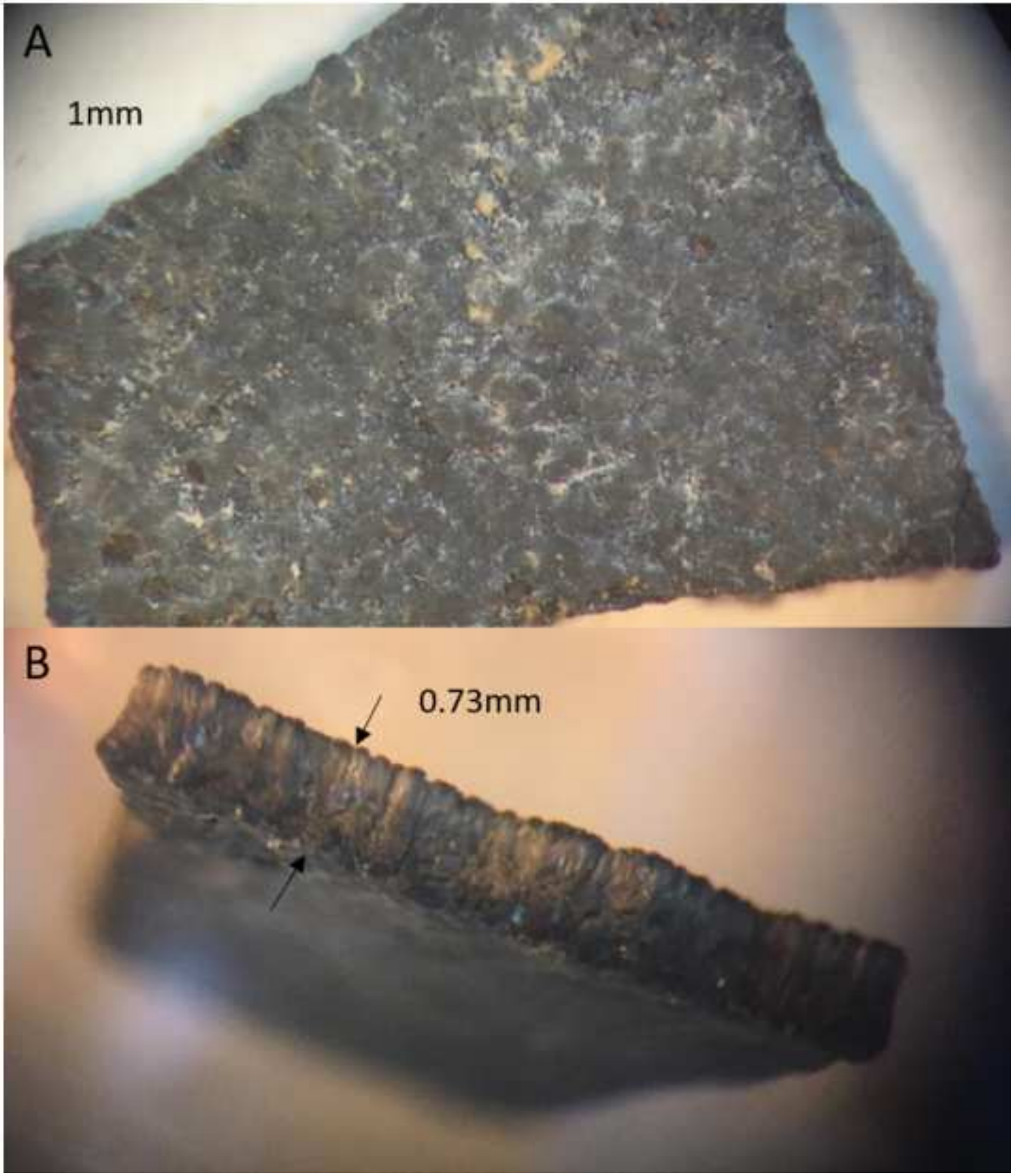


Figure 7

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