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SHORT COMMUNICATION

# Study of archaeological nits/eggs of *Pediculus humanus capitis* by scanning electron microscopy

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## Abstract

This paper presents and discusses archaeological samples of *Pediculus humanus capitis* nits/eggs in Arica, northern Chile, dating between 2000 B.C. and A.D. 500. Eight samples of nits/eggs taken directly from seven mummified bodies of both the valley and the coast of Arica, were collected and studied. Samples were analyzed with scanning electron microscopy (SEM), uncoated, using low and variable pressure modes. The aim was to study the morphology of the nits/eggs, the different degrees of preservation and their research potential. All samples were in good external condition and due to manipulation before SEM analysis, the oldest ones were fractured allowing the observation *in situ* of the hatching *ad portas* of an embryo. This inside view of the egg allowed observation and identification of microstructures of the embryo such as abdominal and thoracic spiracles and claws. In the most recent and best preserved samples, external structures characteristic of the egg such as aeropyles and operculum were observed. SEM can contribute significantly to the study of ectoparasites that affected ancient American populations and in this particular case to illustrate the stages and morphology of Andean archaeological specimens of *P. humanus capitis*.

**Keywords:** *Pediculus humanus capitis*, Chile, Mummies, Variable pressure, Operculum, Spiracles

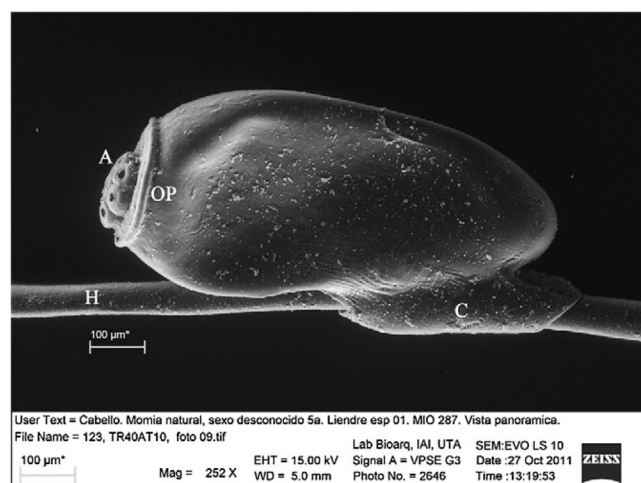
## 1. Introduction

*Pediculus humanus capitis* is the subspecies of the human head louse. The females of *P. humanus capitis* are larger than males, showing clear sexual dimorphism (Campos et al., 2007, p. 29). About a day after mating, females begin laying eggs. Each female lays about 3–10 per day. Over 4–5 weeks, a female lays about 60–100 eggs. The eggs are whitish and about 800 µm long (Atias, 1999, p. 465). The females have terminal portion called gonopod and a uterine gland that secretes cement-like glue, which serves to fix the eggs to the hair during egg laying. These eggs are strongly attached to the hair by this glue, preventing them from easy removal but leaving open the operculum with the aeropyles necessary for respiration of the embryo (Zuñiga and Caro, 2010, p. 57). The glue is composed of proteins similar to hair keratin, but it is more rigid, probably due to tyrosine and phenylalanine residues which hardens the nit/egg sheath (Burkhart and Burkhart, 2005, pp. 130–131).

Season and general climate affects the position of eggs. In cold weather the position of the eggs is closer to the scalp and only one egg per hair is laid. By contrast, in warmer climates more than one egg per hair are laid by a single female (Zuñiga and Caro, 2010, p. 57). Laboratory experiments show that the proximity of the egg to the scalp provides the ideal conditions of temperature (27–31 °C) and relative humidity (45–75%) for

its development (Mougabure et al., 2006, p. 260). With these optimal parameters, the eggs embryonates within 5–10 days and then a very labile larva hatches. The larva must feed quickly to survive (Zuñiga and Caro, 2010) (p. 58). An adult state is reached after three nymphal stages over 6–9 days (Botero, 1998, pp. 387–388), (Heukelbach, 2010, pp. 29–30). The cycle from egg to adult takes about 2–4 weeks and all of this happens in the head of the host (Botero, 1998, p. 388).

The firm adherence of the nits/eggs to the hair and keratinous composition of the shell of the nits/eggs allows them to survive for a long time, even in the archaeological record. This throws some light on the extent of the infestation and the lifestyles of ancient populations. Thus, Araújo et al. (2000, p. 269) have reported the finding of a louse egg on a human hair at an archaeological site in northeastern Brazil dating back to 10,000-years. Also Rivera et al. (2008, p. 31) reported a high degree of infestation in 6 of 7 tested mummies, associated with the late stage of the Chinchorro tradition (ca. 2000 B.C.) of northern Chile. And in the south of Perú in the Chiribaya culture a range of 18% to 71% infestation of *P. humanus capitis* was found between high elevation and coastal sites (Reinhard and Buikstra, 2003, p. 178). Another case in a Maitas Chiribaya mummy from Arica was reported, in northern Chile, dating back to A.D. 760 that was severely infested with *P. humanus capitis* nits/eggs (Arriaza et al., 2012).



**Figure 1.** Specimen TR40AT10. Excellent preserved egg (A: aeropyles; C: cementum; H: hair; OP: operculum).

This study reviews and reports different mummified archaeological nits/eggs samples from various sites in Northern Chile (ca. 2000 B.C. to A.D. 500) providing new data that contributes to the evolutionary study of head lice. Using SEM, in variable pressure mode, the morphological details of the mummified nits/eggs and embryos are identified.

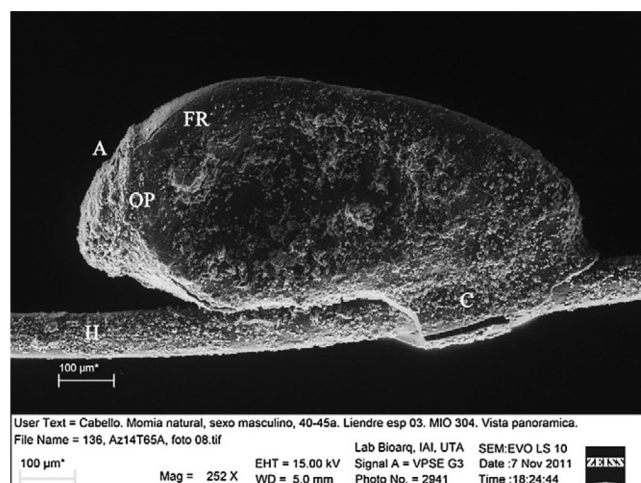
## 2. Materials and methods

We analyzed eight nits/eggs of *P. humanus capitis* extracted directly from bodies that were naturally mummified. The macro analysis was undertaken at the laboratory of physical anthropology of the Archaeological Museum of San Miguel of Azapa of the Universidad de Tarapacá (MASMA). These nits/eggs are from five archaeological sites in the city of Arica, northern Chile: Quiani ( $n = 1$ ), Morro 1 ( $n = 2$ ), Camarones 15 ( $n = 2$ ), Azapa 14 ( $n = 2$ ), TR40A ( $n = 1$ ). The first two sites correspond to the Early Period (Chinchorro Fishermen and Gatherer, ca. 1500–1300 B.C.) and the third to the fifth correspond to the Formative Groups (ca. 1500 B.C.–A.D. 500), (Agüero, 1995; Agüero and Cases, 2004; Arriaza and Standen, 2008; Dauelsberg, 1974; Rivera et al., 2008). Following the methodology proposed by Reinhard and Buikstra (2003, p. 173) an area on the head of 2 cm × 2 cm in the temporal and occipital of the mummified individuals was selected to quantify the presence of nits/eggs. From this area, eight specimens were extracted and are reported in this study. It should be mentioned that the specimens of the nits/eggs analyzed were selected because of being attached to the hair of pre-Columbian individuals and their degree of preservation and integrity.

The collected samples were analyzed at the Instituto de Alta Investigación, Universidad de Tarapacá, Arica, Chile. The specimens were mounted on aluminum stubs on a double contact carbon film and positioned on the stubs using a stereoscopic microscope model SZX-7. Samples were analyzed uncoated using a SEM, model EVO-LS 10 in low vacuum and variable pressure (VP) mode. The chamber pressure was 150 Pa, and column  $2 \times 10^{-5}$  Pa high vacuum was, the working distance was 4–8 mm with a tilt of 0°. The images were recorded at 3024 × 2304 pixels and a scanning speed of 12 min 54 s.

## 3. Results

The nits/eggs revealed excellent preservation, but in a few cases placing them in the stubs created microfractures, which



**Figure 2.** Specimen Az14T65A. Excellent preserved egg, but with superficial debris (A: aeropyles; C: cementum; FR: fracture; H: hair; OP: operculum).

allowed the observation of anatomical structures *in situ*. These features are described below including an intact egg to a hatching egg. Samples were not rehydrated because this SEM model permits to work with variable pressure mode, without altering the morphology of the samples. In the future, fractures might be prevented by rehydrating the remains in 0.5% trisodium phosphate which might make the lice more pliable.

In Figure 1, specimen TR40A T10 of the Formative Period shows a louse egg in excellent condition. The image allows us to identify aeropyles (A), cementing substance (C), hair (H), and operculum (OP). These structures are in very good condition similar to a modern sample. We cannot determine if it is embryonated. Such determination depends on imaging the internal structures of the egg which we can only do by fracturing the egg. Not all of the eggs are embryonated on the mummies.

In Figure 2, specimen Az14T65A, a louse egg from the Formative Period is dirty with indeterminate elements at this magnification. The following structures are visible: A, C, H, and OP. In addition, there is a fracture of the egg, a result of the handling and fragility of the specimen. The morphology of the egg corresponds to *P. humanus capitis*, but we cannot be certain that is embryonated.

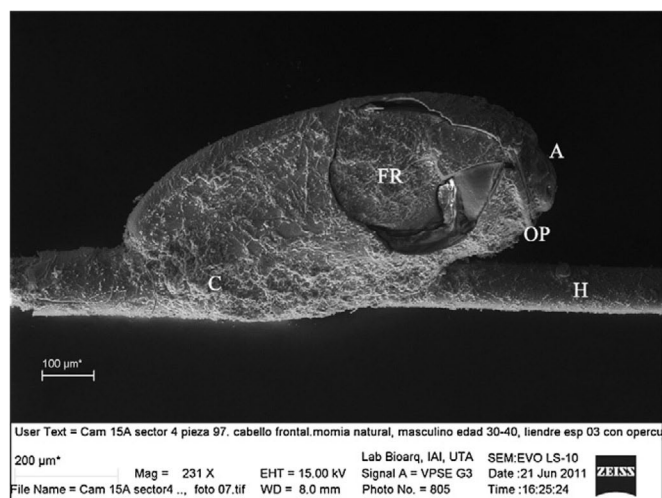
In Figure 3, specimen Cam15A sector 4, 97, a louse egg from the Formative Period, the following structures are visible: A, C, H, and OP. In addition, there is a fracture of the egg resulting from manipulation and positioning the nit/egg onto the stub. The split (microfracture) of the egg is not wide enough to allow the observation of anatomical structures of an embryo of *P. humanus capitis*.

In Figure 4, specimen Az14T16, a louse egg from the Formative Period, the following structures are visible: A, C, and OP. There is a *post-mortem* fracture allowing visualization of the inside of the egg, showing part of a claw (CL). The egg morphology, size and shape are consistent with an embryonated *P. humanus capitis* egg.

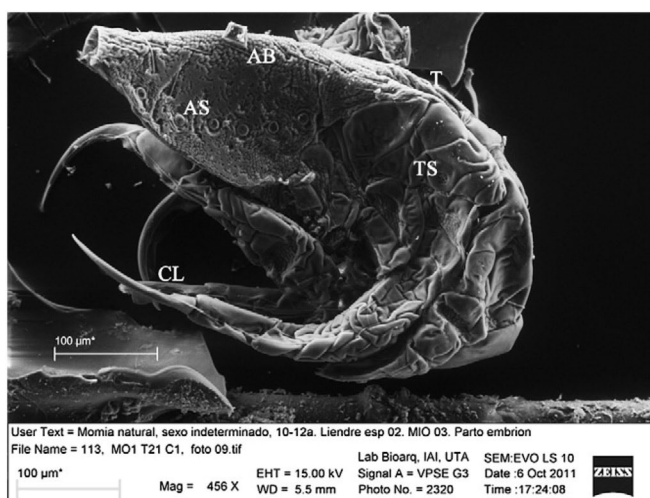
In Figure 5, specimen Quiani7T16A, a louse egg of the Early Period, the following structures are visible: A, C, H, OP, and the *post-mortem* fracture of the egg permitted observation of the abdominal spiracle (AS), abdomen (AB) and embryo's claw (CL). These anatomical structures are characteristic and conclusively demonstrate that the egg is embryonated.

In Figure 6, specimen Mo1T21C1, an egg from the Early Period, was collected from a hair that separated from the head of the mummy during handling. The following structures are visible: abdominal and thoracic spiracles (AS, TS), AB, CL and

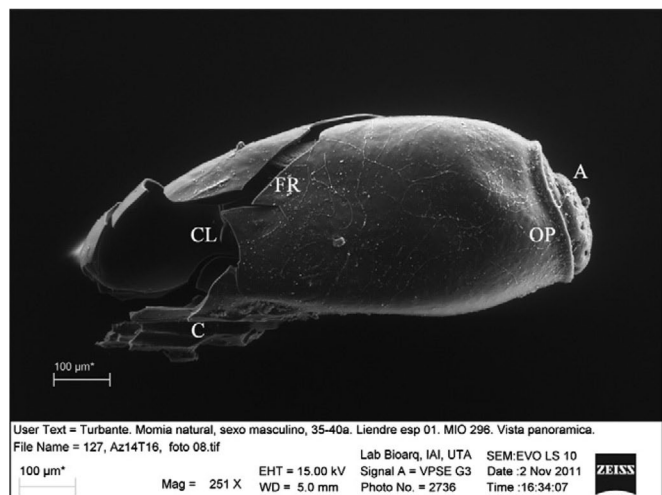




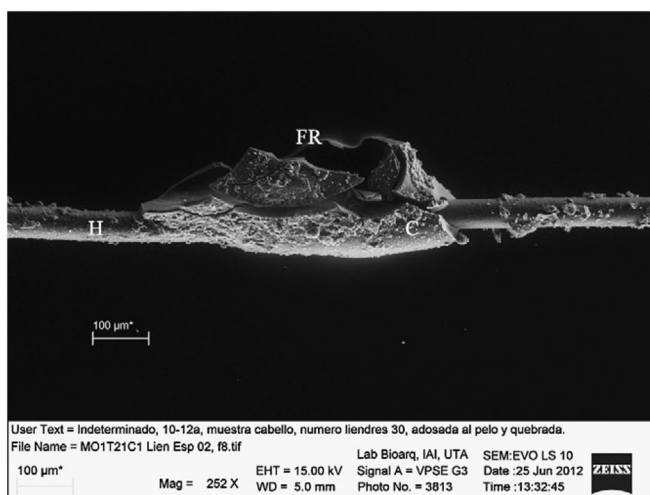
**Figure 3.** Specimen Cam 15A sector 4 Pieza 97. *Post-mortem* fractured egg (A: aeropyles; C: cementum; FR: fracture; H: hair; OP: operculum).



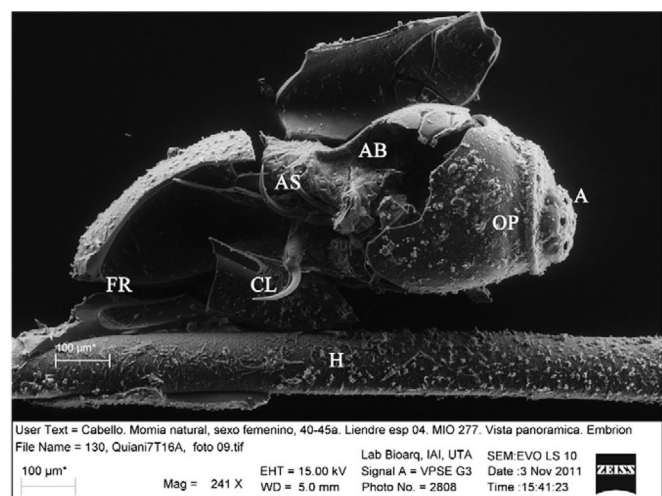
**Figure 6.** Specimen Mo1T21C1. Severe *post-mortem* fractured egg and embryo (AB: abdomen; AS: abdominal spiracle; CL: claw; T: thorax; TS: thoracic spiracle).



**Figure 4.** Specimen Az14T16. *Post-mortem* fractured egg (A: aeropyles; C: cementum; FR: fracture; CL: claw; OP: operculum).



**Figure 7.** Specimen Mo1T21C1. Remains of a fractured egg and cementum (C: cementum; FR: fracture; H: hair).



**Figure 5.** Specimen Quiani7T16A. Severe *post-mortem* fractured egg (A: aeropyles; AB: abdomen; AS: abdominal spiracle; CL: claw; FR: fracture; H: hair; OP: operculum).



**Figure 8.** Specimen Cam15T6. Hair with remains of cementum (C: cementum; H: hair).

thorax (T). The *post-mortem* fracture allowed the observation of anatomical structures that demonstrate the presence of an embryo of *P. humanus capitis*. This ectoparasite is more than 3000 years old, and shows a nymph on the verge of hatching from its egg *in situ*.

In Figure 7, specimen Mo1T21C1 a fractured louse egg from the Early Period. The fracture was likely caused by the combination of the fragility of the specimen, rough manipulation and storage conditions. Even though the louse egg is broken the cementum substance appears intact. The following structures are visible: C, H, and a fracture (FR).

In Figure 8, specimen Cam15T6, hair and cement substance from the Formative Period, shows that minimal remains of the egg/nit are present, represented by glue residue.

#### 4. Discussion

The archaeological samples of nits/eggs have varying degrees of preservation and the images of SEM (Figures 1 & 2) reveal anatomical structures of louse eggs in perfect condition despite the passage of time and their handling during museum curation and laboratory research. In these images we can confirm that the cementing substance is a very strong structure, in spite of time it still continues to hold the nits/eggs to the hair. In Figures 1–3, typical anatomical nits/eggs structures can be seen, but SEM analysis cannot indicate if these eggs are embryonated because these eggs are intact. In contrast, Figures 4–6 show that the eggs are embryonated since the eggs are fractured. Interestingly, the eggs for which the embryo is observed, resulting from the breakdown of the egg, are the oldest archaeological samples. This would indicate that their frailty would be related to the age of the specimen of about 3000 years. In addition, accidental fracture during handling shows an embryo-nymph in very good condition that was on the verge of hatching (Figure 6). In Figures 4–6 we can observe the microstructures characteristics of *P. humanus capitis*, also the degree of internal preservation is much better than the egg itself. In life, the eggshell protects the embryo from different environmental pollutants and manipulation. After thousands of years, the egg gradually becomes weaker and loses its elasticity easily fracturing when subjected to a minimum pressure on a mounting stub. Interestingly, through SEM analysis of uncoated samples, we can not only observe micromorphology, but also preserve the specimen for subsequent analysis such as DNA. The specimens can be mechanically or chemically removed from the stubs because they are not gold or carbon coated. Extraction of ancient DNA is difficult, and contamination is always a problem, thus we also left some unmounted samples for future molecular study, independent of the SEM work. Also, unhatched eggs are protected by their shells.

For quantifying infestation, the residue of the cementum may be most important. In Figure 1 and Figure 3 the cementum substance can be clearly observed. In addition, in Figure 7 and Figure 8, although the egg shell is broken, the cementum still is attached to the hair, showing its resilience and durability. Ancient nit-picking, grooming, and washing may remove the eggs, but traces of the cement glue remain. Therefore, mummies that are apparently nit/egg free should be examined for traces of cementum to document infestation that was controlled by the time of death.

In summary, microscopic studies of archaeological specimens of the Americas are useful to verify the types of ectoparasites that affected the daily lives of ancient populations and in this particular case, they contribute to the study of the stages and micromorphology of *P. humanus capitis*. The samples

shown here demonstrate that nits/eggs can become very brittle with time, but the cementum endures through time. The study of microsamples and SEM analyses can be a very useful tool to shed light onto ancient ectoparasites and human cultural behavior. Our study of louse eggs in ancient mummies reveals heavy infestations by sex, age and cultural periods. Reinhard and Buikstra (2003) showed that louse infestation prevalence was very high in prehistoric Andean villages, sometimes reaching 70% at some villages, but most individuals were lightly infested. Most of the lice were found on a very few individuals. Indeed, 84% of the lice were recovered from 10% of the most heavily infested mummies. Arriaza et al. (2012) demonstrated that in this background in infestation prevalence, individuals could become overwhelmed with lice such that every centimeter of the scalp was covered with lice. Atacama Desert temperatures decrease significantly at night and in antiquity housing raw materials were minimal, thus people lived in small huts and in crowding conditions. Cultural innovation could also be at play. Early fishing populations did not manufacture hair combs making it more difficult to delouse and control head lice infestations. Lastly, most Andeans wore long hair and during agropastoral times farmers had elaborate hairstyles and delousing combs. All these cultural variables influenced the likelihood and degree of head louse infestations. The combined research of these workers shows that cultural patterns such as grooming, headwear, and hair style created unusual epidemiological patterns. Prehistoric Andean prevalence patterns were the opposite of louse infestation in the modern world: children were least infested and men were most infested. Using SEM methods as presented here, we will be able to expand our studies to look for traces of infestation represented by cementum that may reveal prehistoric success at controlling infestations. With archaeological evidence of individual status, we will be able to assess the risk level posed by poverty in prehistoric times. In essence, SEM analysis opens a more detailed method to assess the nuances of infestation related to behavior.

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[Appendix A, with 5 Supplemental Figures, follows.]

#### Glossary

A:	aeropyles
AB:	abdomen
AS:	abdominal spiracle
C:	cementum
CL:	claw
FR:	fracture
H:	hair
OP:	operculum
T:	thorax
TS:	thoracic spiracle



### Appendix A. Supplementary data

All pre-Columbian Arica populations were affected by head lice independently of cultural period, subsistence and geographic location. Early archaic Chinchorro populations (ca. 2000 B.C.) for example had the lowest infestation value with 75% and those that were positive had an average of 8.2 nits/eggs in a 2 cm × 2 cm area of the head and a range of 1-50 eggs/nits. Later populations had frequencies about 90% and developed special combs to delouse (Supplementary Figs. 1-5)



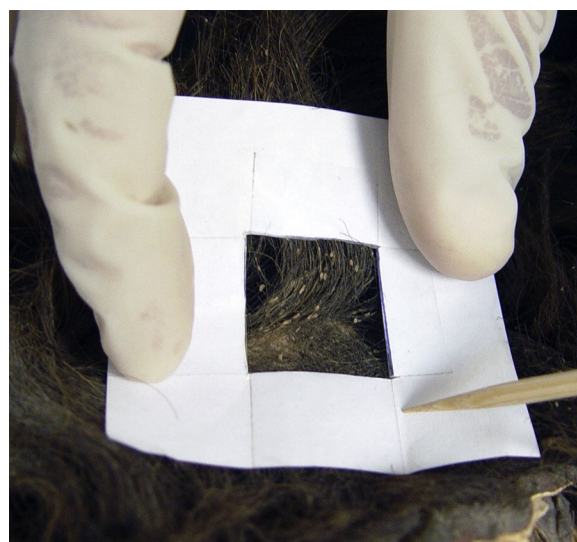
**Supplementary Figure 1.** Chinchorro mummy, infant with artificial mummification and long hair. Circa 2000 B.C.



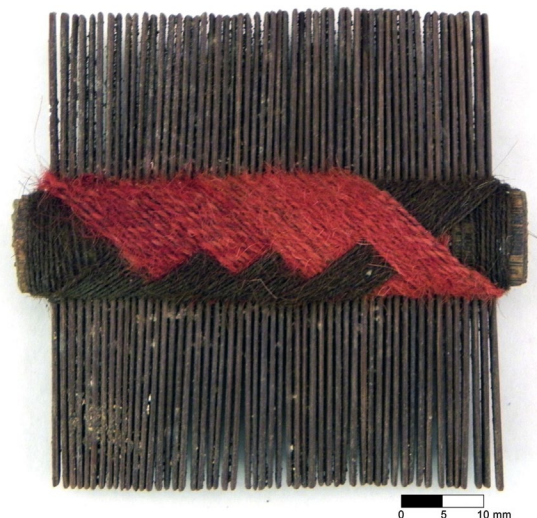
**Supplementary Figure 2.** Various stages of nits/eggs. Two hatched eggs (left: top and bottom), two non-hatched eggs (bottom: center and right) and one hatching egg (top: center). Case Quiani 7 T9. Circa 1300 B.C.



**Supplementary Figure 3.** Severe head lice infestation (nits/eggs) in an agropastoral male. Case AZ140 T75. Circa 1000 A.D.



**Supplementary Figure 4.** Detail of nits/eggs counting methodology. Case AZ140 T75. Circa 1000 A.D.



**Supplementary Figure 5.** Delousing comb. Case PLM4 T171. Circa 1400 A.D.