Manuscript Details

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Title	Incubated eggs in a Roman burial? A preliminary investigation on how to distinguish between the effects of incubation and taphonomy on avian eggshell from archaeological sites
Short title	Distinguish between the effects of incubation and taphonomy on avian eggshell from archaeological sites
Article type	Research Paper

Abstract

Microscopic analyses can be used to determine whether fragments of eggshell come from hatched, incubated or non-incubated eggs. This information is essential for their interpretation since the developmental state of eggs often permits archaeologists to draw conclusions about the function of these finds at a site. However, what has often been neglected in previous studies is the fact that not only incubation but also taphonomy may affect the microstructure of shells. This preliminary study aims to demonstrate that taphonomic processes can in fact imitate site specific dissolution features that are commonly interpreted as traces of incubation. One likely cause of this could be bacteria or other microorganisms. The paper further introduces an approach by which a distinction between taphonomic and embryonic dissolution may be possible. The successful application of this technique on seemingly incubated eggs from a late Roman burial of Ober-Olm (Germany) indicates that these shells were altered only by taphonomy and not by embryonic development as initially assumed. It is finally emphasized that the preliminary data of this investigation need to be validated in future research.

Keywords	avian eggshell; incubation; taphonomy; eggshell quantification; late antiquity; burial sites; ritual

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Suggested reviewers	Marc McKee, Jim Hayward, Werner Müller, Frank D. Steinheimer, Alexander Heising

Submission Files Included in this PDF

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April 15, 2019

Chris O. Hunt
Co-Editor
Journal of Archaeological Science: Reports

Dear Prof. Hunt,

we revised our manuscript and would like to resubmit it with the new title ***"Incubated eggs in a Roman burial? A preliminary investigation on how to distinguish between the effects of incubation and taphonomy on avian eggshell from archaeological sites"***.

We gratefully acknowledge the time and effort that the reviewers and you have put into assessing the previous version of our paper. The reviewers' comments were very valuable and constructive and helped us to considerably improve the manuscript. We have carefully considered all recommendations and comments and below provide responses to the raised issues. Moreover, we included a point by point outline of every change made in the revised manuscript.

We look forward to hearing from you and to respond to any further questions and comments you or the reviewers may have.

Kind regards and happy Easter

Benjamin Sichert
(on behalf of all authors)

REVIEWERS' COMMENTS	RESPONSES	CHANGES MADE
GENERAL COMMENTS		
Reviewer 1: "It was a pleasure to read this manuscript by Sichert et al, and to observe the thinking, underlying logic and evidence for options for eggshell dissolution at archeological sites. Although the notion of taphonomy effects on eggshell dissolution are not new as indicated by the authors, it seems this has nevertheless remained underappreciated, and the authors here find an interesting new way to look at this in a modern compost heap (remarkably seemingly undisturbed over 10 years). Generally I am happy about the paper and the analyses within, and have the following more specific comments."	We gratefully thank the reviewer for this positive feedback.	
Reviewer 2: "This study addresses the question of whether it is possible to determine the developmental stage of avian eggshell found within archaeological remains. The authors compare 1) eggshell uncovered at a human burial site in Germany, 2) modern hatched goose and chicken eggshell, and 3) eggshell retrieved from a modern compost heap. Following a comparison of qualitative and quantitative characteristics of the three eggshell classes, the authors conclude that it is difficult to reliably infer the developmental state of eggs from archaeological sites based on eggshell microstructure. The authors acknowledge this study to be preliminary in nature. Indeed, some features of the study lacked rigor. For example, characterization of the compost heap from which modern eggshell was retrieved was fairly superficial. Nonetheless, I think as a preliminary evaluation of the question posed, the work was carried out carefully and the results were interpreted with caution. The evidence presented supports their conclusion that it is difficult to determine with confidence the developmental stage of eggshell from an archaeological site."	We agree with the reviewer that our study is preliminary in nature. This is all the more reason for us to be happy about his positive feedback.	

TITEL		
Reviewer 1: "1) The title is a little bit off. It alludes only to incubated/hatched eggs, without its comparator (the latter being the nonphysiologic acidic dissolution), and I would drop the "a preliminary investigation" - I think a better title can be concocted."	We have chosen a new title which now includes also taphonomy. However, we would prefer not to drop "preliminary". Our study is still only preliminary and we believe it should be acknowledged as such.	New title: "Incubated eggs in a Roman burial? A preliminary investigation on how to distinguish between the effects of incubation and taphonomy on avian eggshell from archaeological sites"
ABSTRACT		
Reviewer 1: "2) The abstract fails to indicate any results. I don't know of space limitations for the abstract, but a couple statements of results would be nice. As written is too general, like "can in fact imitate features ... (what features?). ... "the paper introduces causes ... (what causes?)."	In accordance with the reviewers' recommendation we have added some results in the abstract.	Page 2, Line 8: "…can in fact imitate features …" revised to "can in fact imitate site specific dissolution features…" Page 2, Line 9-12: "The paper further introduces likely causes and one possible solution to this problem." revised to "One likely cause of this could be bacteria or other microorganisms. The paper further introduces an approach by which a distinction between taphonomic and embryonic dissolution may be possible."
Reviewer 2: "Page 2, Line 10: Here and throughout the manuscript, shouldn't "Roman" be capitalized?"	We thank the reviewer for pointing out this error.	Page 2, Line 13; Page 3, Line 7; Page 5, Line 14; Page 10, Line 27; Page 12, Line 27; Page 13, Line 21; Page 14, Line 30; Page 15, Line 14: "Roman" capitalized
INTRODUCTION		
Reviewer 2: "Page 2, Line 4 under Introduction: ". . . the fact that their microstructure can be used to determine . . ." This paper is questioning this so-called "fact," so it seems that another term (e.g. "belief," "confidence," "conviction," etc.) conveying less certainty should be used here."	We thank the reviewer for this important comment.	Page 2, Line 24: "fact" replaced by "belief"
Reviewer 2: "Page 2, last line: Do Jonuks et al. (2018) really say that the hatching egg "resembles the resurrection of Christ," or do they say it symbolizes that belief? It seems a stretch to me that anyone would think a hatching egg resembles a resurrection!"	Jonuks et al. (2008) in fact say that the hatching egg symbolizes the resurrection of Christ. We thank the reviewer for noticing this error.	Page 2-3, Line 32-2: "The authors further argue that the hatching egg represented a powerful symbol in Christian tradition, since it resembles the resurrection of Christ…" revised to "The authors further argue that in Christian tradition the hatching egg symbolizes the resurrection of Christ…"

Reviewer 2: "Page 3, Line 11: "This theory is partly confirmed . . ." At this stage it would be more appropriate to write "This hypothesis is partly confirmed . . ." A theory is a well-tested assertion about nature, which does not seem to be the case in this situation. The purpose of this paper is just beginning to test this assertion."	We thank the reviewer for pointing out this error. In line with his recommendation, we replaced the word accordingly.	Page 3, Line 13: "theory" replaced by "hypothesis"
BACKGROUND		
Reviewer 1: "3) Is shell "corrosion" the right term to use? This is most frequently used to mean an oxidation (particularly of a metal). Is this a term used in the field? I understand that erosion would mean something else being more mechanical, but what about the term dissolution? This is used sometimes by the authors, as is resorption. Dissolution is usually though used generally for minerals dissolving, and degradation might be used for organics being removed. Anyway, give it some thought. Perhaps dissolution and degradation at least somewhere in the manuscript, thus indicating loss of both mineral and organics, or generally outlining these terms a little better, at least at one place. The shell is of course full of organics across its dimensions. Two relevant papers using these terms and describing related versions of this process, that should be discussed and cited in this work, are eggshell nanostructure and dissolution after egg incubation, and the effects of organics and calcite dissolution. Athanasiadou D, Jiang W, Goldbaum D, Basu K, Pacella MS, Bohm CF, Chromik RR, Hincke MT, Rodriguez-Navarro AB, Vali H, Wolf SE, Gray JJ, Bui KH, McKee MD (2018) Nanostructure, osteopontin and mechanical properties of avian calcitic eggshell. Science Advances 4(3):eaar3219. DOI:10.1126/sciadv.aar3219 Nelea V, Chien YC, Paquette J and McKee MD (2014) Effects of full-length phosphorylated osteopontin and constituent acidic peptides and amino acids on calcite dissolution. Cryst. Growth Design. 14:979-987."	The term "corrosion" in connection with the effects of taphonomy on eggshells from archaeological sites was first used by Philippe Morel (1990). This term has the advantage that it is often better suited for short descriptions of eggshells and their surfaces: For example, "dissolved shell/surface" has a different meaning than "corroded shell/surface". Instead of conveying the image of an eggshell with the typical cratered mammillae, "dissolved" rather gives the impression that the shell/surface has more or less dissolved completely. For this reason we would like to keep the expression "corroded". If this is inadvisable one possible alternative may be "cratered". The term "partially dissolved" (Athanasiadou et al. 2018.) may be another solution, but still has similar disadvantages as "dissolved". In any case we have in line with the reviewers' recommendation tried to better outline the term "corrosion" (Page 6, Line 12-15). We have also cited the study by Athanasiadou et al. (2018). However, we have refrained from using the term "degradation" in the text. It is undeniable that degradation of organic material has taken place. Our study, however, deals only with the visible dissolution of the mineral components of the eggshell.	Page 3, Line 25: "Athanasiadou et al. 2018, 9." cited Page 4, Line 21: "corrosion" replaced by "resorption" Page 5, Line 28-29: „corrosive features" replaced by "areas with mineral dissolution" Page 6, Line 10-14: "After this preparation, each fragment of eggshell was assigned to one of the following five categories:" revised to "After this preparation, the general appearance of the mammillae of each fragment of eggshell was assessed. For this purpose the expression "corroded" was used as neutral and short descriptive term, meaning the typical cratering of the mammillae as a consequence of mineral dissolution (Morel 1990, 146.). Each fragment of eggshell was assigned to one of the following five categories:"

Reviewer 1: "7) A common terminology is air sac, rather than air cell. Where the term "blunt pole" is used in the same figure, wouldn't it be better to use "sharp pole" rather than "apex" as used?"	We appreciate the reviewer's recommendations on the terminology and accordingly replaced "apex" with "sharp pole". We would have changed also "air cell" to "air sack" but the former term appears also in a literal quotation: (Page 4, Line 14-16: "E. Bradley Beacham and Stephen R. Durand (2007) mention that in *"some uncommon instances, the air cell may become dislodged, resulting in inconsistent patterns of resorption."…"* It would be confusing for the reader if we used a different term in the text. If the reviewer strongly recommends the use of "air sac" we offer to change the literal quotation by Beachan/Durand (2007) to a corresponding quotation.	Figure 1 and 2; Page 8, Line 27; Page 8, Line 30: "apex" replaced by "sharp pole"
Reviewer 1: "8) It was annoying that the figures were not numbered."	We are sorry for the inconvenience caused by missing figure numbers.	
Reviewer 1: "9) Figure 1 has an "e" missing from Membran"	We thank the reviewer for pointing out this error.	Figure 1: "membran" revised to "membrane"
Reviewer 2: "Page 4, Line 10: "The dissolution of the mammillary bodies appears at different stages of incubation . . ." Different stages in the same animal, in the same species, or in different species? Please clarify."	Like the passage before it, this sentence refers to incubated eggs in general. To clarify this we added "The described…" at the beginning of the sentence. We hope that this will make it easier to recognize this connection.	Page 4, Line 12: "The dissolution of the mammillary bodies appears at different stages of incubation …" revised to "The described dissolution of the mammillary bodies appears at different stages of incubation …"
MATERIALS AND METHODS		
Reviewer 1: "4) Are areal measurements made from underlying graph paper really the accurate state of the art?"	We agree that there exist more advanced and precise ways to make area measurements. However, the more traditional method applied by us delivers quite accurate results while having the advantage that it is very cost-efficient and easy. Like us, many zooarchaeologists encountered with eggshells will not have the opportunity to use expensive equipment for area measurements.	
Reviewer 1: "5) Shells were washed in water - how? Water jets? flowing tap water" were brushes used?"	The shells were carefully rinsed in flowing tab water. No brushes were used. We added this information in the revised manuscript.	Page 7, Line 16-18: "…they were rinsed carefully in water and dried at room temperature." revised and extended to "…they were rinsed carefully in flowing tab water and dried at room temperature. No brushes were used."

Reviewer 2: "Page 5, Line 4: "Firstly the microscopic appearance of two modern hatched reference shells . . ." Were just two eggshells used, one goose and one chicken, or do the authors mean two KINDS of shells? If only one eggshell of each type was used, this would seem to represent one of the weakest aspects of this study. If the reference shells included only one shell from each species, how do we know they were representative? There is considerable individual variation in avian eggshell."	Indeed only two modern eggshells were used (see also Page 5, Line 20-22: "For each species one successfully hatched egg of natural brood was obtained from small flocks of captive greylag geese (*Anser anser*) and domestic chicken (*Gallus gallus f. domestica*).") We agree with the reviewer that this is the weakest aspect of our study. It is also the main reason why we declare the investigation as preliminary. It is true that there is considerable variation in avian eggshell and this has also been pointed out in the manuscript (Page 4-5, Line 31-1: "Moreover, while details of the above described processes – for example the timing and degree of calcium removal – are known to be variable, the basic principles of embryonic mineral resorption seem to be similar for most or even all avian species (Blom/Lilja 2004, 365-366. Beacham/Durand 2007, 1612-1614. Chien et al. 2009, 537.)." The purpose of the two reference shells was to illustrate and roughly quantify one of these basic principles, namely that the largest part of the inner shell surface is corroded by embryonic resorption during incubation and that only the air cell area remains unaffected by this process. This is an observation that was also made in detailed studies with larger numbers of eggshells (eg. Beacham/Durand 2007, 1612-1614.).	Page 5, Line 6-10: "Firstly the microscopic appearance of two modern hatched reference shells (goose and chicken) will be described. Since it is not the aim of this paper to present a detailed study on modern incubated eggs, the purpose of these two shells is to illustrate and quantify the observations of previous studies…" revised to "Firstly the microscopic appearance of two modern hatched reference shells (one goose and one chicken) will be described. The purpose of these two shells is to merely illustrate and roughly quantify the observations of previous studies (eg. Jakab 1979, 149-151 …" Page 16, Line 7-9: "It has to be stressed that this study is based on a small number of modern reference shells." revised to "It has to be stressed that this study is still very preliminary in nature. For instance, in addition to descriptions from literature (eg. Beacham/Durand 2007, 1612-1614.), only two modern shells were used as reference for hatched eggs."
Reviewer 2: "Page 6, Line 24: The authors state that the studied compost heap was first laid out in the spring of 2009, but they do not state when the heap was studied. So we do not know how long the heap existed before it was examined."	We agree with the reviewer that this is an important information and added the date of excavation.	Page 7, Line 3: "For stratified sampling of the compost heap, a vertical section was cut through the pile." revised to "In November 2017 a vertical section was cut through the compost pile for stratified sampling."
Reviewer 2: "Page 7, Line 3: I do not understand what is meant by the following statement: "Measurements of the <0.5 mm sediment faction of all samples . . ." What are these <0.5 mm sediment factions? I see nothing that defines what these are in the previous text."	The "<0.5 mm sediment faction" refers to the preparation of the samples for chemical analyses. Before the measurements were conducted the compost sediments were dry sieved (mesh size 0.5 mm). We have simplified the sentence and added the relevant reference (Baillard et al. 2004.) for readers seeking more information on the sample preparation.	Page 7, Line 7-8: "Measurements of the <0.5 mm sediment fraction of all samples were taken at the Geoarchaeological Laboratory of the IPAS, University of Basel." revised to "Measurements of the sediment of all samples were taken at the Geoarchaeological Laboratory of the IPAS, University of Basel (Baillard et al. 2004.).

Reviewer 2: "Page 7, Line 29: ". . . remains of a rooster." How do you know it was a rooster and not a hen? Presence of spurs? Size? Other features?"	The identification as a rooster is based both on metric data and the presence of a spur scar on the one recovered tarsometatarsus. Tarsometatarsi with spur scars can be identified as bones of young male animals (eg. De Cupere et al. 2005, 1593 Fig. 7.).	Page 7, Line 33: "… remains of a rooster …" revised to "… remains of a rooster with a spur scar on its one recovered tarsometatarsus …"
RESULTS		
Reviewer 1: "Figure 14 indicating convex shell surface corrosion/pitting. Here you have to be very careful. Shells have variable-thickness (or even absent) cuticles, that vary in form and thickness and texture within the same egg. It even contains a different mineral phase sometimes, calcium-phosphate hydroxyl apatite. thus, it is much more difficult to unambiguously state these may be corrosion/pitting sites. If the authors were to look at more eggshells and more cuticles by SEM, or in the literature, they will see this crusty material from time to time that if you zoom in on, may look like pitting from dissolution. It is also possible that given even environmental ion concentrations can be such that new mineral may precipitate on tops of shell fragments, and give additional surface texture that in its inverse may look like dissolution pitting. A word of caution here, and the authors need to think more about this being cuticle, or that this material in Figure 14 is not actually an integral part of the native shell, but was deposited later."	We agree with the reviewer that great caution is advised when identifying pitting holes. On the archaeological eggshells from the Roman burial we in fact could frequently observe that new mineral was precipitated on top of the shell fragments indicating very complex processes involving both dissolution and precipitation of minerals. Figure 14 shows on of the archaeological shells were we feel confident that the observed features are indeed pitting holes protruding into the shell. Moreover this identification was not only conducted by SEM images but also by stereomicroscopy.	
Reviewer 2: "Perhaps I missed it, but I don't see where the pH of the soil surrounding the eggshells in the Roman burial site is reported. Otherwise, the results are well written. The figures are nicely executed, and the SEM photos are sharp and clear. The figures nicely illustrate the evidence described in the text."	Unfortunately, no soil samples were taken at the excavation of the Roman burial. See also Page 7, Line 30-31: "… no soil samples were taken from the burial …"	
DISCUSSION		
Reviewer 1: "6) No discussion is given to the eggshell pores - wouldn't this be an important favored channel for taphonomic dissolution processes that might help to distinguish physiological (where the pores are not really enlarged) with environmental dissolution?"	This is a very interesting idea which we have not considered so far. In fact, it would certainly be worthwhile investigating this in future studies. However, it needs to be noted that enlarged pores, like many other features, cannot exclude incubation because incubated eggs are also subject to taphonomic factors.	

Reviewer 2: "I think the authors have done a fine job comparing various hypotheses regarding the causes of various eggshell dissolution features. I believe they are correct in asserting the difficulty in determining the stage of egg development from buried eggshell."	We are very grateful for this approval.	
ACKNOWLEDGEMENTS		
		Page 16, Line 24-25: "Finally, we want to thank two anonymous reviewers for their very helpful and valuable comments."
REFERENCES		
	We added Athanasiadou et al. 2018 (cited on Page 3, Line 26) to the reference list.	Page 17, Line 3-7: "Athanasiadou et al. 2018: D. Athanasiadou/W. Jiang/D. Goldbaum/A. Saleem/K. Basu/M. S. Pacella/C. F. Böhm/R. R. Chromik/M. T. Hincke/A. B. Rodríguez-Navarro/H. Vali/S. E. Wolf/J. J. Gray/K. H. Bui/M. D. McKee (2018) Nanostructure, osteopontin, and mechanical properties of calcitic avian eggshell. Science Advances 4 (2018) 1–13." added to the reference list.
	We noticed that we had placed the reference of Blom/Lilja 2004 in the reference list not at its correct position according to the alphabetical order. We moved this reference to its correct position.	"Blom/Lilja 2004 …" moved from Page 17, Line 7 to Page 17, Line 23.
	We added Braillard et al. 2004 (cited on Page 7, Line 3) to the reference list.	Page 17-18, Line 32-3: "Braillard et al. 2004: L. Braillard/M. Guélat/Ph. Rentzel, Effects of Bears on Rockshelter Sediments at Tanay Sur-les-Creux, Southwestern Switzerland. Geoarchaeology: An International Journal 19 (2004) 343–367." added to the reference list.

REFERENCES USED IN THE RESPONSES:

Athanasiadou et al. 2018
D. Athanasiadou/W. Jiang/D. Goldbaum/A. Saleem/K. Basu/M. S. Pacella/C. F. Böhm/R. R. Chromik/M. T. Hincke/A. B. Rodríguez-Navarro/H. Vali/S. E. Wolf/J. J. Gray/K. H. Bui/M. D. McKee (2018) Nanostructure, osteopontin and mechanical properties of calcitic avian eggshell. Science Advances 4 (2018) 1–13.

Beacham/Durand 2007
E. B. Beacham/S. R. Durand, Eggshell and the archaeological record: New insights into turkey husbandry in the American Southwest. Journal of Archaeological Science 34, 2007, 1610–1621.

Braillard et al. 2004
L. Braillard/M. Guélat/Ph. Rentzel, Effects of Bears on Rockshelter Sediments at Tanay Sur-les-Creux, Southwestern Switzerland. Geoarchaeology: An International Journal 19 (2004) 343–367.

De Cupere et al. 2005
B. De Cupere/W. Van Neer/H. Monchot/E. Rijmenants/M. Udrescu/M. Waelkens, Ancient breeds of domestic fowl (*Gallus gallus f. domestica*) distinguished on the basis of traditional observations combined with mixture analysis. Journal of Archaeological Science 32 (2005) 1587–1597.

Morel 1990
P. Morel, Quelques remarques à propos de coquilles d'oeufs découvertes dans une tombe de l'époque augustéenne à Sion Petit-Chasseur. In: J. Schibler/J. Sedlmeier (Ed.), Festschrift für Hans R. Stampfli. Beiträge zur Archäozoologie, Archäologie, Anthropologie, Geologie und Paläontologie (Basel 1990) 141–146.

Highlights

Taphonomic processes can imitate incubation-related changes on avian eggshell

A distinction between embryonic and taphonomic mineral dissolution is difficult

Quantification may be one key to distinguish between the effects of both processes

Incubated eggs in a Roman burial? A preliminary investigation on how to distinguish between the effects of incubation and taphonomy on avian eggshell from archaeological sites

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Abstract

Microscopic analyses can be used to determine whether fragments of eggshell come from hatched, incubated or non-incubated eggs. This information is essential for their interpretation since the developmental state of eggs often permits archaeologists to draw conclusions about the function of these finds at a site. However, what has often been neglected in previous studies is the fact that not only incubation but also taphonomy may affect the microstructure of shells. This preliminary study aims to demonstrate that taphonomic processes can in fact imitate site specific dissolution features that are commonly interpreted as traces of incubation. One likely cause of this could be bacteria or other microorganisms. The paper further introduces an approach by which a distinction between taphonomic and embryonic dissolution may be possible. The successful application of this technique on seemingly incubated eggs from a late Roman burial of Ober-Olm (Germany) indicates that these shells were altered only by taphonomy and not by embryonic development as initially assumed. It is finally emphasized that the preliminary data of this investigation need to be validated in future research.

Keywords

avian eggshell; incubation; taphonomy; eggshell quantification, late antiquity; burial sites, ritual

1. Introduction

Various studies in the past have demonstrated that the analysis of avian eggshells from archaeological sites offers unique observations about human-animal interactions. What makes the inconspicuous remains often particularly appealing for detailed investigation is the belief that their microstructure can be used to determine whether the eggs were hatched, incubated or non-incubated. This information is important in archaeological reconstruction and sometimes can even be the basis for unexpected conclusions. For instance, Tõnno Jonuks et al. (2018) recently suggested that the function of two eggs in two 12th to 13th century AD burials of Kukruse (Estonia) may be closely connected with their developmental state. The fact that the eggs from the burials showed signs of advanced incubation is seen as an indicator of allegorical significance, possibly symbolizing beliefs about rebirth through the image of a bird emerging from its shell (Jonuks et al. 2018, 118-119). The authors further argue that in Christian tradition the hatching egg symbolizes the resurrection of Christ (Jonuks et al. 2018, 118.). In analogy to this, or also to closely-related pagan concepts, incubated eggs may have been chosen deliberately for burials at Kukruse.

The case of Kukruse is not unique. Eggs have been found in a range of grave deposits across different cultures and periods and some of these likewise show traces of incubation (eg. Jakab 1979.). One example is the late Roman burial site of Ober-Olm (Germany), which we will discuss in this paper. Similarly to the early medieval eggs from Estonia, it could be argued that the incubated state is the expression of an allegoric significance of these gifts. However, another possibility would be that the features that are currently interpreted as traces of incubation are not necessarily the result of embryonic development but may originate also from taphonomic processes after the egg's burial. This hypothesis is partly confirmed by the earlier but relatively unknown findings of Werner Müller and Philippe Morel (Morel 1990. Morel/Müller 1997. Werner Müller, personal communication, June 2016.) and prompted the present investigation about eggshell taphonomy. The principal aim of this preliminary paper is not to present finished and comprehensive research on this problem but to sensitize archaeobiologists about the pitfalls of identifying incubated eggs and introduce them to one possible methodological solution.

2. Background: Biological principles and the identification of incubated eggs

During the incubation period the avian eggshell acts as a major source of calcium for the growing embryo (Chien et al. 2009, 527. Burley/Vadehra 1989, 284-286. Athanasiadou 2019, 9.). This is possible because the shell is primarily made up of calcite (approx. 96%), the most stable form of calcium carbonate ($CaCO_3$) (Chien et al. 2008, 84.). The embryonic resorption of shell calcium is based on its dissolution under acidic conditions (Chien et al. 2009, 528.). The chorioallantoic membrane (CAM), an extra-embryonic tissue (**Fig. 1**), which initiates mineral resorption after coming into contact with the inner shell membrane around the beginning of the second half of the incubation period, coordinates this process (Burley/Vadehra 1989, 284-286. Chien et al. 2009, 528-535. Beacham/Durand 2007, 1614.). The subsequent calcium dissolution visibly changes only the mammillary tips, resulting in their characteristic ‘cratering’ during incubation (**Fig. 2**). The reason for this peculiar pattern is the fact that the organic component (approximately 4%) of the eggshell, which is assumed to facilitate and guide the acid-based calcium dissolution by weakening the shells mineral structure, is mainly present in the mammillary bodies (Chien et al. 2008, 85. Chien et al. 2009, 535-537.). It is important to note that it is not the entire eggshell surface that is affected by this process. The small area beneath the egg’s air cell, usually located at the blunt end of the shell, is outside of the CAM’s sphere of action (**Fig. 1**). Therefore, shielded by the air cell, this zone remains unaffected from calcium resorption while the mammillae of the remaining, considerably larger, part of the shell become increasingly cratered due to mineral loss (**Fig. 2**; Jakab 1979, 150-151. Morel 1990, 144. Beacham/Durand 2007, 1612.).

The described dissolution of the mammillary bodies appears at different stages of incubation, usually in a uniform pattern (Marc D. McKee, personal communication, March 2016. Beacham/Durand 2007, 1614 and 1615 Fig. 3.). It should be noted, however, that certain exceptions to this usual appearance of embryonic calcium resorption may occur due to developmental disorders and variations; E. Bradley Beacham and Stephen R. Durand (2007) mention that in *“some uncommon instances, the air cell may become dislodged, resulting in inconsistent patterns of resorption.”* (Beacham/Durand 2007, 1614.). Moreover, for instance, a failure to turn eggs sufficiently during breeding can impede the CAM in advancing evenly across the shell-membrane, possibly causing a patchy pattern of resorption but then also leading in most cases to the death of the embryo (Tullett/Deeming 1987, 242-247.). Both anomalies seem to represent rather exceptional cases and a failure to turn eggs was reported to leave recognizable traces on shells (Romanoff 1960, 1134.).

Changes to the eggshell microstructure during incubation (or the lack of them) are already visible by low-magnification stereomicroscopy (30x - 40x magnification), and it has been proposed that the identification of an egg’s developmental state during incubation can be achieved not only for modern eggs but also for archaeological finds (e.g. Jakab 1979. Morel 1982. Morel 1990. Morel/Müller 1997. Beacham/Durand 2007. McGovern et al. 2006. Conrad et al. 2016. Lapham et al. 2016. Jonuks et al. 2018.). Moreover, while details of the above described processes – for example the timing and degree of calcium removal – are known to be variable, the basic principles of embryonic mineral resorption seem to be similar for most or even all avian species (Blom/Lilja 2004, 365-366. Beacham/Durand 2007, 1612-

1614. Chien et al. 2009, 537.). For the following preliminary study we therefore propose that intra-avian comparisons are permitted to a certain extent. However, this premise needs to be validated in future investigations.

3. Materials and Methods

This study is based on the comparison of three groups of eggshells. Firstly the microscopic appearance of two modern hatched reference shells (one goose and one chicken) will be described. The purpose of these two shells is to merely illustrate and roughly quantify the observations of previous studies (eg. Jakab 1979, 149-151. Beacham/Durand 2007, 1612-1614.). The two modern hatched shells will be compared with known non-incubated chicken eggs from a modern compost heap. Changes observed on the latter's microstructures must be exclusively due to taphonomy. Finally, a comparison will be conducted with archaeological goose eggs from the late Roman burial of Ober-Olm (Germany).
As has been outlined in the background section we assume in this preliminary investigation that the basic principles both of embryonic mineral resorption and taphonomic processes are comparable across different avian species.

3.1 Modern reference shells

For each species one successfully hatched egg of natural brood was obtained from small flocks of captive greylag geese (*Anser anser*) and domestic chicken (*Gallus gallus f. domestica*). To compare these reference shells with the archaeological specimens, they were prepared as follows. Firstly, membranes that blocked the view on the eggshell's internal surface had to be removed chemically while leaving the mineral structure intact. For this reason, shells were placed in glass containers with 5% sodium hydroxide solution and heated for 10 to 20 minutes in a boiling water bath as it is suggested for eggshells by Bušs and Keišs (2009, 91.). Afterwards, the shells were rinsed in water and dried at room temperature. Initially, the shells were observed in this state by stereomicroscopy. That way the position of areas with mineral dissolution or their absence could be noted. Then, for reasons of comparability, the eggshells were randomly broken by hand to achieve roughly similar fragment sizes as those observed for the modern shells of the compost heap and the archaeological shells of burial 19.

The assessment of the broken shells was conducted in a standardized manner also applied to the compost eggshells and archaeological specimens (see sections 3.2 and 3.3): In a first step, the area in square millimeters (mm^2) of each fragment was determined. This was conducted by placing the shell on millimeter paper and counting the number of covered squares. For pieces with a strong curvature, the actual area had to be estimated. Both the number of shell fragments and their total area in square millimeters (sum of individual shell fragment areas) served later as foundation for quantification. This was necessary because of the large variability of shell fragment sizes within the archaeological assemblages and the compost heap.

After this preparation, the general appearance of the mammillae of each fragment of eggshell was assessed. For this purpose the expression "corroded" was used as neutral and short descriptive term, meaning the typical cratering of the mammillae as a consequence of mineral dissolution (Morel 1990, 146.). Each fragment of eggshell was assigned to one of the following five categories:

'*surface uncorroded*'

'*surface mostly uncorroded with corroded zone(s)*'

'*surface mostly corroded with uncorroded zone(s)'*

'*surface uniformly corroded*'

'*not assessable*'.

Finally, a number of fragments was selected for nano-imaging in a Nova NanoSEM 230.

3.2 Eggshells from a modern compost heap

Reference samples of exclusively taphonomically altered eggshells were recovered from a modern compost pile (site coordinates: 48°03'54.9"N 7°36'16.6"E), located 210 km to the south of Ober-Olm on the upper Rhine plane at the foot of the Kaiserstuhl hills near Freiburg i. Br. (Baden-Wuerttemberg, Germany).

The studied heap had been laid out in spring 2009 on formerly ploughed farmland and underneath a walnut tree. It was framed by a block construction of alternately longitudinal and transversely stacked, wooden logs, forming in top view a square of 1,15m. From its initial setting up the accumulating pile was never dug over or changed in any other way. It served a four-person-household for discarding mostly organic kitchen refuse, including eggshells, but almost no other animal remains.

Moreover, small amounts of charcoal and ash were disposed on the compost heap. The eggshells came exclusively from non-incubated eggs, used for food preparation.
In November 2016 a vertical section was cut through the compost pile for stratified sampling. Samples of sediment and a minimum of 100 eggshell fragments were extracted by hand from each layer of the structure (**Fig. 3**). An additional soil sample was taken from the pile's eggshell-free subsoil.
Measurements of the sediment of all samples were taken at the Geoarchaeological Laboratory of the IPAS, University of Basel (Baillard et al. 2004.). The analyzed parameters were the total carbonate content (by production of carbonic gas after reaction with HCl, i.e. Müller's calcimeter), the humus content (by colorimetric method using sodium fluoride as reagent), and the pH (with a pH-meter in a KCl solution). In addition, the organic content (loss on ignition; Davies 1974.) and the phosphate content (by colorimetric method using ammonium molybdate as reagent; Lorch 1940.) were measured.
The collected eggshells were handled with great care and any unnecessary chemical and physical strain was avoided. However, to remove attached sediment, they were rinsed carefully in flowing tab water and dried at room temperature. No brushes were used. A chemical removal of shell membranes was not conducted.
Assessment and quantification of the compost shells was identical to that carried out on the modern hatched reference shells described in section 3.1 (*Modern reference shells*). One additional recorded feature was the presence or absence of organic shell membranes.

3.3 Archaeological eggshells

The archaeological eggshells were recovered during excavations in 2001 from the burial of a middle-aged female (burial 19) at the late Roman cemetery of Ober-Olm (coordinates 49°57'14.8"N 8°12'22.3"E). The site dates between the second half of the 4th and the first half of the 5th century AD (Machura/Sichert 2015, 79.) and is proposed to belong to a rural estate nearby (Machura in prep.). **Figure 4** shows the location and arrangement of the eggshells in burial 19 upon recovery. Two eggs, still recognizable but heavily fragmented, lay slightly isolated at the deceased's left hand side (**Fig. 4:** ④ **and** ⑥). In the same area, but some centimeters closer to the dead, there was a chaotic assemblage of an unknown number of eggshells, partly covered by the remains of a rooster with a spur scar on its one recovered tarsometatarsus

(**Fig. 4:** ⑤). During the excavation, shells were collected by hand. No sieving was applied and no soil samples were taken from the burial.

A preliminary identification by macroscopic comparison with modern reference eggs by Frank D. Steinheimer (ZNS Halle) suggests that the shells ④ and ⑥ come from *Anser anser f. domestica* or its wild ancestor. Although shell thicknesses and curvatures of some fragments in assemblage ⑤ indicate the presence of one or more smaller sized egg(s) they likewise seem all to come from domestic or wild goose (Frank D. Steinheimer, personal communication, October 2018.). Peptide mass fingerprinting (PMF) was conducted on eight eggshell fragments from all assemblages of burial 19 using the methods detailed in Presslee et al. (2017). The analyses confirmed the identification of all eggshell fragments as Anseriformes (see spectrum in supplementary figure I).

The assessment of the eggshell fragments by stereomicroscopy was conducted in the above-described standardized manner by recording the number of fragments and their area in square millimeters (mm^2) (see 3.1 *Modern reference shells*).

4. Results

4.1 Modern reference shells

The modern hatched eggs of goose and chicken were dominated by uniformly corroded surfaces both by number of fragments and their total areas in square millimeters (mm^2) (**Tab. 1; Fig. 5**). However, the number and total area of uncorroded shells was higher in the chicken egg. Apart from this and other minor variations, it is notable that the two eggs of two different species show roughly similar percentages of uniformly corroded, uncorroded and patchily corroded surfaces (**Fig. 5**). Uncorroded shells came always from the air cell area at the egg's blunt poles. Fragments that displayed both corroded and uncorroded zones (**Fig. 2**) originated mostly from the small zone of transition between air cell area and the remaining surface. Only few shells from the eggs sharp pole areas had patches of uncorroded mammillae within corroded zones. These shells were classified accordingly (*'surface mostly corroded with uncorroded zone(s)')*. Apart from that, the surfaces at the lateral sides and sharp poles of both eggs were always corroded uniformly. Occasionally small spots of one to five intact mammillae occurred in between otherwise entirely uniformly-corroded shells. Since these small spots were rare and isolated, shells with this appearance were classified as '*uniformly corroded*'.

4.2 Modern compost heap

The excavation of the compost pile revealed an approximately 30 - 40 cm thick sequence of four layers, still framed by the pile's rotting lower wooden logs (**Fig. 3**). Below this zone was the sandy, slightly silty former topsoil-horizon on which the pile had been set. The location of the samples is marked in **figure 3**.

Although some bioturbation by roots and small mammals was observable in the section, it did not seem to have significantly affected the heap's stratigraphy. In fact, the comparatively low amount of turbation and the relative coherence of each sampling unit were also indirectly visible in the recovered fragments of eggshell: The shells were slightly more fragmented in the bottom layers of the heap than at the top (**Tab. 2**). Moreover, in the upper parts of the pile, the inner sides of the eggshells were often still covered by organic shell membranes (**Fig. 6; Fig. 7.1**). This hindered their microscopic assessment, particularly on specimens of layer I. With increasing depth the organic membranes became progressively decomposed and less frequent and eventually disappeared from shells in sample IV (**Fig. 6**).

The spatial timeline of fragmentation and organic decomposition described above was paralleled by signs of corrosion on the eggshells. Already the upper, youngest, sample contained some fragments that featured corrosion marks on mammillary tips (**Tab. 2; Fig. 5**). With increasing depth of samples, the finding of corroded shells became more frequent. Uniform dissolution patterns (**Fig. 7.2**) were observed in all samples, however, usually only at low frequencies and total areas in square millimeters (mm^2). The majority of corroded shells displayed patchy patterns of corroded and uncorroded surfaces (**Fig. 7.3**).

Geochemical soil samples overall underlined the relatively low amount of turbation described above (**Tab. 3**). For instance, loss on ignition and humic substances were with 42% and 1,5 c.u. highest in sample I from the uppermost und thus least decomposed layer of the compost pile. Both values decreased with increasing depth – only loss on ignition in sample III with 20% breaks slightly ranks. Geochemical analysis further showed comparatively high calcium carbonate values in all layers (8-21%). Finally, measurements of the pH indicated an alkaline milieu (7,8-8,6) in the entire compost heap and below.

4.3 Archaeological eggshells

The microscopic structure of eggshells of all assemblages of burial 19 displayed signs of, often strong, calcium carbonate dissolution (**Tab. 4; Fig. 5; Fig. 8**). These corrosive features, however, often differed considerably from those on shells of the modern hatched reference eggs and reports about modern incubated eggs from literature and personal communications. *Uniform* corrosion, which appears most often in embryonic resorption was present only at very low frequencies and total areas (mm^2) in all assemblages (**Tab. 4; Fig. 5; Fig. 9.1**). Instead, mostly *irregular* patterns of corrosion were observed (**Fig. 5, Fig. 8**; **9.2).** These patchy surface appearances, which hatched reference shells displayed only at relatively low percentages, characterized the largest proportion of the shells from all assemblages of burial 19 (**Tab. 4; Fig. 5**).

Not only did the state of calcium dissolution vary strongly for neighboring mammillae in archaeological shells, some specimens from burial 19 also exhibited patches of excessively flattened mammillae (**Fig. 9.3**). In addition, mineral dissolution had affected some external surfaces of shells, which showed sporadically deep depressions on their external (convex) sides (**Fig. 9.4**).

Moreover, both on the inner and the outer sides of many shells, roots of plants had formed shallow grooves. There was no visible correlation between these grooves and specific states of the surrounding mammillary tips, which sometimes appeared uncorroded (**Fig. 9.5**) and sometimes corroded (**Fig.9.6**).

5. Discussion

In the following discussion the likely reasons for taphonomic calcium dissolution on eggshells will firstly be presented and debated. Afterwards, approaches to gain more certainty for the distinction between taphonomic corrosion and embryonic mineral resorption will be introduced. In this section, the eggshells from the late Roman burial 19 of Ober-Olm will serve as example of application. The results of the analysis will finally be evaluated for their interpretive implications considering the function of the eggs during funeral rituals.

5.1 Causes of taphonomic corrosion

In 1990, Philippe Morel drew attention to the possibility that the dissolution of the mammillary tips of archaeological eggshells may not only be a result of embryonic resorption but also of taphonomic processes (Morel 1990, 144-146.). This hypothesis was experimentally reinforced by Werner Müller, who applied droplets of acid on shells of modern non-incubated eggs and discovered that specimens treated that way displayed the same crater-like dissolution features as shells of hatched birds (Morel 1990, 144-146. Werner Müller, personal communication, June 2016.). The samples from a modern compost heap examined in this study now complement those pioneering findings, which unfortunately went unnoticed in later studies, and provide data from an actualistic environment that might be closer to the archaeological reality than artificial laboratory conditions.

Chicken eggshells from the compost pile clearly showed evidence of site-specific mineral loss and a subsequent cratering of mammillary tips, allegedly typical for incubation. This is because the centers of the mammillary bodies represent structural weak spots that are vulnerable to any corrosive attack, irrespective of whether caused by embryonic action during incubation or taphonomic weathering. As explanation for this taphonomic corrosion, Philippe Morel and Werner Müller suggested slightly acidic soil conditions (Morel/Müller 1997, 96). Yet, chemical analyses of the compost sediments indicate an alkaline environment in all layers of the pile (**Tab. 3**). This is even more surprising when considering that with the increasing depth of the samples, and thus with increasing age of recovered shells, the percentage of taphonomically altered fragments increases (**Fig. 5**). Corrosive processes thus seem to have occurred not only during the chemically-active phase, when the organic waste discarded together with eggshell started to decay, but also at later times. If the corrosion of the mammillary tips was based on acidic dissolution, this must have happened at a scale too small and too localised to have had an impact on the overall alkaline sediment. But how can such a localized process be explained?

In paleontologically-motivated experiments, Denise L. Smith and James L. Hayward (2010) investigated the possible role of bacteria in triggering eggshell deterioration. Their results and subsequent explanatory model was that *“bacterial decomposition of the eggshell protein matrix produces organic acids, which, in turn, dissolve the* $CaCO_3$ *of the shell. The dissolved* $CaCO_3$ *and* NH_3 *from protein degradation increase* *the pH of the surrounding sediment."* (Smith/Hayward 2010, 324.). Another consequence of these processes is an increase in soil calcium carbonate (Smith/Hayward 2010, 320.).

This model may also be applicable to processes inside the compost heap. For instance, *B. subtilis* and *P. fluorescens*, which belong to the group of bacteria tested by Smith & Hayward, are known to exist in compost sediments (Tuomela et al. 2000, 173. Boulter et al. 2002, 665-669.).

To a certain extent the model above may also partially explain both the high pH and the soil calcium carbonate values in the pile. Yet, it is likely that these conditions cannot be explained by eggshell decomposition alone. In general, most compost heaps, after a first phase where pH decreases, turn alkaline when progressively more ammonium is released due to the decomposition of proteins by microorganisms (Tuomela et al. 2000, 173. Bilitewski/Härdtle 2013, 404-406.). Moreover, it should be remembered that in our compost heap ash had been disposed of, likely leading to an additional rise of soil calcium carbonate and alkaline pH. Nevertheless, bacterial action on the eggshells may have contributed to the overall measured values in the compost sediments and may have also been responsible for site specific dissolution features both on the shells of the modern pile and archaeological assemblages.

At the beginning of this study, the involvement of plant roots was considered to be another possible explanation for eggshell corrosion: It is well known that roots can change the pH in their surrounding sediment (Hinsinger et al. 2003, 43.). In the compost heap, visible roots were in fact present, however limited and rather confined to its lateral sides and the area underneath the structure. Moreover, root groves on shells could not be observed, making this a less likely cause for direct mammillary dissolution on compost eggshells.

On the contrary, imprints of plant roots were frequent features on specimens from the Roman burial. However, since no consistent correlation between zones of cratered mammillae and root grooves could be established in this case a connection is also currently excluded.

To summarize, both embryonic resorption and taphonomic corrosion are likely based on acidic dissolution processes, driven and modulated by the organic matrix of the shell, or by the empty channels it left behind. At this stage, we suggest that bacteria or other microorganisms are the most likely cause of taphonomic mineral loss. Due to the complexity of such processes, however, our results cannot be considered final and we invoke more research efforts from interdisciplinary teams.

5.2 Strategies for distinguishing taphonomic and embryonic corrosion

Notwithstanding the precise causes of taphonomic corrosion, the shells from the compost heap demonstrate that the visible effects of these processes cannot be differentiated easily from embryonic calcium resorption. Nevertheless, it would be the wrong step to dismiss the possibility of assessing the developmental state of archaeological eggshells. In fact, Philippe Morel has previously presented approaches to distinguish both causes of mineral loss. In the following section these methods will be reevaluated and complemented with new results and strategies.

One of the techniques developed by Philippe Morel to identify the taphonomic origin of corrosion was based on the fact that an eggshell's blunt pole is usually shielded from the CAM by the air cell and in consequence from embryonic calcium resorption. He reasoned that the corrosion he observed on the blunt pole of archaeological shells thus could not be related to incubation (Morel 1990, 146.). This approach requires very favourable conditions of preservation and low degrees of fragmentation since it is necessary to assign shell fragments to their original position on the egg. Unfortunately, in most archaeological assemblages, including the burial of Ober-Olm, these requirements are not met. However, other ways to verify that taphonomic corrosion had taken place also exist: For instance, in the case of the Roman grave finds, dissolution features observed on the shells' external (convex) sides (**Fig. 9.4**) likewise could not be related to incubation (Simone Häberle, personal communication, January 2017). Also, patches of excessively flattened mammillae (**Fig. 9.3**) may indicate weathering processes. Finally, according to Philippe Morel, extensive irregular corrosion patterns in general are also a result of taphonomy rather than incubation (Morel 1990, 146.).

All three of the above described features may allow the detection of taphonomic mineral loss. However, they do not exclude incubation. This is because shells of incubated and hatched eggs are also subject to taphonomic processes. For instance, it is possible that the originally uncorroded air cell area of a hatched egg becomes corroded during and after its embedding in archaeological structures. This secondary taphonomic calcium carbonate dissolution may then cause, for example, inconsistent patterns of corrosion. Likewise, corrosive features at the shells external (convex) sides and patches of flattened mammillae can be observed as taphonomic dissolution on shells of hatched eggs (Smith/Hayward 2010, 320.). For this reason, additional approaches are needed to not only prove the occurrence of taphonomic processes but also to exclude prior incubation. One key to success in this matter may at least partly be an issue of quantification. This can be demonstrated with the eggshells from burial 19 (Ober-Olm): Although the shells of all eggs beside the dead largely bore marks that, according to the degree of mamillary corrosion, seemed to be related to advanced incubation or even hatching, the large total areas in square millimeters (mm^2) with irregular patterns of dissolution were not consistent with the observations from modern hatched specimens (**Fig. 5**). Even when assuming that parts of the archaeological eggs may be missing, the recorded surface appearances cannot be brought into accordance with the proportions known from the reference specimens.

For earlier stages of incubation, the current data situation is less clear: It cannot yet be entirely excluded that early embryonic calcium dissolution may manifest itself with different corrosion patterns and proportions than those observed in the hatched reference specimens. However, observations by other scientists indicate that early embryonic resorption also appears according to uniform patterns (Marc D. McKee, personal communication, March 2016. Beacham/Durand 2007, 1614 and 1615 Fig. 3.). Furthermore, developmental variations and disorders, another possible cause of the encountered inconsistencies on the shells, are rather exceptional cases and seem unlikely to occur in more than one egg of the burial.

In combination with the fact that taphonomic corrosion is likely to have taken place, there are no firm arguments that point towards incubation. Indeed, the observed proportions of surface appearances show similarities with shells of non-incubated eggs from the modern compost heap (Fig. 5). The shells with both corroded and uncorroded areas appear in both groups more frequently and at larger total areas in square millimeters (mm^2) than uniformly cratered shells.

For this reason, at this stage, all data indicate that the shells of the late Roman burial 19 were not incubated at the time of the funeral or at least not incubated long enough to leave traces on the shell. However, the following section will show that this does not simplify the interpretation of these grave gifts.

5.3 The developmental state of eggs and their function during funerary rituals

Tõnno Jonuks et al. (2018) suggested that information about the developmental state of eggs in burial deposits may help to identify their function during the funerary rituals. Based on this hypothesis it could be argued that shells of non-incubated eggs might have symbolized culinary gifts and remains of incubated or hatched eggs more abstract concepts instead. In fact, Christian writers in late antiquity have often emphasized the image of emerging life from the avian egg and drawn parallels to resurrection:

"The buried chick calls out loud from the egg's inside; at this sound the grave splits open and its body rises (to live). That is to say, also the chick in its egg is a corpse. Its body promises our body resurrection."

Epraem, Carmina Nisibena 103,65,18-19 (translated by Beck 1963, 92.)

It is possible that this image was integrated into late Roman funerary rituals. However, it has to be noted also that this model may rather mirror modern western concepts and not necessarily past realities. For instance, it cannot be ruled out that partly incubated eggs may have been consumed, similar to practices in parts of Asia today (Magat 2002, 63.). On the other hand, a symbolic significance related to abstract concepts does not necessarily require physical incubation of the egg, for example, the custom of decorating children's coffins with golden eggs, symbolizing life that is only temporarily trapped within the grave, was testified at the Swiss parish of Stammheim (Kt. Zürich) (Gattiker/Gattiker 1989, 44-45.). Despite this highly emblematic concept, the egg's developmental state seemed to have been of no relevance for this ritual.

To summarize, it can be stated that information regarding whether an egg was not-incubated, incubated or hatched is indeed important but not the only aspect to be considered in the very complex discussion regarding their function during funeral rituals.

6. Conclusion

The study of eggshells from archaeological sites has a real potential to clarify aspects of people's lifeways and deathways. However, the methodological limits of such studies need to be well understood. The analysis of eggshells from a modern compost pile has shown that changes in the microstructure of shells are not necessarily related to incubation, as frequently assumed, but can also be 'imitated' by taphonomic processes. This makes it difficult to reliably identify the developmental state of eggs from archaeological sites. Quantifying corrosive appearances by means of shell fragment areas in square millimeters (mm^2) may be one key to a more reliable identification of the developmental state.

It has to be stressed that this study is still very preliminary in nature. For instance, in addition to descriptions from literature (eg. Beacham/Durand 2007, 1612-1614.), only two modern shells were used as reference for hatched eggs. The presented results thus need further verification and possible corrections. Future research will show if our approach for distinguishing between the effects of taphonomy and incubation proves to be successful.

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References

Athanasiadou et al. 2018

D. Athanasiadou/W. Jiang/D. Goldbaum/A. Saleem/K. Basu/M. S. Pacella/C. F. Böhm/R. R. Chromik/M. T. Hincke/A. B. Rodríguez-Navarro/H. Vali/S. E. Wolf/J. J. Gray/K. H. Bui/M. D. McKee (2018) Nanostructure, osteopontin and mechanical properties of calcitic avian eggshell. Science Advances 4 (2018) 1–13.

Beacham/Durand 2007

E. B. Beacham/S. R. Durand, Eggshell and the archaeological record: New insights into turkey husbandry in the American Southwest. Journal of Archaeological Science 34, 2007, 1610–1621.

Beck 1963

E. Beck, Des heiligen Ephraem des Syrers Carmina Nisibena. Corpus Scriptorum Christianorum Orientalium Scriptores Syri 103 (Louvain 1963).

Bilitewski/Härdtle 2013

B. Bilitewski/G. Härdtle, Abfallwirtschaft. Handbuch für Praxis und Lehre[4] (Berlin 2013).

Blom/Lilja 2004

J. Blom/C. Lilja. A comparative study of growth, skeletal development and eggshell composition in some species of birds. Journal of Zoology 262, 2004, 361-369.

Boulter et al. 2002

J. I. Boulter/J. T. Trevors/G. J. Boland, Microbial studies of compost. bacterial identification, and their potential for turfgrass pathogen suppression. World Journal of Microbiology & Biotechnology 18, 2002, 661–671.

Braillard et al. 2004

L. Braillard/M. Guélat/Ph. Rentzel, Effects of Bears on Rockshelter Sediments at Tanay Sur-les-Creux, Southwestern Switzerland. Geoarchaeology: An International Journal 19 (2004) 343–367.

Burley/Vadehra 1989
R. W. Burley/D. V. Vadehra, The avian egg. Chemistry and biology (New York 1989).

Bušs/Keišs 2009
A. Bušs/O. Keišs, Method for identification of avian species by eggshell microstructure: Preliminary study. Acta Universitatis Latviensis 753, 2009, 89–98.

Chien et al. 2008
Y.-C. Chien/M. T. Hincke/H. Vali/M. D. McKee, Ultrastructural matrix-mineral relationships in avian eggshell, and effects of osteopontin on calcite growth in vitro. Journal of Structural Biology 163, 2008, 84–99.

Chien et al. 2009
Y.-C. Chien/M. T. Hincke/M. D. McKee, Ultrastructure of avian eggshell during resorption following egg fertilization. Journal of Structural Biology 168, 2009, 527–538.

Conrad et al. 2016
C. Conrad/E. L. Jones/S. D. Newsome/D. W. Schwartz, Bone isotopes, eggshell and turkey husbandry at Arroyo Hondo Pueblo. Journal of Archaeological Science: Reports 10, 2016, 566–574.

Davies 1974
B. E. Davies, Loss-on-ignition as an estimate of soil organic matter. Soil Science Society of America Proceedings 38, 1974, 150–151.

Gattiker/Gattiker 1989
E. Gattiker/L. Gattiker, Die Vögel im Volksglauben. Eine volkskundliche Sammlung aus verschiedenen europäischen Ländern von der Antike bis heute (Wiesbaden 1989).

Hinsinger et al. 2003

P. Hinsinger/C. Plassard/C. Tang/B. Jaillard, Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant and Soil 248, 2003, 43–59.

Jakab 1979
B. Jakab, Vergleichende Analyse der anlässlich von Ausgrabungen in Ungarn freigelegten Eierschalen-Funden, Acta archaeologica Academiae Scientiarum Hungaricae 31, 1979, 147–162.

Jonuks et al. 2018
T. Jonuks/E. Oras/J. Best/B. Demarchi/R. Mänd/S. Presslee/S. Vahur, Multi-method Analysis of Avian Eggs as Grave Goods: Revealing Symbolism in Conversion Period Burials at Kukruse, NE Estonia. Environmental Archaeology 23, 2018, 109-122.

Lapham et al. 2016
H. A. Lapham/G. M. Feinman/L. M. Nicholas, Turkey husbandry and use in Oaxaca, Mexico. A contextual study of turkey remains and SEM analysis of eggshell from the Mitla Fortress. Journal of Archaeological Science: Reports 10, 2016, 534–546.

Lorch 1940
W. Lorch, Die siedlungsgeographische Phosphatmethode. Die Naturwissenschaften 28, 1940, 633–640.

Machura, Sichert 2015
M. Machura/B. M. Sichert, Nischengräber des spätantiken Bestattungsplatzes von Ober-Olm (Lkr. Mainz-Bingen). Berichte zur Archäologie in Rheinhessen und Umgebung 8, 2015, 79-89.

Machura in prep.
M. Machura, Mittelkaiserzeitlicher und spätantiker Bestattungsplatz von Ober-Olm (Lkr. Mainz-Bingen).

Magat 2002

M. Magat, Balut: Fertilized Duck Eggs and their Role in Filipino Culture. Western Folklore 61, 2002, 63–96.

McGovern et al. 2006
T. H. McGovern/S. Perdikaris/Á. Einarsson/J. Sidell, Coastal connections, local fishing, and sustainable egg harvesting: Patterns of Viking Age inland wild resource use in Mývatn district, Northern Iceland. Environmental Archaeology 11, 2006, 187–205.

Morel 1982
P. Morel, Bestimmung eines Eierschalenfragments am Löffel Nr. 133. In: E. Riha/W. B. Stern, Die römischen Löffel aus Augst und Kaiseraugst. Forschungen in Augst 5 (Augst 1982) 10–11.

Morel 1990
P. Morel, Quelques remarques à propos de coquilles d'oeufs découvertes dans une tombe de l'époque augustéenne à Sion Petit-Chasseur. In: J. Schibler/J. Sedlmeier (Ed.), Festschrift für Hans R. Stampfli. Beiträge zur Archäozoologie, Archäologie, Anthropologie, Geologie und Paläontologie (Basel 1990) 141–146.

Morel/Müller 1997
P. Morel/W. Müller, Hauterive-Champréveyres 11. Un campement magdalénien au bord du lac de Neuchâtel. Étude archéozoologique. Archéologie neuchâteloise 23 (Neuchâtel 1997).

Presslee et al. 2017
S. Presslee/J. Wilson/J. Woolley/J. Best/D. Russell/A. Radini/R. Fischer/B. Kessler/R. Boano/M. Collins/B. Demarchi, The identification of archaeological eggshell using peptide markers. STAR: Science & Technology of Archaeological Research 3, 2017, 89-99.

Ridlen/Johnson 1964

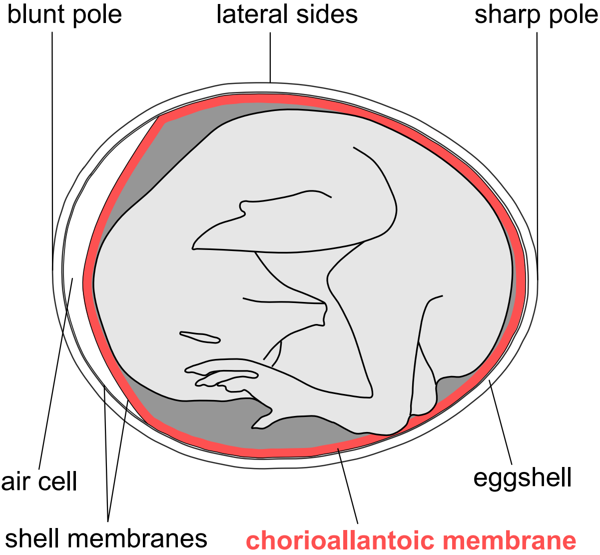
S. F. Ridlen/H. S. Johnson, From Egg to Chick. A Guide to the Study of Incubation and Embryonic Development. Circular (University of Illinois, Agricultural Experiment Station) 878, 1964, 1-16.

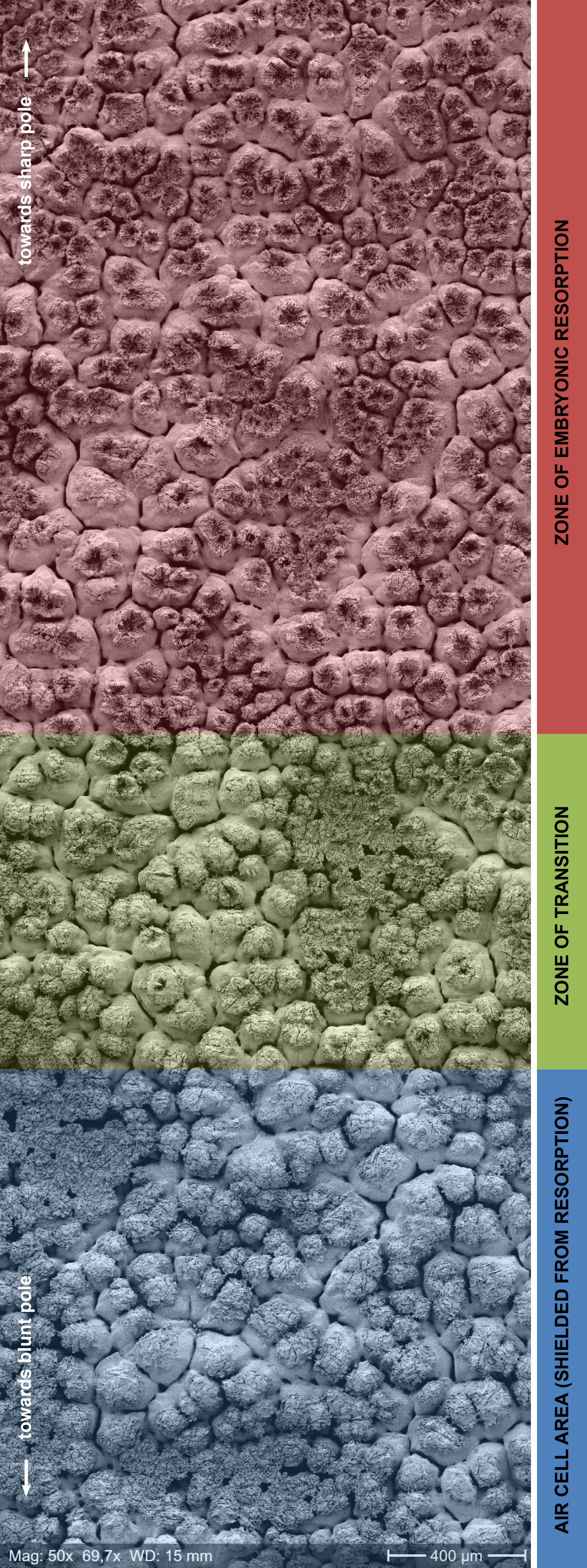
Romanoff 1960
A. L. Romanoff, The avian embryo. Structural and functional development (New York 1960).

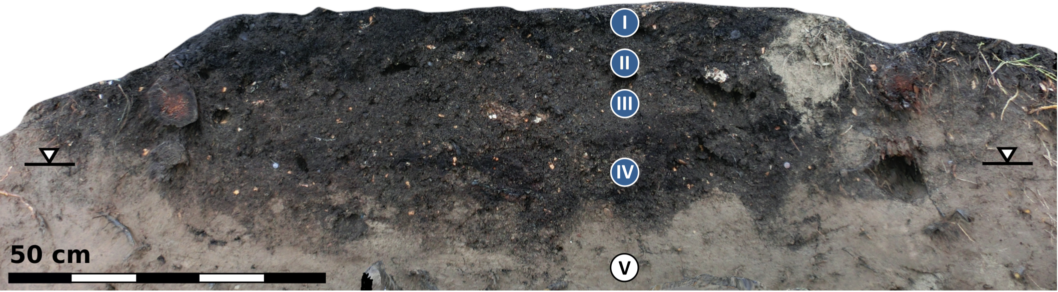
Smith/Hayward 2010
D. L. Smith/J. L. Hayward, Bacterial decomposition of avian eggshell: A taphonomic experiment. Palaios 25, 2010, 318–326.

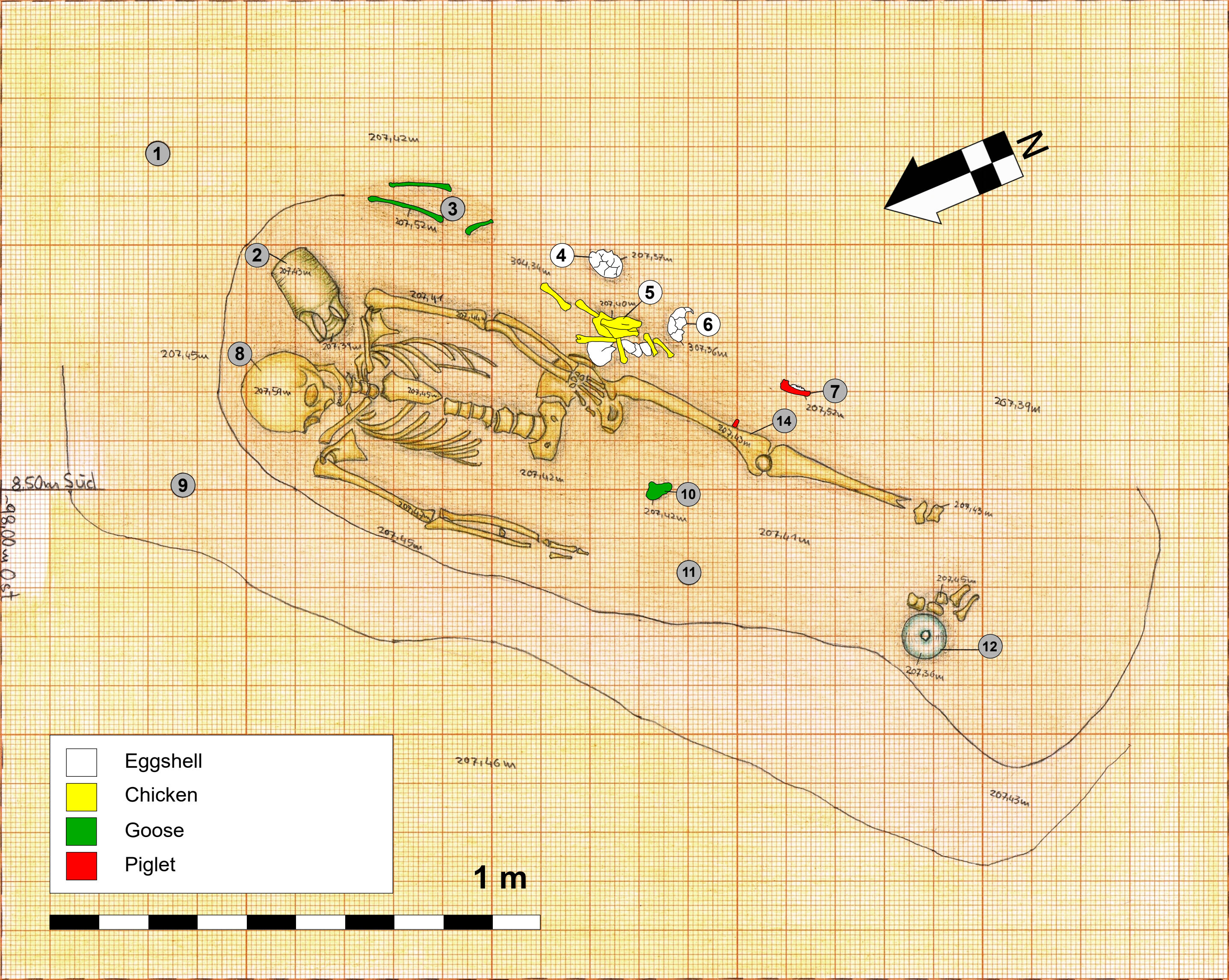
Tullett/Deeming 1987
S. G. Tullett/D. C. Deeming, Failure to turn eggs during incubation: Effects on embryo weight, development of the chorioallantois and absorption of albumen. British Poultry Science 28, 1987, 239–243.

Tuomela et al. 2000
M. Tuomela/M. Vikman/A. Hatakka/M. Itävaara, Biodegradation of lignin in a compost environment: A review. Bioresource Technology 72, 2000, 169–183.

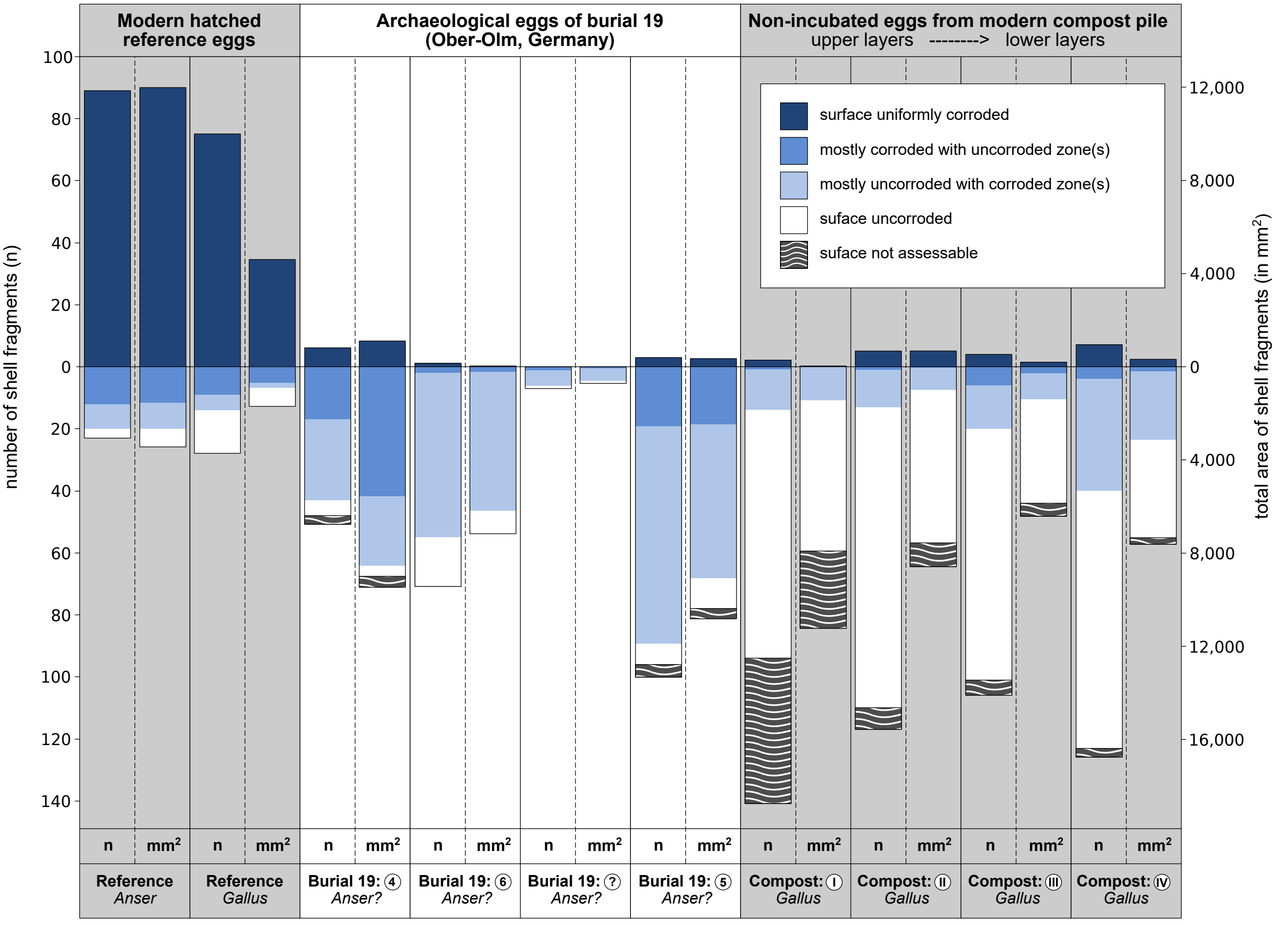

blunt pole
lateral sides
sharp pole
air cell
shell membranes
eggshell
chorioallantoic membrane

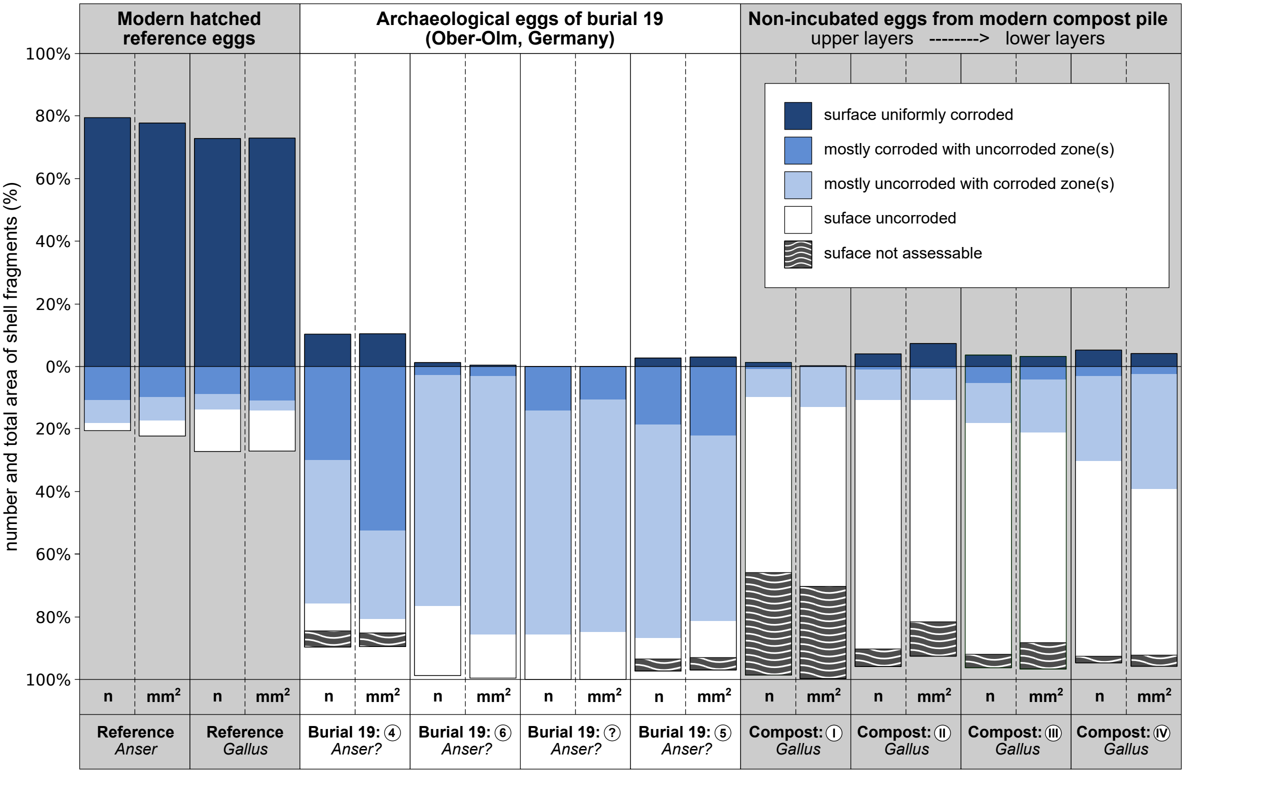

towards sharp pole
ZONE OF EMBRYONIC RESORPTION
ZONE OF TRANSITION
AIR CELL AREA (SHIELDED FROM RESORPTION)
towards blunt pole
Mag: 50x 69,7x WD: 15 mm
400 µm

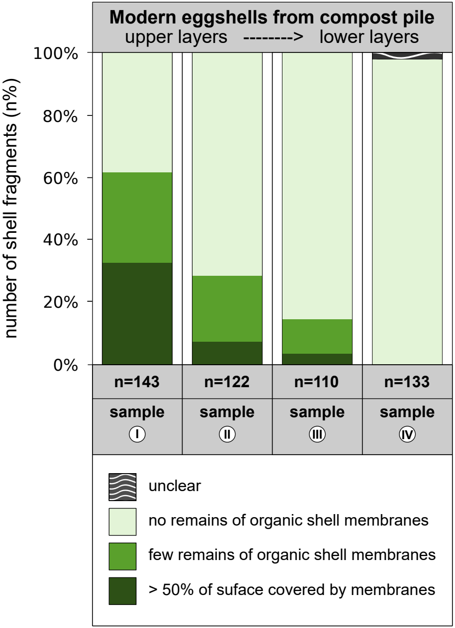

I
II
III
IV
V
50 cm

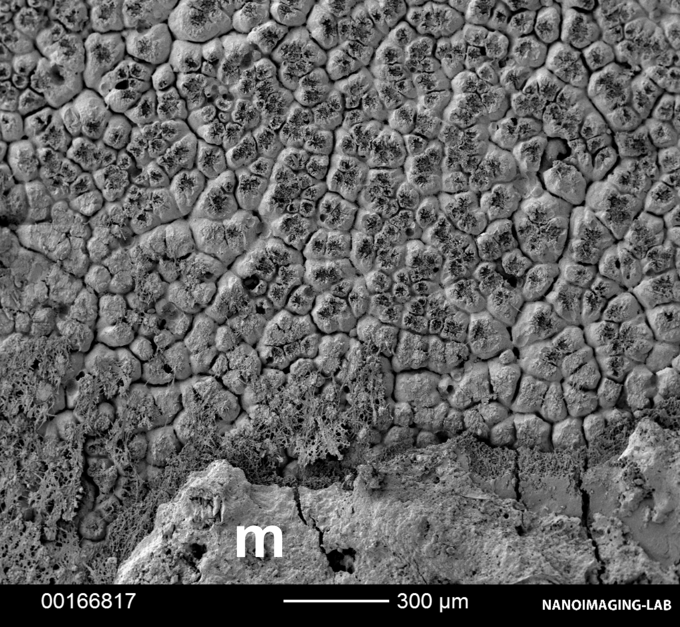


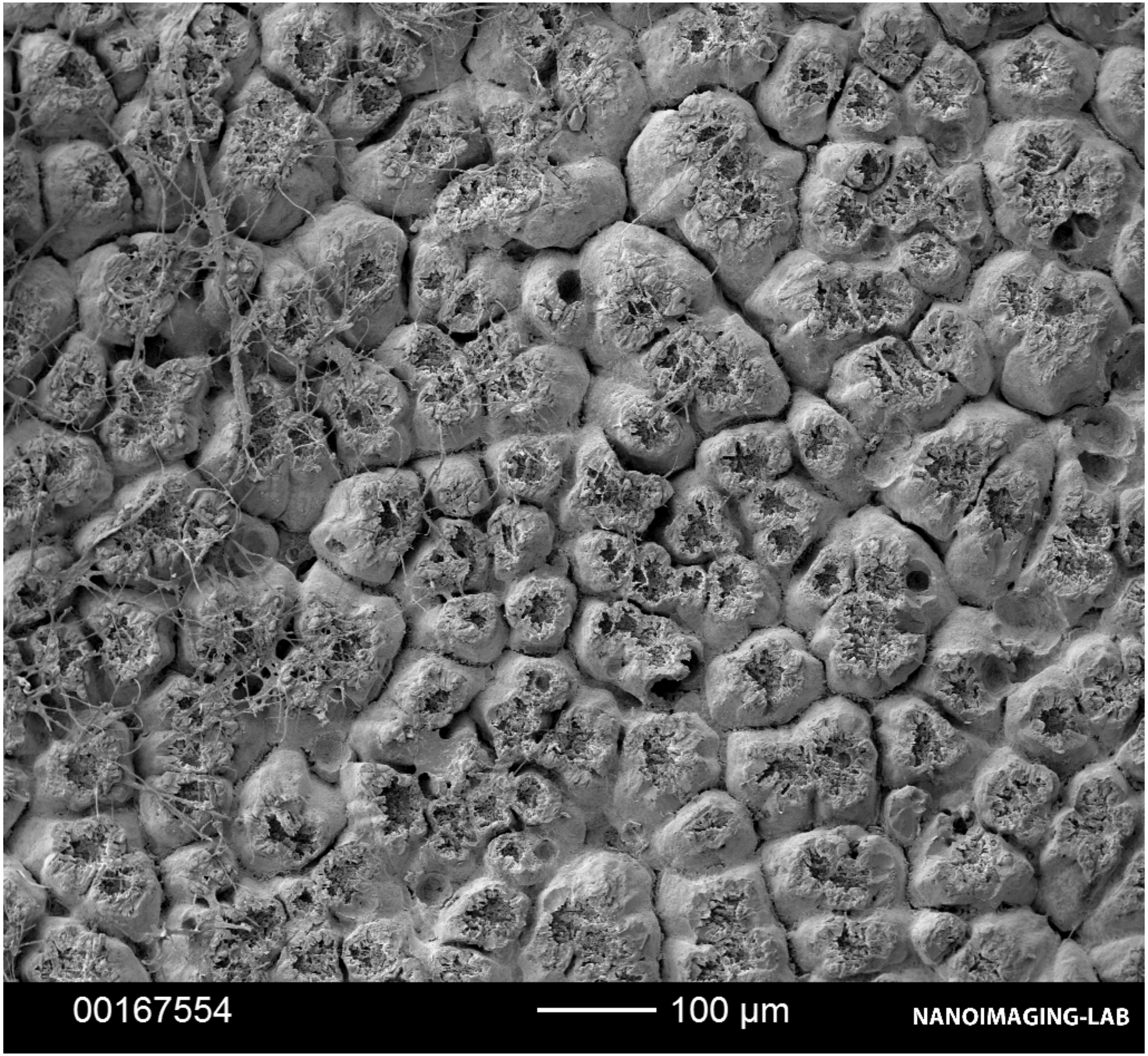
1
2
3
4
5
6
7
8
9
10
11
12
14
N
8,50m Süd
98,00m Ost
207,42m
207,52m
207,43m
207,39m
207,41
207,45m
207,59m
207,40m
207,37m
207,36m
207,52m
207,39m
207,43m
207,42m
207,41m
207,45m
207,36m
207,46m
207,43m
Eggshell
Chicken
Goose
Piglet
1 m

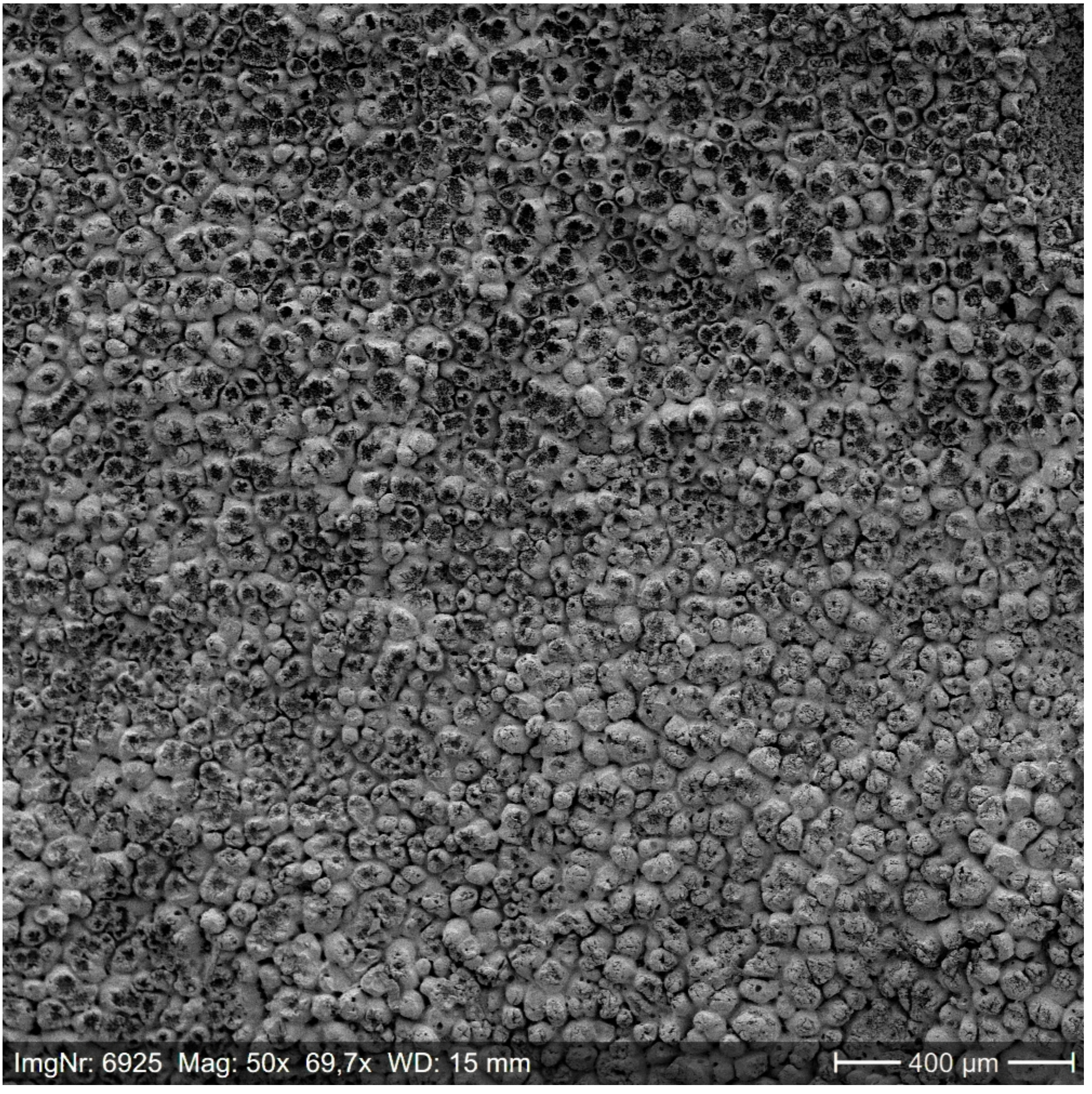

Modern hatched reference eggs
Archaeological eggs of burial 19 (Ober-Olm, Germany)
Non-incubated eggs from modern compost pile
upper layers --------> lower layers
surface uniformly corroded
mostly corroded with uncorroded zone(s)
mostly uncorroded with corroded zone(s)
suface uncorroded
suface not assessable
number of shell fragments (n)
total area of shell fragments (in mm²)
100
80
60
40
20
0
20
40
60
80
100
120
140
12,000
8,000
4,000
0
4,000
8,000
12,000
16,000
n
mm²
Reference Anser
Reference Gallus
Burial 19: ④ Anser?
Burial 19: ⑥ Anser?
Burial 19: ⑦ Anser?
Burial 19: ⑤ Anser?
Compost: Ⓘ Gallus
Compost: Ⓘ Gallus
Compost: Ⓘ Gallus
Compost: Ⓘ Gallus


Modern hatched reference eggs
Archaeological eggs of burial 19 (Ober-Olm, Germany)
Non-incubated eggs from modern compost pile
upper layers --------> lower layers
surface uniformly corroded
mostly corroded with uncorroded zone(s)
mostly uncorroded with corroded zone(s)
suface uncorroded
suface not assessable
number and total area of shell fragments (%)
100%
80%
60%
40%
20%
0%
20%
40%
60%
80%
100%
n
mm²
Reference Anser
Reference Gallus
Burial 19: ④ Anser?
Burial 19: ⑥ Anser?
Burial 19: ⑦ Anser?
Burial 19: ⑤ Anser?
Compost: Ⓘ Gallus
Compost: Ⓘ Gallus
Compost: Ⓘ Gallus
Compost: Ⓘ Gallus


Modern eggshells from compost pile
upper layers --------> lower layers
number of shell fragments (n%)
100%
80%
60%
40%
20%
0%
n=143
n=122
n=110
n=133
sample I
sample II
sample III
sample IV
unclear
no remains of organic shell membranes
few remains of organic shell membranes
> 50% of suface covered by membranes


m
00166817
300 µm
NANOIMAGING-LAB


00167554
100 µm
NANOIMAGING-LAB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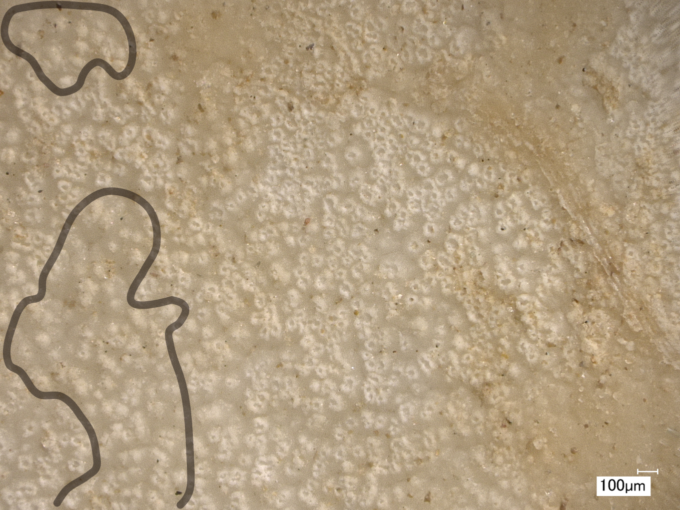
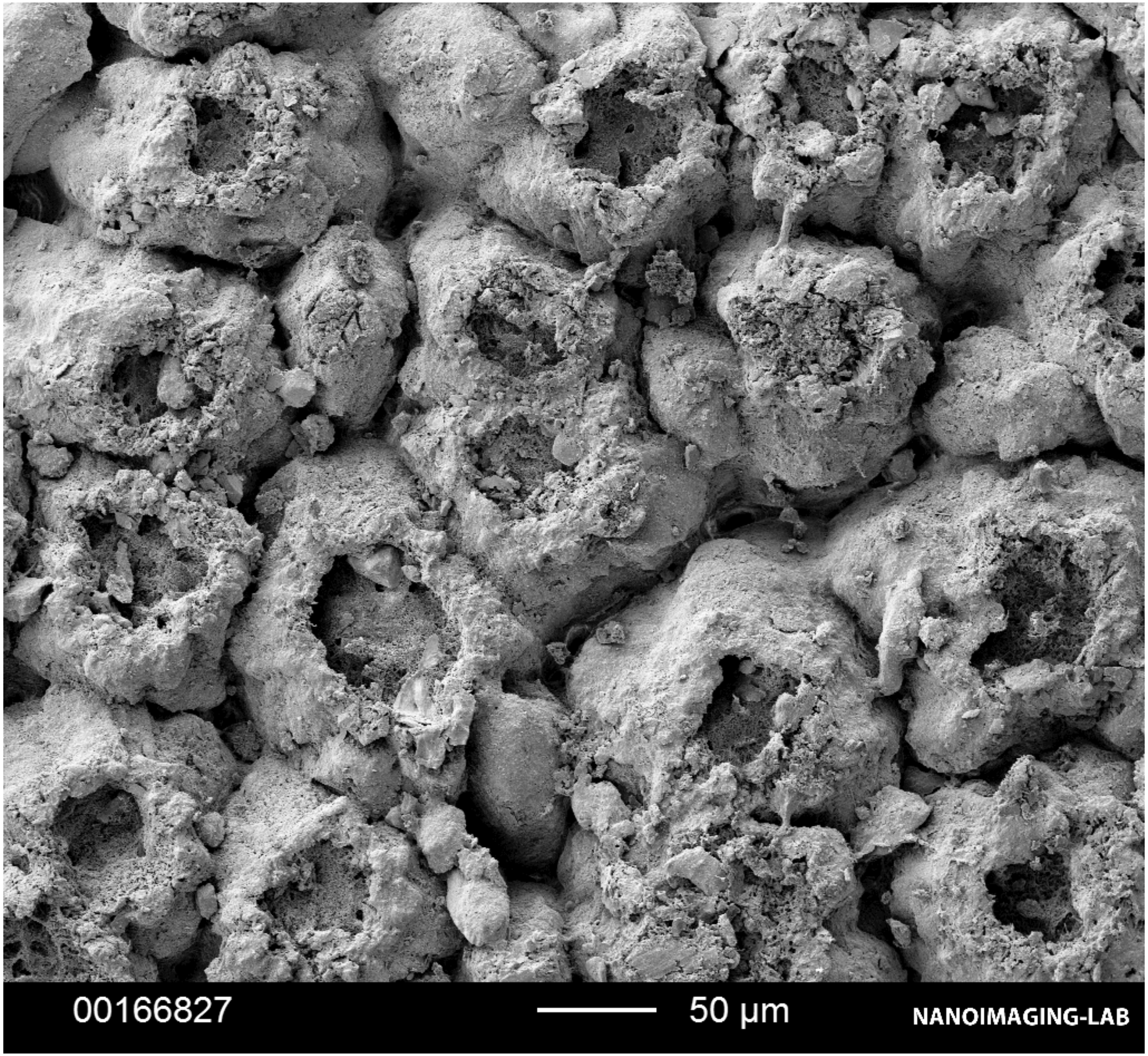
400 µm


100μm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50 µm
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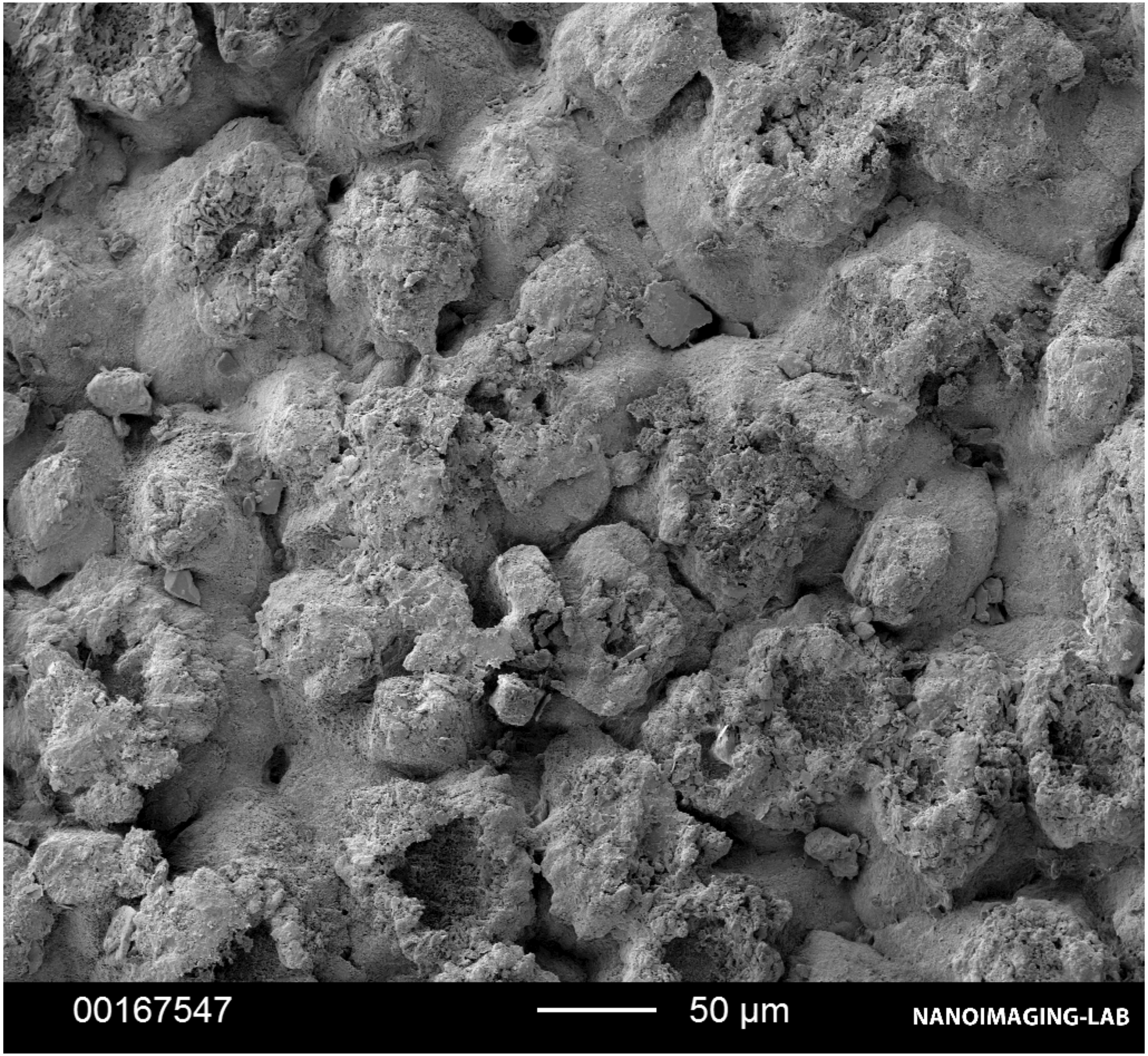
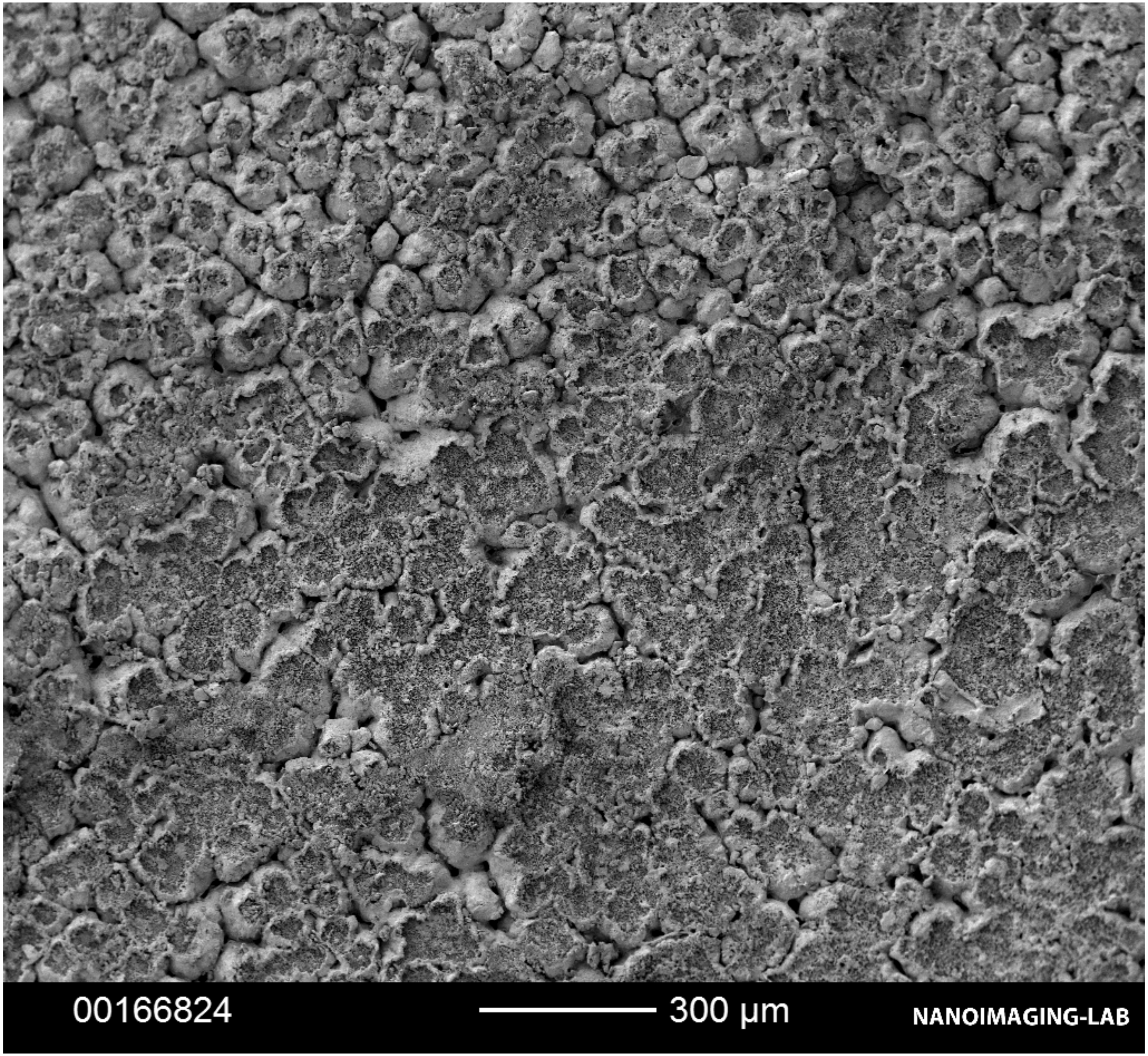
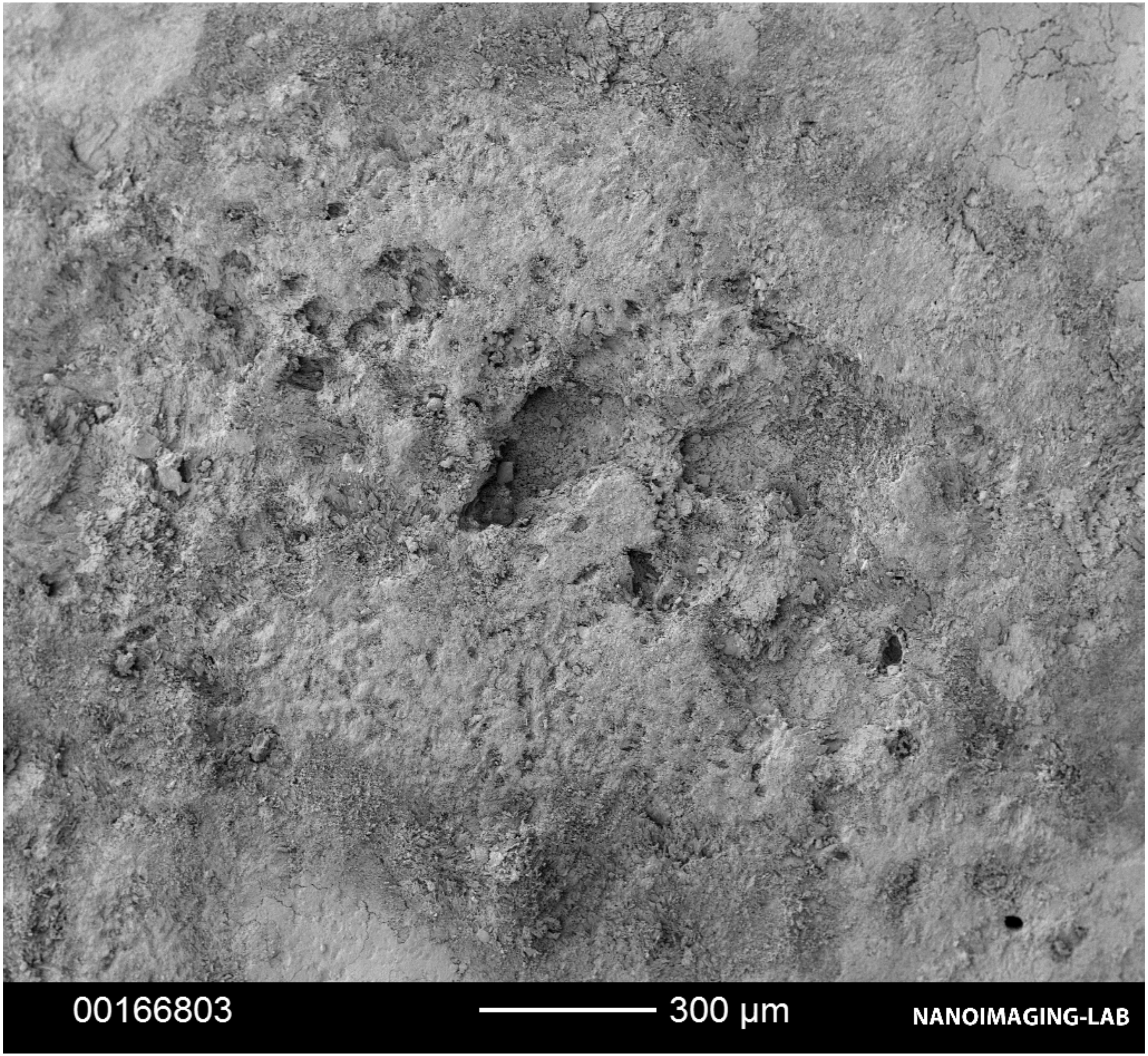
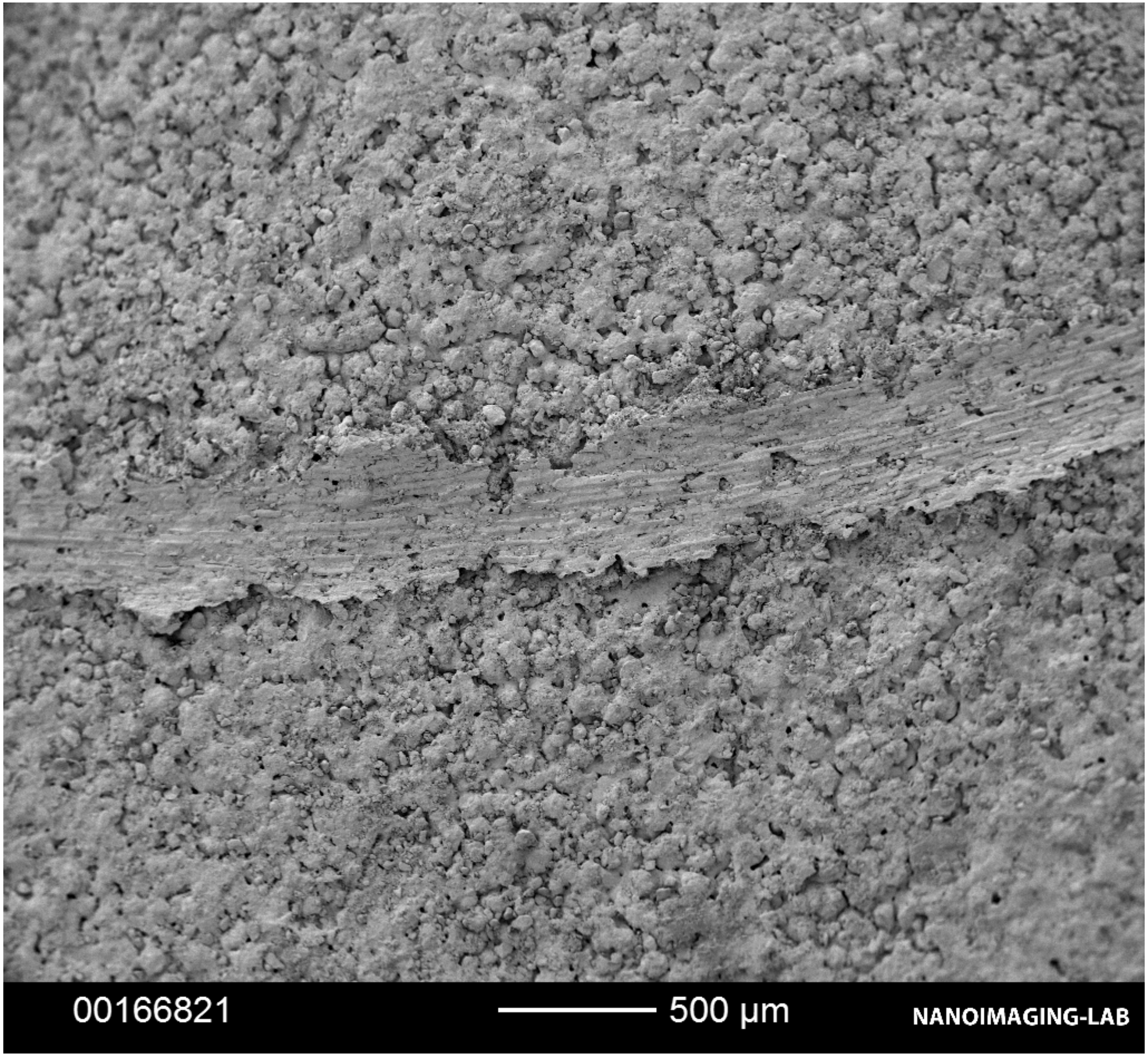

00167547
50 µm
NANOIMAGING-LAB


00166824
300 µm
NANOIMAGING-LAB


00166803
300 µm
NANOIMAGING-LAB


00166821
500 µm
NANOIMAGING-LAB

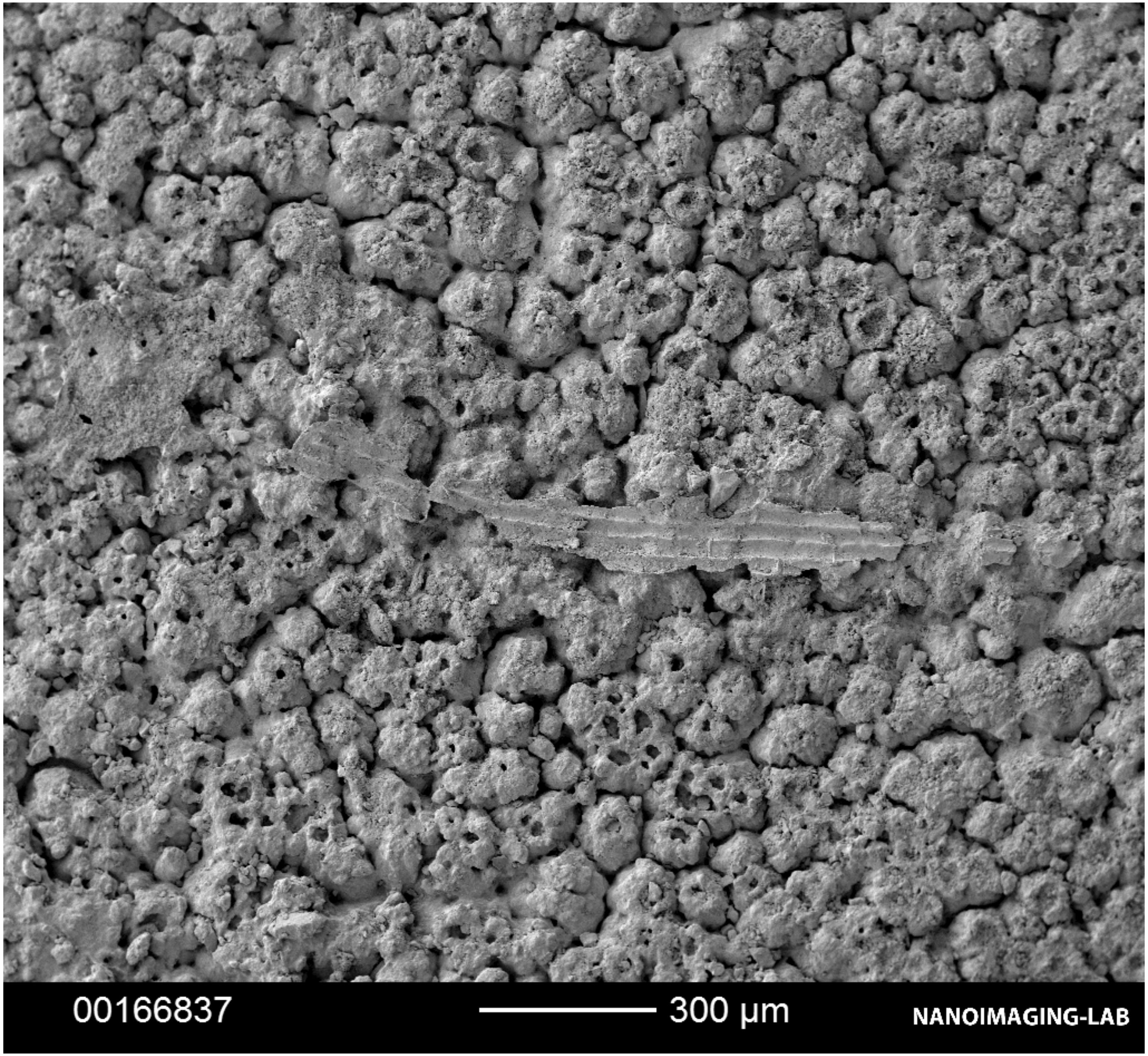

00166837
300 µm
NANOIMAGING-LAB

Figure captions

Fig. 1: Schematic drawing of an egg at an advanced stage of incubation (Figure by B. Sichert, based on a figure by A. L. Romanoff cited by Ridlen/Johnson 1964, 13 Fig. 9.).

Fig. 2: Modern hatched reference shell of *Anser anser* (Figure by O. Fischer and B. Sichert).

Fig. 3: Section through the compost pile and location of the samples. Sample V (former subsoil) contained no eggshells (Figure by B. Sichert).

Fig. 4: Burial 19 (Ober-Olm, Germany) (Figure by M. Vitucci and B. Sichert).

Fig. 5: Corrosion patterns on modern hatched reference shells, archaeological shells of burial 19 (Ober-Olm, Germany) and modern compost eggshells quantified by number of fragments (n) and total shell fragment areas in square millimeters (mm^2). Above: absolute numbers/areas. Below: percentages (Figure by B. Sichert).

Fig. 6: Remains of organic shell membranes on compost eggshells (Figure by B. Sichert).

Fig. 7: Modern compost eggshell (*Gallus gallus f. d.*). Corroded mammillae and remains of the organic shell membrane (m) (Figure by E. Bieler and B. Sichert).

Fig. 8: Modern compost eggshell (*Gallus gallus f. d.*). Uniformly corroded shell (Figure by E. Bieler).

Fig. 9: Modern compost eggshell (*Gallus gallus f. d.*). Mostly corroded shell with uncorroded zone (bottom right) (Figure by O. Fischer).

Fig. 10: Stereomicroscopic image of an eggshell (*Anser anser?*) from burial 19 (Ober-Olm, Germany). Mostly corroded shell with two uncorroded zones (left side) (Figure by O. Fischer and B. Sichert).

Fig. 11: Archaeological eggshell (*Anser anser?*) from burial 19 (Ober-Olm, Germany). Uniformly corroded shell (Figure by E. Bieler).

Fig. 12: Archaeological eggshell (*Anser anser?*) from burial 19 (Ober-Olm, Germany). Corroded and uncorroded mammillae (Figure by E. Bieler).

Fig. 13: Archaeological eggshell (*Anser anser?*) from burial 19 (Ober-Olm, Germany). Zone of excessively flattened mammillae (lower half) (Figure by E. Bieler).

Fig. 14: Archaeological eggshell (*Anser anser?*) from burial 19 (Ober-Olm, Germany). Corrosive feature on external (convex) side (Figure by E. Bieler).

Fig. 15: Archaeological eggshell (*Anser anser?*) from burial 19 (Ober-Olm, Germany). Root groove and uncorroded shell (Figure by E. Bieler).

Fig. 16: Archaeological eggshell (*Anser anser?*) from burial 19 (Ober-Olm, Germany). Root groove and mostly corroded shell wit uncorroded zones (Figure by E. Bieler).

		Anser anser		*Gallus gallus f. dom.*
	n	mm^2	n	mm^2
surface uniformly corroded	89	12002	75	4617
mostly corroded with uncorroded zone(s)	12	1530	9	686
mostly uncorroded with corroded zone(s)	8	1136	5	204
surface uncorroded	3	789	14	823
Total	112	15457	103	6330
Degree of fragmentation		138.0 mm^2/ fragment		61.5 mm^2/ fragment

Tab. 1: Modern hatched reference shells (*Anser anser* and *Gallus gallus f. d.*) quantified by number of fragments (n) and total shell fragment areas in square millimeters (mm^2). Corrosion patterns and degree of fragmentation.

	Sample I		Sample II		Sample III		Sample IV		Sample V	
	n	mm^2	n	mm^2	n	mm^2	n	mm^2	n	mm^2
surface uniformly corroded	2	38	5	696	4	212	7	330		
mostly corroded with uncorroded zone(s)	1	20	1	59	6	282	4	203		
mostly uncorroded with corroded zone(s)	13	1424	12	924	14	1106	36	2920		
surface uncorroded	80	6474	97	6578	81	4471	83	4222		
not assessable	47	3341	7	1029	5	570	3	300		
Total	143	11297	122	9286	110	6641	133	7975	no eggshells	
Degree of fragmentation		79.0 mm^2/ fragment		76.1 mm^2/ fragment		60.4 mm^2/ fragment		60.0 mm^2/ fragment		

Tab. 2: Modern compost eggshells (*Gallus gallus f. d.*) quantified by number of fragments (n) and total shell fragment areas in square millimeters (mm^2). Corrosion patterns and degree of fragmentation.

	Sample I	Sample II	Sample III	Sample IV	Sample V
Calcium carbonate	21%	17%	17%	8%	20%
(dolomite)	(2%)	(2%)	(2%)		(2%)
Loss on ignition	42%	35%	20%	33.5%	3.5%
Phosphates	6.9 c.u.	4.7 c.u.	6.1 c.u.	5.6 c.u.	4.3 c.u.
Humic substances	1.5 c.u.	1.3 c.u.	1.3 c.u.	1.1 c.u.	0.2 c.u.
pH	7.8	7.8	7.8	8.1	8.6

Tab. 3: Geochemical measurements of the compost sediments (<0.5 mm fraction).

	assemblage 4		assemblage 5		assemblage 6		not assignable	
	n	mm^2	n	mm^2	n	mm^2	n	mm^2
surface uniformly corroded	6	1121	3	361	1	48	0	0
mostly corroded with uncorroded zone(s)	17	5547	19	2460	2	213	1	79
mostly uncorroded with corroded zone(s)	26	2988	70	6612	53	5953	5	554
surface uncorroded	5	464	7	1310	16	1022	1	114
not assessable	3	480	4	463	0	0	0	0
Total	57	10600	103	11206	72	7236	7	747
Degree of fragmentation		186.0 mm^2/ fragment		108.8 mm^2/ fragment		100.5 mm^2/ fragment		106.7 mm^2/ fragment

Tab. 4: Archaeological eggshells (*Anser anser?*) quantified by number of fragments (n) and total shell fragment areas in square millimeters (mm^2). Corrosion patterns and degree of fragmentation.