JOURNAL OF
Pacific
Archaeology

– RESEARCH REPORT –

A new sedimentary microfossil type: Squamous epithelium, discovered at the archaeological sites of Carpenters Gap 3, in the Kimberley, Western Australia and Pangpang, on Efate Island in Vanuatu

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Abstract

During recent microfossil analyses of sedimentary deposits from two archaeological sites in Oceania, Carpenters Gap 3 in Western Australia (a rockshelter with a c. 30,000 yr BP record of human occupation) and Pangpang in Vanuatu (an open site assumed of immediately post-Lapita age, c. 2800 yr BP), an unusual type of microfossil was discovered. This comprised layered sheets of epithelial tissue, which had the morphological features of stratified squamous epithelium, a type of animal epidermal tissue. It is a fundamental tissue type found across most multicellular animal groups, especially mammals, adapted for rapid diffusion or protection from abrasion. The squamous epithelium described here represents a new type of sedimentary microfossil. In environmental history contexts, at the very least this microfossil can provide an additional or alternative line of evidence of former animal (roosting/sleeping/nesting or hunted and butchered) and human presence, being especially useful if no animal macrofossils are preserved. In addition, although DNA is not located in the cell membrane, if nuclei are preserved within cells in recovered epithelium (uncertain in this case), ancient DNA analysis can be carried out to potentially provide high resolution taxonomic resolution.

Keywords: Oceania; Melanesia; middens; rockshelters

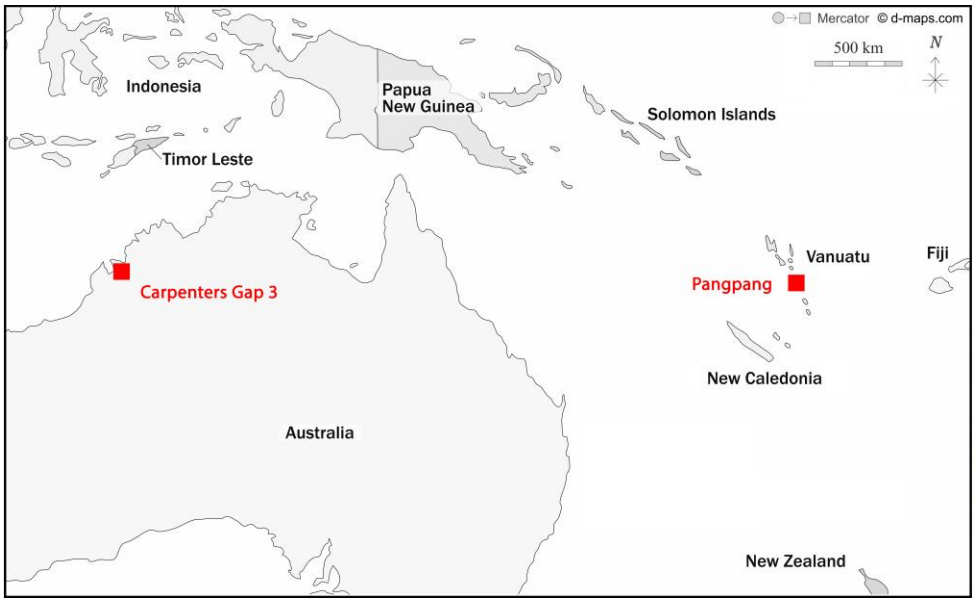
To cite this article:
Horrocks, M., Bedford, S., and O'Connor, S. 2026. A new sedimentary microfossil type: Squamous epithelium, discovered at the archaeological sites of Carpenters Gap 3, in the Kimberley, Western Australia and Pangpang, on Efate Island in Vanuatu. Journal of Pacific Archaeology, 16(1): Article 6:1-11. DOI: https://doi.org/ 10.70460/jpa.v16i1.408

Submitted: 25/04/2026, Accepted 25/08/2026, First online 27/08/2026

1. Introduction

The study of microfossils includes those from animals (e.g., helminth eggs and helminth larvae), plants (e.g., pollen grains, fern spores, phytoliths, starch grains and amyloplasts, calcium oxalate crystals, phenolic inclusions, xylem cells, single layer epithelial sheets), fungi (e.g., spores, mycelium), protists (e.g., algal material, euglenoid cysts), zooplankton and phytoplankton, and sponge spicules (e.g., Reichert 1913; Foged 1979; Large and Braggins 1991; Moore *et al*. 1991; Kondo *et al*. 1994; Moore 2000; Macphail and Stevenson 2004; Piperno 2006; Flexner *et al*. 2019; Horrocks *et al*. 2023; Horrocks, Allen and Fox 2025; Horrocks, O'Connor and Dilkes-Hall 2025; Horrocks *et al*. 2026). Strictly, these are sub-fossils as they have not undergone complete permineralization but by convention are referred as fossils. Studies are usually carried out via light microscopy or scanning electron microscopy. This broad range of biological material has over many decades of scientific research provided a very large amount of proxy evidence for determining environmental and vegetation histories during the Quaternary, including human activity.

During recent microfossil analyses of sedimentary deposits from two archaeological sites in Oceania, Carpenters Gap 3 in the Kimberley, Australia (Horrocks *et al*. 2025) and Pangpang on the island of Efate, Vanuatu (Bedford *et al*. 2026) (Figure 1.), another type of microfossil was discovered. This comprised microscopic, layered sheets of epithelial tissue, with the morphological features of stratified squamous epithelium, a type of animal epidermal tissue (Figure 2). Our aim here is to present a detailed description of this sedimentary microfossil type and outline its potential usefulness as an environmental proxy, for any particular age period, and possible origins in the context of the two sites.



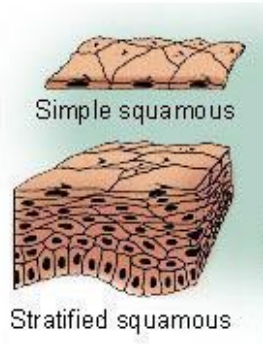


Figure 1 (Left): Map showing site locations (www.d-maps.com/conditions.php?lang=en).

Figure 2 (Right): Two types of animal epithelium (Author: US Government). The type in this study is stratified squamous epithelium.

2. Study areas and sites

2.1 Carpenters Gap 3

Carpenters Gap 3 rockshelter is on the northern margin of the Windjana Gorge National Park in the Napier Range, south central Kimberley, Western Australia (Figure 1). The range is a 350-million-year-old remnant Devonian limestone reef complex (Playford *et al*. 2009). The site is an extensive limestone shelter approximately 30 m above the savannah plain and has a deep lower cave extending at least 30 m into the range. Excavations have been carried out in the lower cave approximately 17 m inside the drip line (O'Connor *et al*. 2014).

The main deposit is within the cave and has extensive surface cracking, reflecting seasonal or periodic wetting and drying. Carpenters Gap 3 was first excavated by O'Connor in 1993. ^{14}C estimates obtained on charcoal, seeds, and freshwater shell showed a c. 30,000-year occupation. Although since incorporated into other work (e.g., O'Connor and Veth 2006:36), the excavation details were never fully reported. Further excavation was carried out in 2012 and along with the findings from 1993, reported by O'Connor *et al*. (2014). O'Connor *et al.*'s (2014) study provided evidence for low level occupation following first settlement, with an occupational hiatus during the Last Glacial Maximum. As with most Australian sites, evidence for occupation increased sharply from the mid-Holocene. Faunal remains, interpreted mainly as the remains of people's meals, suggested foraging of the immediate surroundings throughout the entire period of occupation. Results of analysis of faunal bones and teeth from the 2012 excavation are given in the work of Dodd (2019).

More recently, Horrocks, O'Connor and Dikes-Hall (2025) carried out starch (and euglenoid cyst) analysis in sediments from Carpenters Gap 3, revealing processing of gathered native plants. Finally, this year a helminth parasite study was carried out (Horrocks *et al*. 2026).

Another rockshelter, Carpenters Gap 1, is 3 km away overlooking the same plain and has been similarly researched (see Horrocks, O'Connor and Dikes-Hall (2025) for a summary). A major difference between the two shelters is that Carpenters Gap 1 lacks a cave extension.

2.2 Pangpang

The Pangpang archaeological landscape is located on the east coast of Efate Island, Vanuatu. It comprises a series of sites dating from first arrival to the historic period (Bedford *et al*. 2026). A colonising Lapita site sealed immediately by post-Lapita Erueti deposits is located adjacent to the Forari River on uplifted river terraces in the area known as Etok.

Excavations undertaken on Mounds B and C at Etok revealed deeply stratified and well-preserved deposits (up to almost 2 m deep in Mound B) spanning the Early Erueti to Lapita periods, c. 3000-2800 yr BP. Microfossil analysis was recently carried out on samples from Mounds B and C (Horrocks 2026).

3. Materials and methods

For both sites, density separation was carried out on excavated sediment samples using the method outlined in the work of Horrocks (2020), which we summarise here. This method, originally developed for starch grain separation, can also recover squamous epithelium (and helminth eggs).

For each sample, approximately 4-5cm^3 of material was added to a 10 ml disposable plastic test tube (with attached push-on cap). For deflocculation and clay removal, 6 ml of a 5% Calgon (sodium polymetaphosphate) solution was added, the tube shaken vigorously, then left overnight or for at least six hours. With caps firmly on, the tubes were shaken vigorously from time to time during this period.

The next step, to remove heavy particles, involved shaking the tube vigorously one final time, topping it up with distilled water, shaking it again, and pouring the sample into a 50 ml beaker (one at a time). The tube was then rinsed with distilled water. The beaker was then swirled and paused for five seconds to allow the heaviest particles to settle, then the liquid fraction was decanted back into the tube. The settled fraction was then discarded and the beaker rinsed with distilled water. When this step was finished for all samples, they were centrifuged at 2000 rpm for three minutes and decanted. Distilled water was added to each sample, then the sample was stirred and centrifuged at 2000 rpm for three minutes and decanted. This water wash was repeated until the supernatant was clear.

For the separation step, 2 ml of a 1.8 specific gravity sodium polytungstate solution was added to each sample and mixed thoroughly. The samples were then centrifuged at 1500 rpm for three minutes but not decanted (NB, the centrifuge speed is lower for this part, and only for this part). Holding the test tube at eye level in good light, the upper part of the top layer (i.e., the layer of liquid containing any microfossils above the pellet) was then gently and carefully stirred with a disposable 3 ml Pasteur pipette without disturbing the pellet, and, again without disturbing the pellet, the liquid was transferred into the pipette which was then placed upside down in the test tube rack. The test tube was then cleaned with distilled water to receive the fluid material in the pipette. This was topped up with distilled water to dilute the heavy liquid so the organic residues could then be centrifuged to the base of the tube. With cap firmly on, the tube was shaken vigorously, centrifuged at 2000 rpm for three minutes, and decanted. Distilled water was then added, the tube centrifuged at 2000 rpm for three minutes, and decanted. Separated material was then mounted on slides with glycerol jelly for microscopic examination.

Photomicrographs were taken with a Canon EOS 600D camera mounted on a Nikon E400 microscope. Measurements were made using a calibration slide. Fossil and modern reference micrographic figures are presented in Figures 3 and 4, and Figure 5, respectively.

4. Results

4.1 Carpenters Gap 3

Ten specimens of layered sheets of epidermal cells were found in one sample at Carpenters Gap 3, from Spit 24, dated 13,447-13,201 calBP (O'Connor *et al*. 2014). The cells had an enclosing thin membrane, known as the "cell membrane", typifying animal cells (Connor 2000) (Figure 3A-C, Figure 5). This allowed plant epithelium to be ruled out, as cells of the latter have both a cell membrane and an outer, thicker wall comprised of cellulose and sometimes also lignin, known as the "cell wall" (Connor 2000).

The Carpenters Gap 3 specimens measured up to approximately 550 µm on the longest axis (Figure 3A-C). Some of the cells in the specimen were in layers (i.e., underlying cells could be seen through cells and at different focal planes), indicating stratified squamous epithelium. The cells were

irregularly polygonal shaped, and a few of the cells each had a small object visible which could be a preserved nucleus. (Figure 5). These objects were spherical to sub-spherical in shape and mostly hada fine-grained appearance. Their resolution however was insufficient for unequivocal nucleus identification.

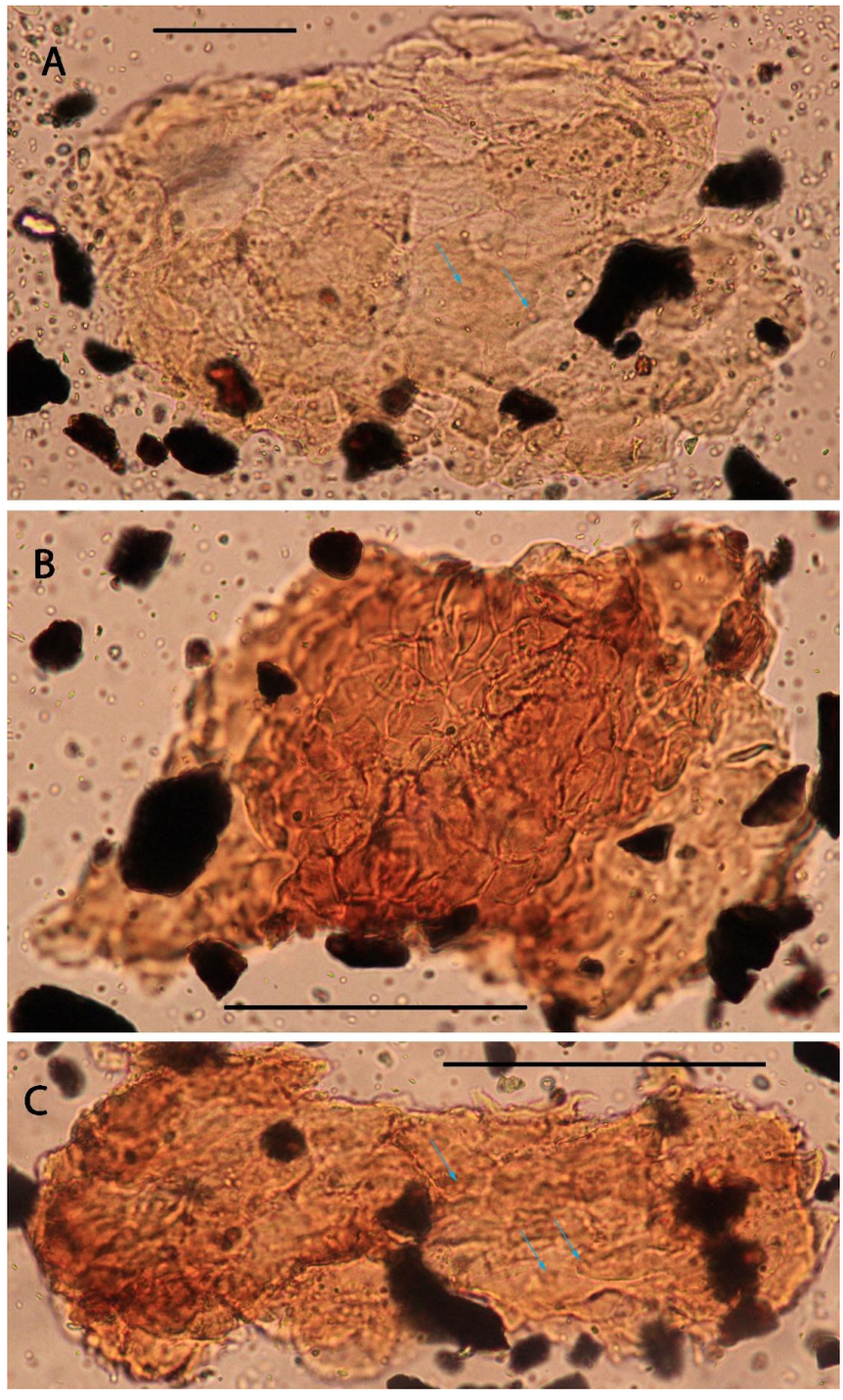


Figure 3: Stratified squamous epithelium from Carpenters Gap 3. (A 400x, B, C 600x; scale bars = 100 µm). A-C Degraded, discoloured cell sheets with characteristic irregular, polygonal shaped cells, and with some scattered small, spherical to sub-spherical possible nuclei visible in low resolution (e.g., arrows) (cf. modern reference stratified squamous epithelium, Figure 5). It is difficult to show sharp outlines of nuclei if they are in deeper layers and at different levels of stratified tissue, as is mostly the case here.

4.2 Pangpang

A single layered sheet of epidermal cells was found in one sample at Pangpang, Sample 144 from Mound B. There is currently no chronometric dating available for the Pangpang site, but the tissue comes from a deeply stratified, well-preserved layer associated with post-Lapita Erueti sherds overlying Lapita deposits (Bedford *et al*. 2026). The cells had an enclosing thin membrane (the cell membrane), typifying animal cells (Figure 4A, B; Figure 5), as observed with the Carpenters Gap specimens (Figure 3A-C)

The Pangpang specimen measured approximately 485 µm on the longest axis and comprised a degraded and discoloured, layered sheet of cells (Figure 4, B). Morphological features were more or less the same as that for Carpenters Gap; and similarly, a few of the cells each had a small object visible which could be a preserved nucleus. Again, we emphasise that the resolution was insufficient for unequivocal nucleus identification.

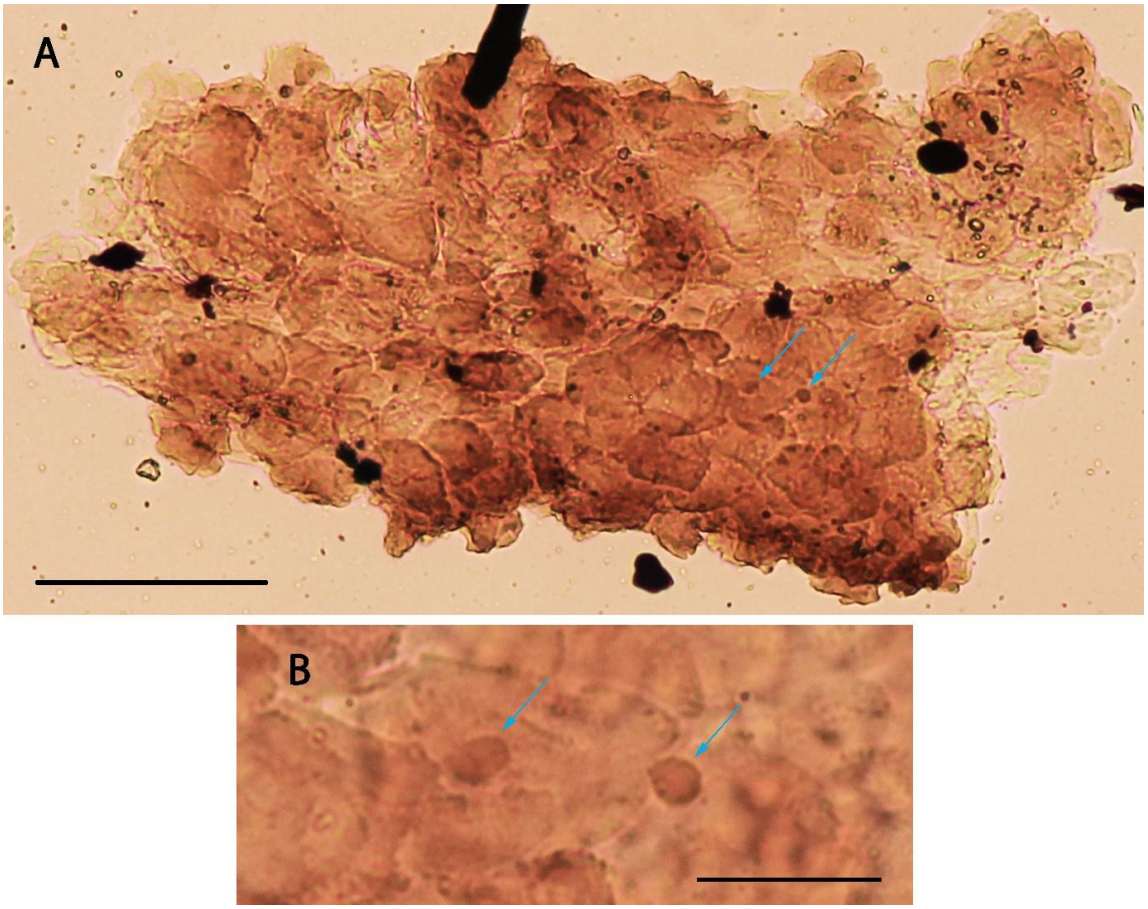


Figure 4: Stratified squamous epithelium from Pangpang. (A 400x, scale bar = 100 µm; B 600x, scale bar = 25 µm). A: A degraded, discoloured cell sheet with characteristic irregular, polygonal shaped cells, and with some scattered small, spherical to sub-spherical possible nuclei visible almost all in low resolution (arrows point out two higher resolution examples) (cf. modern reference stratified squamous epithelium, Figure 5). B: The two possible nuclei shown with arrows in A, micrographed at the higher magnification and enlarged, (cf. modern reference nuclei, Figure 5). It is difficult to show sharp outlines of nuclei if they are in deeper layers and at different levels of stratified tissue, as is mostly the case here.

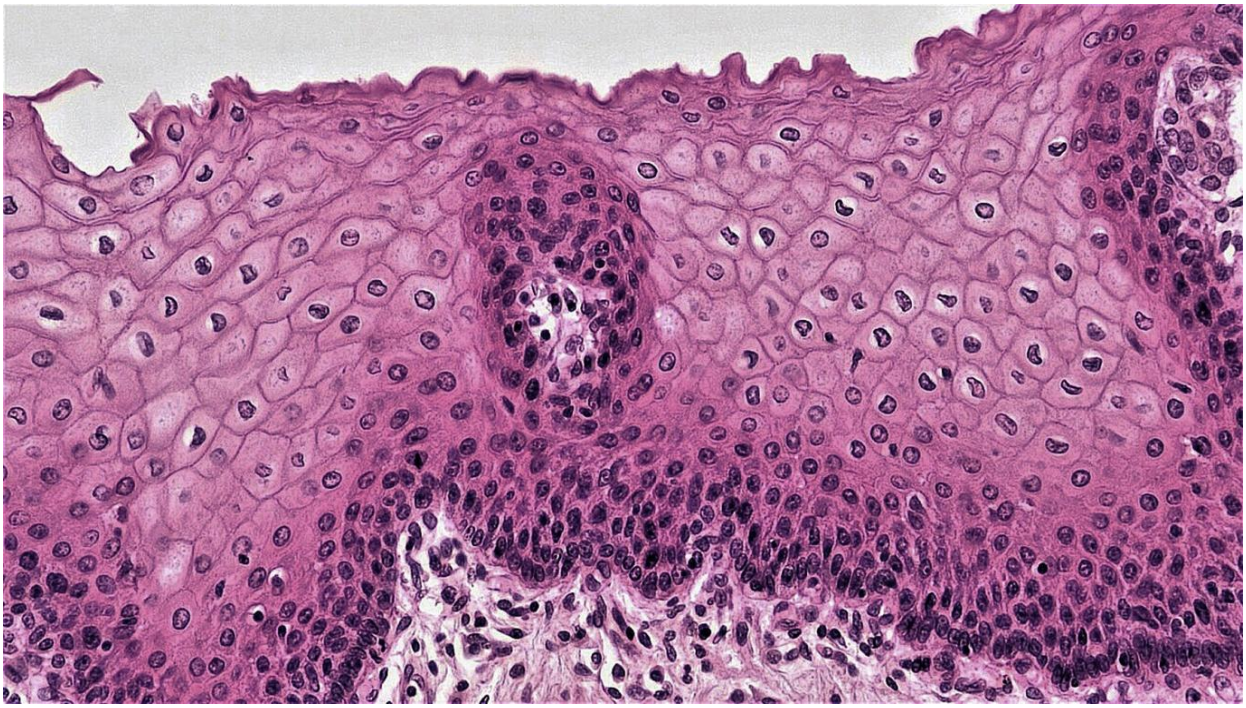


Figure 5: Modern reference stratified squamous epithelium with nuclei visible (200x; Author: Berkshire Community College Bioscience Image Library, 10 May 2018). Such tissues are commonly stained pink with eosin to enhance features; fresh unstained material is usually more or less translucent with little if any colour (although fossil material is often stained by the enclosing substrate and/or discoloured with decay, as in this study).

5. Discussion and conclusions

Squamous (Latin for scaly) epithelium refers to thin, flat, scale-like cells (simple squamous epithelium, Figure 2) that form protective barrier surfaces in the body, such as skin (epidermis) and internal linings. It is a fundamental tissue type found across most multicellular animals, adapted for rapid diffusion (e.g., oxygen exchange) or protection from abrasion. The adaptation for abrasion is layering of cells with a high mitotic division and turnover rate (stratified squamous epidermis, Figure 2). Cells in the stratified tissue are progressively thicker nearer the basal layer. The morphology and function of these tissues, which are generally similar across animal groups, have been intensively researched; full details can be found in numerous publications (e.g., Ross and Pawlina 2010; Ovalle and Nahirney 2020).

All the main animal groups possess squamous epithelium. Mammals have an extensive presence, including the skin, mouth, oesophagus, penis, and vagina (stratified squamous, Figure 2), and lungs and blood vessels (simple squamous, Figure 2). Mammals, birds, and reptiles have keratinised stratified squamous epithelium in their skin, the outermost layer of which (the stratum corneum) is composed of dead cells filled with a tough, fibrous waterproof protein (keratin). Keratinised cells are constantly shed (desquamation) and replaced by new cells formed in the lower layers. Amphibians have simple squamous epithelium in the peritoneum and lung alveoli, and stratified types in the oesophagus. Fish possess simple squamous epithelium in blood vessel lining and body cavities. Many invertebrates also have squamous-like epithelial cells.

Regarding the origin of the tissue at our sites, the possibilities are broad. The faunal macrofossil analysis (bone/teeth from sediments and regurgitated owl pellets) for Carpenters Gap 3 revealed a large array of animal taxa, namely murids, kangaroo rats, possums, echidnas, dasyurids, birds, and reptiles (Dodd 2019). Preliminary faunal identifications from the Pangpang mounds, a rich midden site, also present a broad range of possible origins, including pigs, rats, birds, and reptiles. A human origin could also be inferred, most likely from the hands and feet, especially if sharp lithics were in use. One of the sharpest lithics is obsidian (volcanic glass), a major universal component of early tool sets, including those in Vanuatu (Galipaud *et al*. 2014) and sites on Efate (Bedford *et al*. 2026; Reepmeyer *et al*. 2011). One of us (MH) once received a sample comprising a large number of freshly washed obsidian flakes to check for plant material lodged in crevices; the only extraneous material found comprised numerous microscopic fragments of fresh (modern) keratinised squamous epithelium, obviously derived from recent handling (Horrocks, unpubl. data).

In conclusion, the squamous epithelium discovered at Carpenters Gap 3 and Pangpang represents a new type of sedimentary microfossil. As animal cells lack cell walls (Connor 2000), they can be differentiated from those of plants. In environmental history contexts, at the very least this microfossil type can provide an alternative or additional line of evidence of former animal presence, being especially useful if no animal macrofossils are preserved. In addition, although DNA is not located in the cell membrane, if nuclei are present in recovered epithelium, ancient DNA analysis can be carried out to potentially provide high resolution taxonomic resolution (e.g., Pääbo 1985; Keller et al. 2012; Schuenemann et al. 2017).

Funding

Funding for the Pangpang excavation and analysis came from the Australia and Pacific Science Foundation (APSF23027) and an ARC Discovery Grant (DP250102214). We thank the Green Foundation for Polynesian Research for funding biosecurity and shipping costs for the Carpenters Gap 3 material.

Data Availability Statement.

Data is available on request from the authors.

Partnerships

This research did not use any primary data from Indigenous contexts.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization, M.H.; methodology, M.H.; formal analysis, M.H.; investigation, M.H.; resources, M.H., S.B., S.O.; data curation, M.H., S.B., S.O.; writing—original draft preparation, M.H., S.B., S.O.; writing—review and editing, M.H., S.B., S.O.; visualization, M.H.; funding acquisition, M.H.

Acknowledgments

We thank the Pangpang community for their permission, support, and collaboration.

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