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Late Pleistocene coprolites from Qurta (Egypt) and the potential of interdisciplinary research involving micromorphology, plant macrofossil and biomarker analyses

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ABSTRACT

As part of a rock art dating project at Qurta (Upper Egypt), samples were collected from an organic deposit and from an accumulation of individual faecal pellets. Radiocarbon dating of these relatively well-preserved materials indicates an unexpectedly old age of ca. 45,000 BP or older. In order to identify the biogenic nature of these deposits and to reconstruct the palaeo-environment at the time of their formation, micromorphological, palaeobotanical, and biomarker analyses were carried out. All data indicate that the organic deposit and the pellets were produced by different species. The presence of a novel biomarker, which only occurs in animal urine (hippuric acid), contributed to the conclusion that the organic deposit most likely represents the remains of a rock hyrax (*Procavia capensis*) latrine, whereas the pellets stem from small bovids. Plant macroremains from the pellets indicate that the animals browsed in the more vegetated areas, presumably near the Nile, although the general environment was probably mainly arid and open. Combined with the dates, this suggests that the pellets date to MIS 3 or 4. Our results demonstrate the great potential of an interdisciplinary approach to the study of Quaternary coprolite deposits, allowing for more adequate and more complete interpretation.

1. Introduction

1.1. Coprolite research: methods and applications

Coprolites, i.e., fossil or subfossil excrements, can provide a wealth of information to archaeologists and palaeontologists. They constitute palaeodietary records of humans and animals (Reinhard and Bryant 1992; Ghosh et al. 2008; van Geel et al. 2008; Riley 2012) and contain valuable palaeoenvironmental and palaeoclimate proxies, especially in arid areas where few other archives are available (Scott and Woodborne 2007; Ghosh et al. 2008; Mercuri 2008; Linseele et al. 2010; Marinova et al. 2013; Cremaschi et al. 2014; Carr et al. 2016). In addition, they are informative on the digestive processes (Gill et al. 2010; van Geel et al. 2011), health status and sanitary conditions (Reinhard 1992; Schelvis 1992) of extant and extinct species.

Coprolite research traditionally entails macroscopic and microscopic analysis of plant macrofossils, pollen, phytoliths, spherulites and intestinal parasites (Brochier et al. 1992; Reinhard and Bryant 1992; Bryant and Dean 2006; Shahack-Gross 2011), although micromorphological studies of undisturbed dung materials in thin section are scarce (see Macphail and Goldberg 2018). Dung deposits and related zoogenic accumulations in rock shelters constitute a unique source of information for palaeoecological reconstructions due to the long-term accumulation of various organic materials (Savinetsky et al., 2012). Botanical remains in these deposits not only represent dietary components, they also represent a considerable part of the vegetation that was accessed by the animals and may thus be exploited for reconstructing palaeoenvironment and palaeoclimate, especially in arid areas where few other archives are available (Scott and Woodborne 2007; Ghosh et al., 2008; Mercuri 2008; Linseele et al. 2010; Marinova et al. 2013).

Recently, these approaches have been increasingly supplemented with biomolecular and isotopic analyses. Lipid biomarkers have proven particularly valuable in identifying suspected faecal remains in contexts where macroscopic evidence is lacking, including manured soils, latrines or putative animal pens (e.g., Simpson et al.

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1999; Bull et al. 2001; Baeten et al. 2012). Among lipid biomarkers, 5β-stanols and bile acids received much attention as they are not only diagnostic for faeces, they also provide information about the biological source of the excrements (Bull et al. 2002; Gill et al. 2010; Hofmann et al. 2010). In that respect, recent analysis of modern reference materials contributed to a greater discriminative power of faecal steroids (Prost et al. 2017; Zocatelli et al. 2017). Faecal 5β-stanols ratios also indicate the degree of plant consumption (van Geel et al. 2008, 2011), and recently contributed to a better understanding of the evolution of human diets (Sistiaga et al., 2014a, 2015). Aside from these faecal steroids, coprolites often contain other lipid biomarkers such as alkanes, alkanols or triterpenoids, which generally represent dietary components of the animal's last meal (van Geel et al. 2008, 2011; Gill et al. 2009; Carr et al. 2010). Ancient DNA analysis provides precise taxonomic identification of the source animal and the coprolite's organic content (e.g., Poinar et al. 1998, 2001), while stable isotope analysis provides insights into the source animal's dietary components and trophic level (Ghosh et al. 2003; Carr et al. 2010, 2016), as well as palaeoclimatic issues (e.g., Chase et al. 2009; 2010, 2012; Meadows et al. 2010).

1.2. The Qurta rock art and the find of the coprolite deposits

The circumstances of the discovery of the Qurta rock art and its broader environmental and cultural Late Pleistocene context have already been detailed in previous publications (e.g., Huyge 2008; 2009; Huyge and Claes 2008; Huyge and Ikram 2009) and will not be reiterated here. Since 2005, three rock art sites have been identified at Qurta in Upper Egypt: Qurta I, II and III (henceforth QI, QII and QIII; Fig. 1). A major breakthrough in obtaining absolute dates for the Qurta rock art, was the discovery in 2008 of some partly buried rock art panels at QII (Fig. 2). The hillslope deposits covering the rock art have been dated directly using optically stimulated luminescence (OSL) and indirectly using radiocarbon dating of bioapatite from microvertebrate faunal remains within these sediments (Huyge et al. 2011). The OSL dates give a minimum age for the covered petroglyphs of between 10 and 17 ka. The two radiocarbon dates on animal bones are congruent, although not entirely internally consistent, with the OSL dates: 12,130 ± 45 ^{14}C yr BP (KIA-41532) and 10,585 ± 50 ^{14}C yr BP (KIA-40546) (Huyge et al. 2011). The discrepancy may result from different events having been dated and/or some exchange of carbonate between the bone material and its environment.

Further fieldwork at QII in March 2011 (Huyge and Vandenberghe, 2011) led to the discovery of several more buried petroglyphs at different levels, and, at QII location 4 (QII.4), of the massive organic deposit (QII.4.3) and the faecal pellet accumulation (QII.4.2) that are the subject of this contribution (Fig. 2). Both are unrelated to the rock art, but their study was initiated because of the broad interest of our group in interdisciplinary coprolite research (Linseele et al. 2013). The massive organic deposit covers a different part of the rock face, and the faecal pellet accumulation was found in sediments covering the rock face with the art (QII.4), but well below the level on which the rock art was executed. Micromammal bones were also found, in sediment below the level of the faecal pellets. The dating of the organic deposits and of the bones (see Section 3.2.) therefore bears no relevance for the age of the rock art.

1.3. Aims of the coprolite research at Qurta

In this paper, we describe and evaluate the dates we obtained on the massive organic deposit, the faecal pellet accumulation and the micromammal bones in relation to the previously published dating information from Qurta. We report on the nature and the content of the massive organic deposit and the faecal pellet accumulation, as we have determined through the analysis of lipid biomarkers, micromorphology, and plant fossils. DNA analysis has not been attempted because of previous experiences with subfossil dung pellets from a Holocene cave site in Egypt, where insufficient DNA was preserved for amplification (Linseele et al. 2013). Our aims were as follows: (1) verify the biogenic source of the massive organic deposit and the faecal pellet accumulation; (2) examine the dietary components of both materials and relate this information to the palaeoenvironment through the palaeovegetation record preserved in the deposits; and (3) evaluate the efficiency of the adopted multidisciplinary approach with respect to objectives 1 and 2.

2. Material and methods

2.1. Description of the samples and their find context

The rock art sites of Qurta are located on the east bank of the Nile, along the northern edge of the Kom Ombo Plain, ca. 40 km south of Edfu and ca. 15 km north of Kom Ombo, on the higher parts of the Nubian sandstone (Said 1962, p. 318) scarp bordering the Nile floodplain, at an altitude of ca. 120–130 m above sea level (i.e., ca. 35–45 m above the current floodplain).

The massive organic deposit (QII.4.3) was found in a near-vertical position, stuck to the upper part of a rock face, within a deep cleft between the sandstone rocks bearing rock art panels QII.4.2 and QII.4.3 (Fig. 2). This cleft was completely filled up with loose, fine, sandy sediment. The massive organic deposit is ca. 5 cm thick and covers a surface of ca. 0.5 m^2. Its total weight is estimated at ca. 1.5–2 kg. It contained some localised remains of faecal pellets that, though much degraded, were macroscopically recognisable as such (see Fig. 3c). The deposit showed some traces of animal burrows and lacked any distinctive layering. The label "massive" refers to this lack of structure of the deposit. About half of the total volume was sampled (Fig. 3a).

The faecal pellet accumulation (QII.4.2) was found at a much lower level, in loose, fine, sandy, wind-blown sediment, ca. 1.8–2 m below the massive organic deposit and ca. 0.5–0.7 m below rock art panel QII.4.2 (Figs. 2 and 3b). All pellets were collected. They are ca. 200 in number and are ovoid in shape. Some well-preserved pellets were present (Fig. 3d), but the surface of most of them seems to have been affected by degradation processes that may also have altered their appearance (Fig. 3e).

The collected organic deposit and the faecal pellets were stored at the Royal Belgian Institute of Natural Sciences after analysis.

2.2. Radiocarbon dating

In preparation for radiocarbon dating of macrobotanical inclusions, one sample of the massive organic deposit and one sample of the faecal pellet accumulation were pre-treated through a series of washes, each lasting ca. 30 min, with hot HCl, NaOH and HCl (1%) alternating with, and ending with, water. With the exception of plant fibres, representing ca. 5% of the initial sample, the pre-treatment removed everything, including humus-like materials (Bonneaul et al., 2011). The micromammal bones from below the pellet accumulation, which were identified as belonging to at least three individuals of mouse (cf. *Mus musculus*), did not contain any collagen. Therefore, the bone apatite was dated. The pre-treatment of the bones consisted of soaking in 1% acetic acid for 24 h. After pre-treatment samples

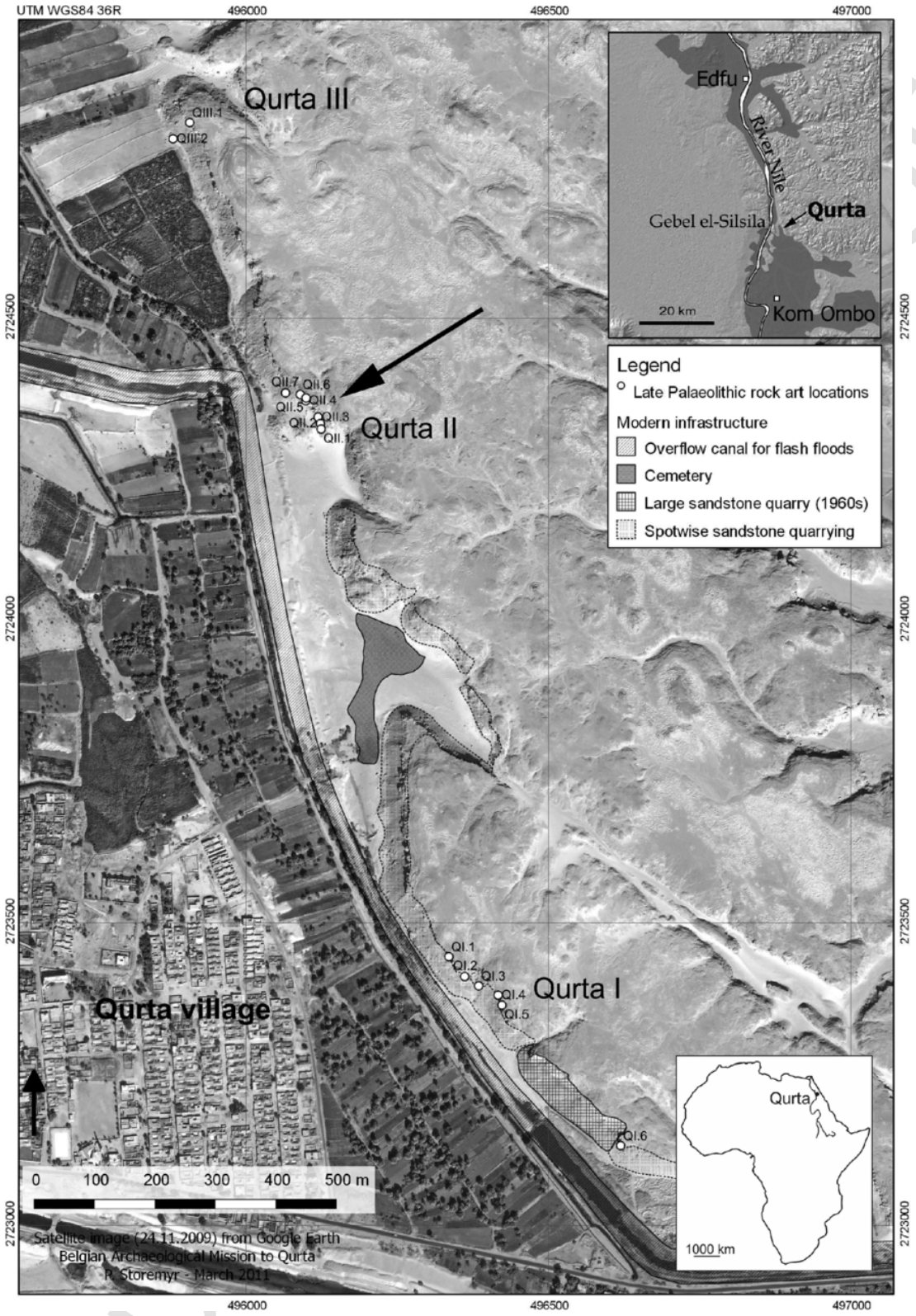

Fig. 1. Satellite image showing the location of rock art sites and locations at Qurta, Egypt. Location QII.4, where the massive organic deposit and the faecal pellet accumulation were discovered in 2011, is indicated with an arrow (modified after Google Earth 2009).

were graphitized using H_2 over a Fe catalyst. Targets were prepared at the Royal Institute for Cultural Heritage in Brussels (Belgium) (Van Strydonck and van der Borg 1990–1991) and ^{14}C concentrations

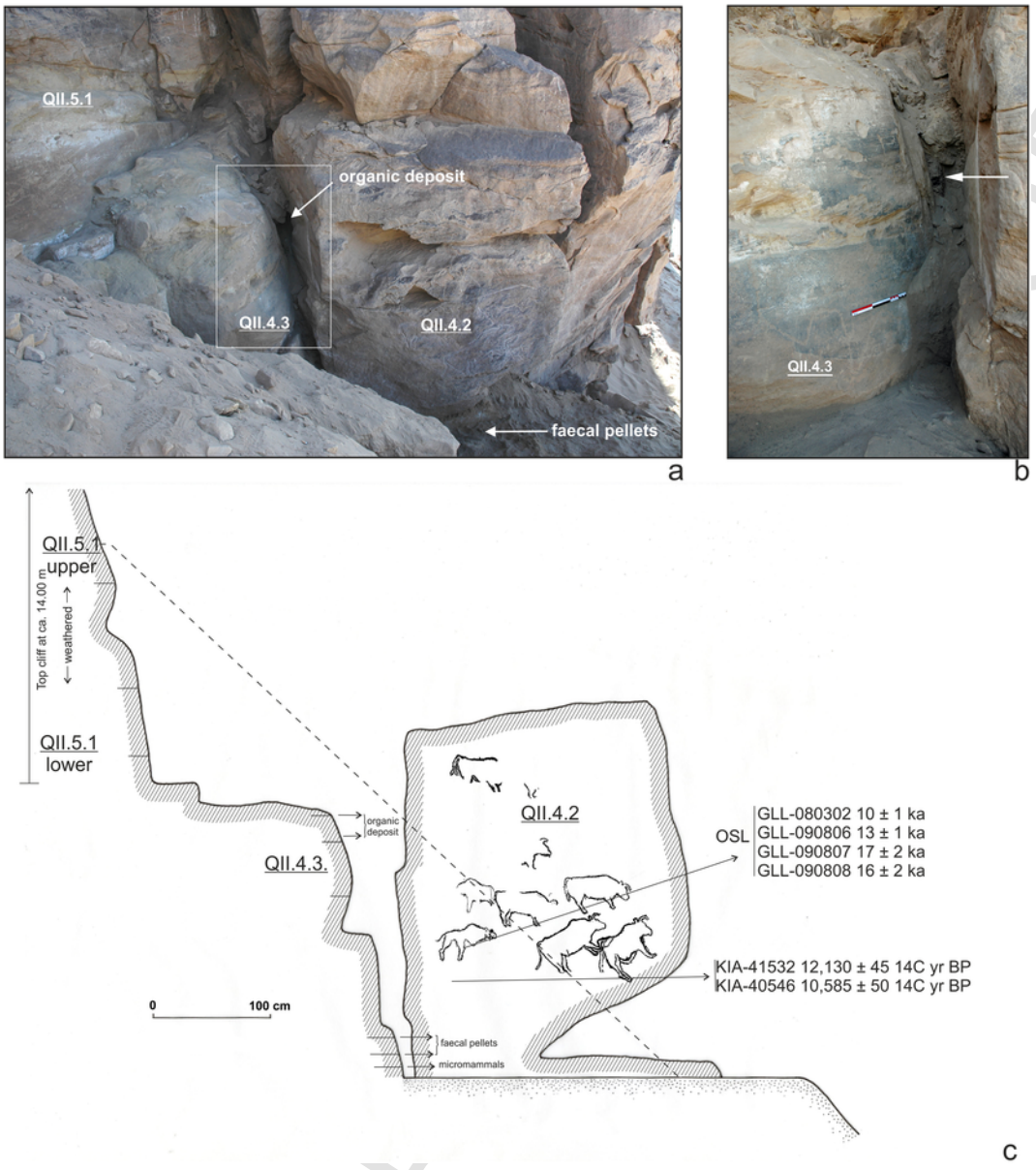

Fig. 2. (a) Photo showing the Nubian sandstone rock face at QII.4 with localization of the massive organic deposit, the faecal pellet accumulation level and rock art panels QII.4.2, QII.4.3 and QII.5.1. The rectangle shows the approximate location of the detail shown in Fig. 2b; (b) Detail photo showing the location of the massive organic deposit (indicated by an arrow) within the cleft between the sandstone rocks bearing rock art panels QII.4.2 and QII.4.3; (c) Schematic profile of QII.4, with localization of the massive organic deposit, the faecal pellet accumulation level, rock art panels QII.4.2, QII.4.3 and QII.5.1, and the 14C and OSL dates obtained at this locality (see Sections 1.2. and 3.2 for details, dates are published in Huyge et al. 2011). The dashed line indicates the top of the hillslope sediment accumulation that was removed to expose the (partly) buried rock art panels.

were measured with accelerator mass spectrometry (AMS) at the Leibniz Labor für Altersbestimmung und Isotopenforschung in Kiel (Germany) (Nadeau et al. 1998). The Leibniz-Labor AMS system is a 3 Million Volt Tandetron 4130 AMS system from High Voltage Engineering designed for the analysis of ^{14}C.

2.3. Micromorphological analyses

The massive organic deposit was sampled at two levels, representing the upper and basal parts of the occurrence. For the pellet accumulation, a random subsample of the available uniform batch of undisturbed pellets was used. Thin sections, measuring 2 × 4 cm, were prepared following standard procedures (Benyarku and Stoops 2005), including impregnation with a cold-setting polyester resin, and they were described using the concepts and terminology of Stoops (2003). To identify specific compounds in the lower sample of the massive deposit, we used powder X-ray diffraction (XRD) analysis (Bruker D8 Advance ECO system), chemical analysis of 1:5 extracts (ion chromatography; Dionex ICS-2000 instrument), and energy-dispersive spectrometry (EDS) analysis using undisturbed fragments and an uncovered thin section (Noran Vantage microanalysis system, Jeol JSM-6400 scanning electron microscope).

2.4. Palaeobotanical analysis

Plant macrofossil analysis was performed on 25 individual pellets and on 10 cm³ subsamples of the massive deposit samples. The

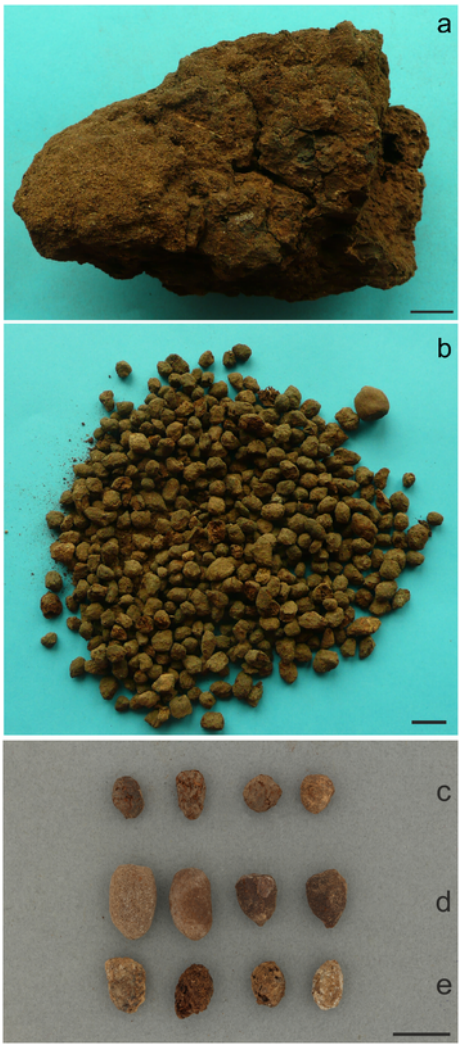

Fig. 3. (a) Sample from the massive organic deposit at Qurta (QII.4.3) before analysis (basal part)); (b) Faecal pellet accumulation from Qurta (QII.4.2) before analysis; (c) Faecal pellets from the organic deposit in Qurta; (d) Faecal pellets from the pellet accumulation in Qurta with a clearly preserved shape; (e) Faecal pellets from the pellet accumulation in Qurta of which the shape is not well preserved (all scales 1 cm).

pellets were gently broken up into 2–3 fragments and observed under a microscope (Carl Zeiss. Stemi 2000, up to 800 × magnification) with reflected light, in order to isolate and study identifiable plant remains. The pellet fragments were then soaked in distilled water for 2–3 h to dissolve the matrix and isolate the plant macrofossils. This was followed by further examination and identification using a binocular microscope (up to 70 × magnification) with cold incident light. The massive deposit was treated in the same way, but in this case it took more than 24 h of soaking to dissolve the matrix. Identification of macrofossils was achieved by comparison with the reference collections of the Centre for Archaeological Sciences (KU Leuven), and the results of the identifications were quantified by counting absolute numbers of items. We applied correspondence analysis (PAST, Hammer et al. 2001) to compare the macrofossil assemblages per pellet and, where possible, to identify (ecological) groups.

Pollen preservation was tested on four sub-samples (each measuring 5 cm^3) from each of the two deposits. The samples were processed following Faegri and Iversen (1989). Clay contamination was removed by ultrasonic sieving (5 µm), and we acknowledge that this procedure may have resulted in the loss of some pollen grains. Although potentially interesting, phytolith analysis and identification were beyond the scope of this study.

2.5. Analysis of lipid biomarkers

Two samples each from the massive organic deposit and the pellet accumulation were finely ground using a pestle and mortar. In one sample (ca. 1 g) from each of the deposits, lipids were extracted according to a protocol involving Soxhlet extraction in dichloromethane: methanol (2:1 v/v), saponification of extracted lipids and subsequent separation in neutral and polar fractions by liquid–liquid extraction (Baeten et al. 2012). In another sample (ca. 1 g) from each of the deposits, lipids were extracted according to a different protocol, based on Gill et al. (2009), in order to enable analysis of chemically bound lipids, i.e., lipids not readily extractable with Soxhlet. Subsamples were first saponified with 2 N potassium hydroxide in 90% methanol for 1 h at 100 °C. After centrifugation and filtration (0.45 µm), the lipid extract was separated in a neutral and polar fraction by liquid–liquid extraction following Baeten et al. (2012). Aliquots of both fractions were derivatised with N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) for 1 h at 60 °C. After removal of excess reagent and solvent (evaporation under a gentle stream of nitrogen), neutral and polar fractions were dissolved in hexane or ethyl acetate, respectively, a known amount of internal standard (*n*-hexatriacontane) was added and analysed with gas chromatography coupled to mass spectrometry (GC–MS). Analyses were performed on an Agilent 6890 N GC instrument, equipped with a HP5-MS capillary column (30 m × 0.25 mm × 0.25 µm) and coupled to an Agilent 5973 N mass spectrometer. One microlitre of each fraction was injected in the splitless mode at a temperature of 290 °C. The oven temperature was held at 50 °C for 1 min, then increased to 150 °C at 10 °C min^{-1} and further ramped to 320 °C at 4 °C min^{-1}, followed by an isothermal hold for 20 min. The transfer line and ion source were held at a temperature of 340 and 230 °C, respectively. The mass spectrometer was operated in scan mode, with spectra recorded between m/z 50 and 700, as well as in selected ion monitoring mode, for detection of sterols and stanols (Baeten et al. 2012). Peak assignments were made by comparison with mass spectral libraries (NIST11), mass spectral deconvolution, retention times of authentic standard compounds and literature mass spectra.

3. Results and discussion

3.1. Source animal taxonomic identification based on macroscopic observations

Based on initial macroscopic observations, the massive organic deposit was tentatively attributed to rock hyrax (*Procavia capensis*), as it is known that in rocky areas of Egypt large deposits of dung are formed in places were colonies of these animals urinate and defecate over a long period of time (Osborn and Helmy 1980, p. 465). However, the deposit differs from rock hyrax middens elsewhere in Africa in two respects. Middens elsewhere can be considerably larger (Chase et al. 2012), and while the faecal pellets within the deposit can plausibly be ascribed to rock hyrax, the measurable specimens are clearly smaller than those of modern hyrax (Fig. 4).

The 21 well-preserved pellets within the faecal pellet accumulation have the typical appearance of artiodactyl pellets, namely, pointed at one end and concave at the other (Walker 1996; Chame 2003) (Fig. 4b). The greatest breadth and length of these pellets, as

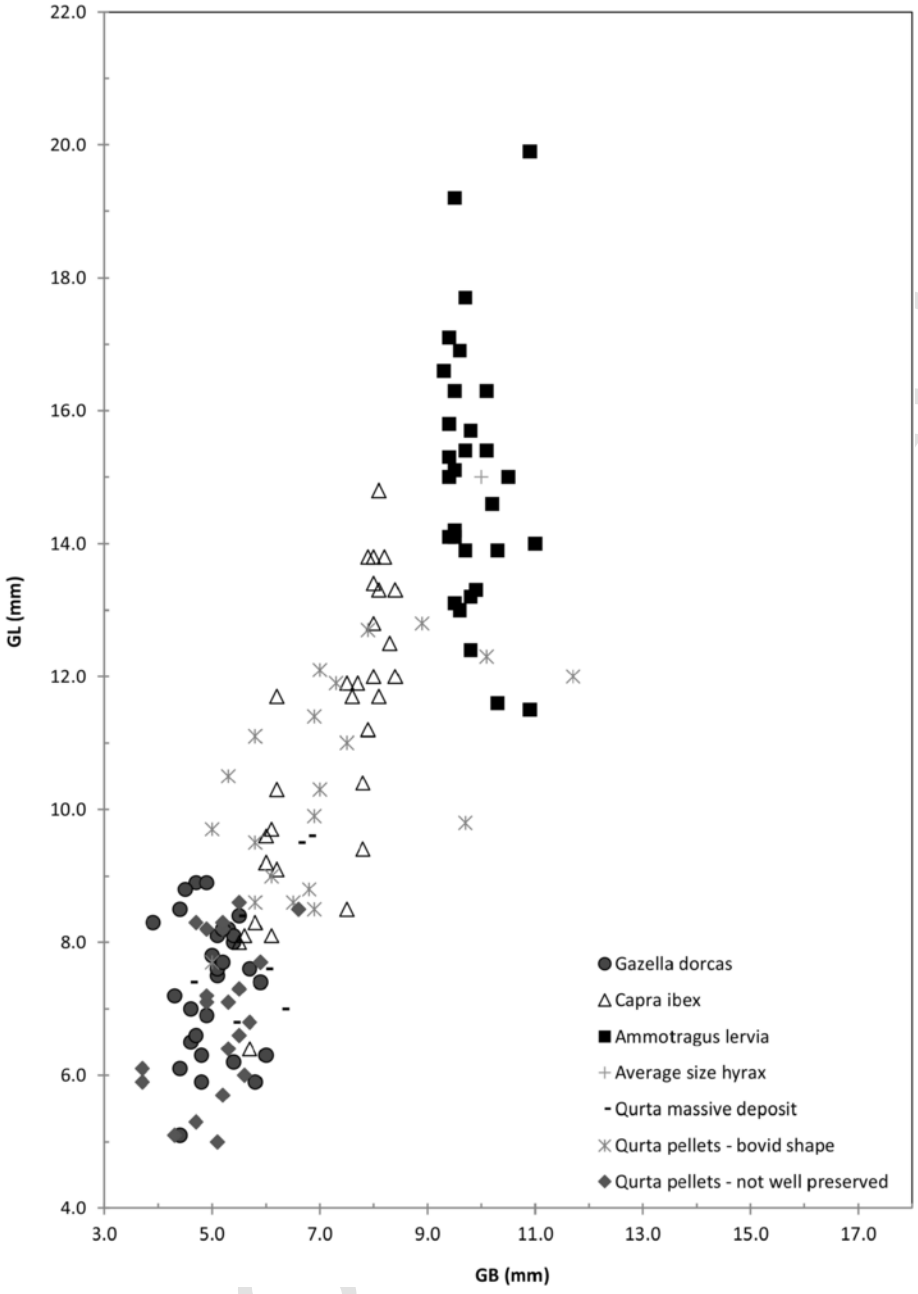

Fig. 4. Greatest length (GL) and greatest width (GB) of the faecal pellets from Qurta compared with those same measurements taken on recent small bovid droppings (Linseele et al. 2010) and the average size of hyrax (*Procavia capensis* as well as *Heterohyrax brucei*, which apparently produce similar sized faecal pellets) excrements (Chame 2003).

well as of a random sample of 21 other, less well-preserved, pellets, was measured and plotted in Fig. 4, together with those taken on recent dung of Egyptian artiodactyls producing pellets of comparable size, namely, small gazelle (mainly *Gazella dorcas*), ibex (*Capra ibex*) and Barbary sheep (*Ammotragus lervia*). The 21 well-preserved pellets mostly overlap in size with ibex, while the largest may be of Barbary sheep. The size of the poorly preserved pellets matches Dorcas gazelle, the largest of which in the modern sample overlap in size with the few faecal pellets from the massive organic deposit. However, the macroscopic appearance of the poorly preserved faecal pellets does not allow us to exclude other species.

3.2. Dates in relation to the other dates from Qurta

The radiocarbon date on the massive organic deposit is 44,780+1970/–1580 ^{14}C yr BP (KIA-45491). That on the faecal pellet accumulation is 45,170+2080/–1650 ^{14}C yr BP (KIA-45492). It is possible that both samples are the same age and that they extend beyond the ^{14}C analytical range. The massive organic deposit is undoubtedly in primary position, but the faecal pellet accumulation may not be. The pellets are certainly not so badly deformed or damaged that transport or reworking needs to be supposed. But the mouse bones from immediately below the pellet accumulation were radiocarbon dated on bioapatite to 25,660±190 BP (KIA-45490), an age

that, stratigraphically speaking, is in clear contradiction with the (much older) age obtained for the overlying faecal pellets (Fig. 2c). We are unsure of the cause of this anomaly, but it cannot be excluded that the pellets were in secondary position, having moved down to a lower level from higher up the slope or even from the edge of the Nubian sandstone scarp, which is at ca. 16 m above the current location of the faecal pellet accumulation. Alternatively, the mouse remains may represent the remains of animals that burrowed into the deposits and died there. At the same time, it cannot be ruled out that the mouse bones (KIA-45490) were affected by exchange of younger carbonates with the environment, leading to a "rejuvenation" of the dated bioapatite. Despite careful sample preparation, that possibility must be considered, especially given the slight inconsistency in the radiocarbon dates that were previously obtained on bioapatite from a higher level at this location (samples KIA-41532 and KIA-40546; see Section 1.2 and Fig. 2c). Bioapatite has been shown to be extremely prone to recrystallisation, especially in regions that underwent multiple climatic changes from arid to humid conditions and back again (e.g., Dal Sasso et al. 2014 for Central Sudan).

3.3. Micromorphological analyses

The massive deposit and the pellet deposit each consist of two main types of organic components: plant remains and fine organic material. In addition, the deposits contain an admixture of detrital mineral grains and other mineral components, as described below (Plates I and II).

3.3.1. Plant remains

The plant remains are up to a few mm in length in both deposits. In the massive deposit, they typically have a dense fibrous texture resembling lignified tissue, which is most pronounced in the lower sample (Plate I, 1). In the pellet deposit, the plant remains are characterised by a less dense tissue structure (Plate II, 1). The main differences between the two deposits are the much greater amount of recognisable plant remains and their better state of preservation in the pellet deposit. The pellet deposit also includes fragments with a tissue type that is not recognised for the massive deposit (Plate II, 2). No full taxonomical identification of plant remains in thin section was attempted for this study, which includes macrofossil analysis by other methods, but a cursory examination shows that the dominant components are vegetative parts of dicotyledonous herbs, and that monocotyledon stems and various likely Cyperaceae remains are present as well.

3.3.2. Fine organic material

In the upper sample of the massive deposit, the fine organic material, without recognisable cell structure, is characterised by an orange-brown colour and an undifferentiated b-fabric (Plate I, 1). It has a mainly dotted aspect, but it includes homogeneous limpid fragments. Similar limpid material also occurs inside some of the plant remains, as infillings of cells. In the lower sample of the same deposit, the nature of the fine material is largely masked by a crystalline component (see Section 3.3.6), and it is most clearly observed where it occurs as large aggregates of homogeneous limpid material. In the pellet deposit, the fine organic material is mainly dark brown, with a weak stipple-speckled b-fabric, and it is orange-brown in only part of some pellets. The concentration or density of fine material is commonly higher in the outer part of the pellets (Plate II, 3, see also Plate I, 1). In both deposits, fluorescence under blue light is lacking or very weak for the fine organic material, which is also the case for the plant remains. SEM/EDS analysis confirmed that this fine material is not phosphatic.

A major difference between both deposits is that the pellet deposit contains a much lower relative amount of fine organic material than does the massive deposit. In the pellet deposit, this results in a structure with a large packing void volume. In contrast, the structure of the other deposit is massive but fragmented. It is least fragmented in the lower sample, whose macropore volume, which is nevertheless significant, includes partially deformed channels. In both samples of the massive deposit, no pellet structure or large excrement features were recognised, at the scale of the thin section. One possible exception are porous aggregates in the lower sample.

3.3.3. Phytoliths

Within the plant remains, crystals with high birefringence commonly occupy part of the cells, especially in the outer part. These phytoliths, which are known to survive digestion (Shahack-Gross 2011), are found in both deposits inside various morphological types of plant remains (Plate I, 2 and Plate II, 4). Clusters of similar crystals, with the same size and morphology as those in the plant remains, also occur within the fine organic material (Plate II, 5 In the massive deposit, they typically occur in close association with the plant remains (Plate I, 1), and they are more dispersed in the pellet deposit. Based on XRD analyses, the compound is identified as whewellite ($Ca(C_2O_4).H_2O$), which is compatible with its optical properties. The pellet deposit also contains clusters of stacked opal phytoliths within the tissue of one plant remain (Plate II, 6). A similar aggregate is recognised for the massive organic deposit, outside the plant fragments (Plate I, 3).

3.3.4. Calcite spherulites

Both samples of the massive deposit contain small to large zones with abundant spherulitic calcite aggregates of variable size, marked by sharp extinction lines (Plate I, 4). Aggregates of this type commonly form within the intestines of animals, particularly herbivores (Canti 1999; Shahack-Gross 2011). At Qurta, the spherulites are generally dispersed in the fine organic groundmass, but their occurrence is locally limited to the outer part of dense fragments. The same type of spherulitic aggregates is absent from the pellet deposit.

3.3.5. Detrital mineral grains

Both deposits contain silt- to sand-sized detrital mineral grains, which are mostly clustered rather than uniformly distributed. In the massive deposit, the detrital grains mainly occur within the fine organic groundmass (Plate I, 5), whereas in the pellet deposit, these grains typically occur as clusters without admixture of fine organic material. In the upper sample of the massive deposit, the detrital mineral content varies between parallel vertical lenses or bands. The same sample also includes a wedge-shaped sand-rich structure with a darker micromass than the main part of the deposit. In both deposits, the detrital fraction is characterised by a large relative amount of components other than quartz, including hornblende, biotite, glauconite and calcite grains (Plate I, 5), which is typical of the local Late Pleistocene Nile deposits. The massive deposit also contains several small sandstone fragments, which are quartz-dominated and show partial cementation by sparitic calcite or opaque material, as in the Nubian Sandstone formation that is exposed along the local escarpment.

3.3.6. Other mineral components

The basal sample of the massive deposit contains a large quantity of a compound that occurs as wide, elongated crystals, partly in the

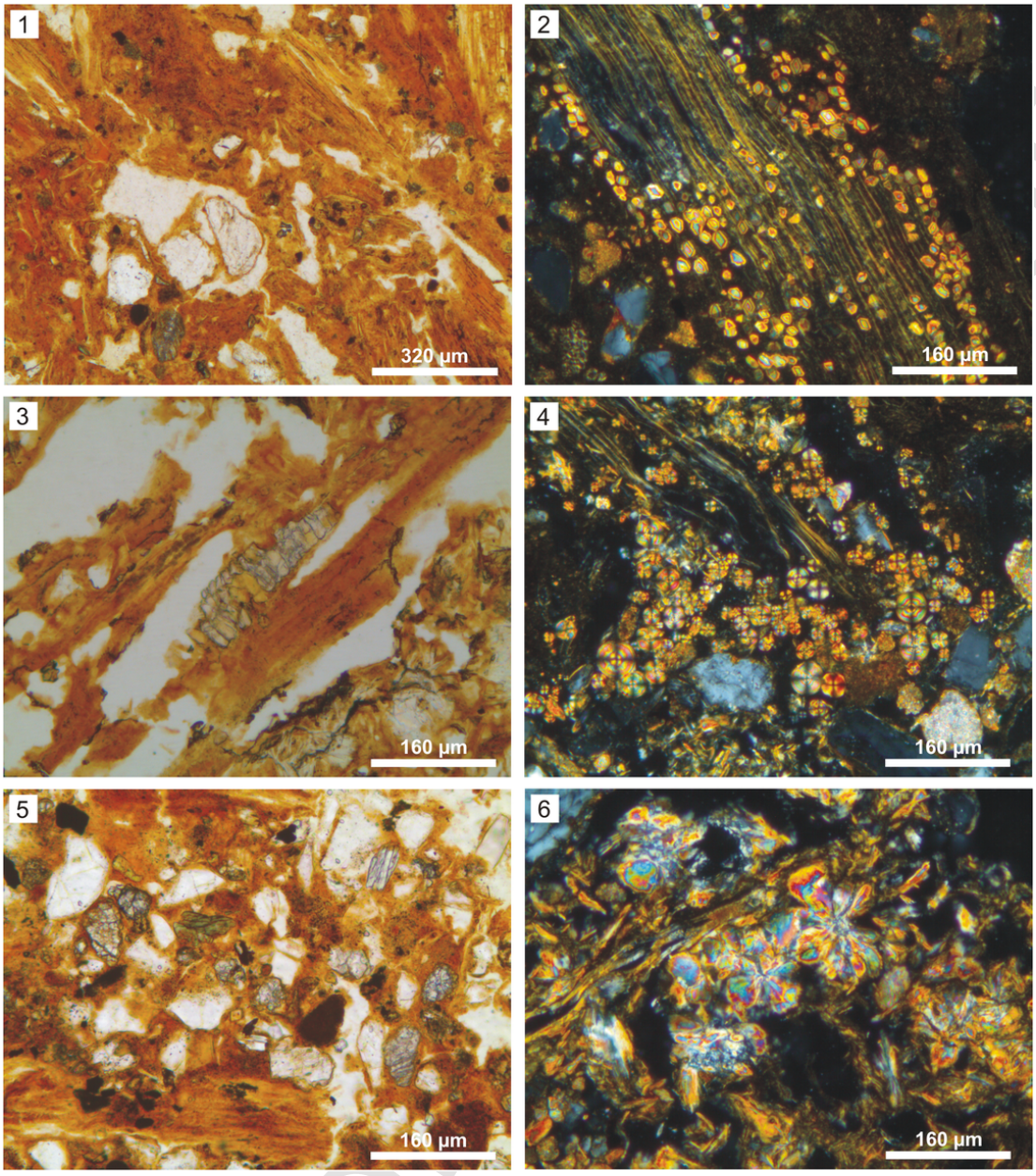

Plate I. Micromorphological features of the massive deposit (QII.4.3). (1) elongated plant remains and fine organic material (plane-polarised light, PPL), (2) oxalate phytoliths inside plant cell remains (cross-polarised light, XPL), (3) cluster of opal phytoliths (PPL), (4) spherulitic aggregates (XPL), (5) sand admixture with common components other than quartz (PPL), (6) radial aggregates of wide elongated crystals of an unidentified compound (XPL).

form of fan-like or radial intergrowths (Plate I, 6). It occurs mainly within the fine organic groundmass, in varying concentrations, locally with great purity along the sides of plant remains or sand grains. No occurrences associated with macropores are recognised, in the form of coatings, infillings or impregnative zones. The compound locally occurs inside plant remains, without a size or form that is imposed by cell morphology. Based on the results of XRD analysis, SEM/EDS analysis, and chemical analysis of extracts, combined with optical properties, the compound could not be identified. It seems to be a Mg- and Na-rich mineral, which may be either inorganic or organic.

3.4. Plant macrofossil analysis

The massive deposit contained no pollen suitable for further analysis. Only few palynomorphs were found and all showed signs of corrosion or degradation. The macrofossils preserved in this deposit were represented only by broadly distinctive vegetative plant parts, such as stems of herbal vegetation, small woody fragments (< 2 mm) or lignified plant tissue fragments possibly originating from mechanical plant tissues of perennial herbs. Those fragments most probably correspond to the plant tissues with dense fibrous texture (Plate I, 1) observed in the micromorphological sections of the same deposit. A big portion of the organic matter showed a high degree of mineralisation, which prevented more precise identification of the plant macro-

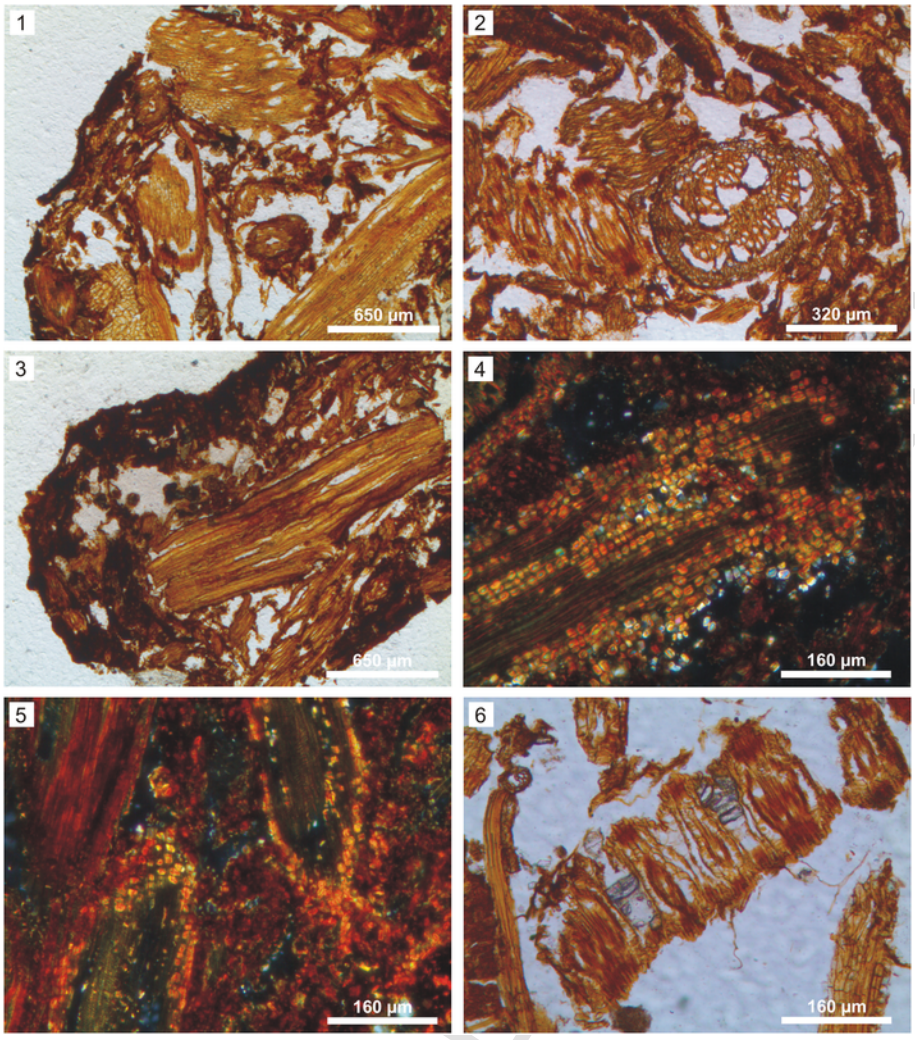

Plate II. Micromorphological features of the faecal pellet accumulation (QII.4.2). (1) pellet with abundant well-preserved plant remains and large pore volume (PPL), (2) plant remain with bimodal cell size distribution (PPL), (3) pellet with high concentration of fine organic material in the outer part (PPL), (4) oxalate phytoliths inside plant remains (XPL), (5) oxalate phytoliths in outer part of plant remains and within fine organic material (XPL), (6) plant remain with groups of opal phytoliths (PPL).

fossils. Therefore, the deposit was not further studied. None of the pellets from the accumulation showed any satisfactory pollen preservation either. In contrast, all of the studied samples (25 in total) contained numerous well-preserved plant macrofossils (Plate III). Most of the plant material consists of lignified stems of herbaceous plants. The absolute number of identifiable fragments per pellet is listed in Table 1. Unidentifiable plant tissue remains were not quantified. The seed/fruit remains were fully quantified. A considerable number of pellets showed rather abundant wetland indicators, especially fruits of fringe-rush (*Fimbristylis bisumbellata*) (Plate III, 1), bristle club-rush (*Isolepis setacea*) (Plate III, 2), damascisa (*Glinus* cf. *lotoides*) (Plate III, 3), Nile peppercress (*Coronopus* cf. *niloticus*) (Plate III, 4) and aquatic buttercup (*Ranunculus* subg. *Batrachium*) (Plate III, 5). Stem fragments of grasses (Poaceae) and a few grains of what is probably savanna grass (cf. *Pennisetum*) (Plate III, 6) were found. These plant species are consistent with an open desert vegetation. The small unidentifiable seeds (Plate III, 7), relatively common in some of the pellets, probably belong to this group as well. Woodland is also represented in a few pellets and comprises thorns and leafs of acacia (*Vachellia/Senagalia*) (Plates III, 8 and 9) and leaves or upper branches of tamarisk (*Tamarix* sp.) (Plate III, 10). Such branches could be one of the sources of woody tissues in the coprolites. In many pellets, vegetative parts from the Asteraceae family make up a considerable part of the macrofossil content (Plate III, 11). Inflorescences attached to some of them made identification possible.

3.5. Lipid biomarker analysis

In both samples, apolar lipids (e.g., alkanols, alkanes and steroids) made up a minor fraction of the extracted lipids, viz. about 7% for the massive deposit and 9% for the pellets. Conversely, polar lipids (e.g., aliphatic acids, phenolic compounds, benzoic acids and N-containing aromatic acids) were much more abundant, making up the major part of the lipid extracts of both samples. The aliphatic lipid and steroid profiles are displayed in Fig. 5. Chromatograms of both samples are displayed in Fig. 6, and a list of all identified lipids, along with retention and quantitative data, is given in supplementary Table S1. A summary of all diagnostic lipids is provided in Table 2. The results of the individual lipid biomarkers are discussed in detail in the following sections.

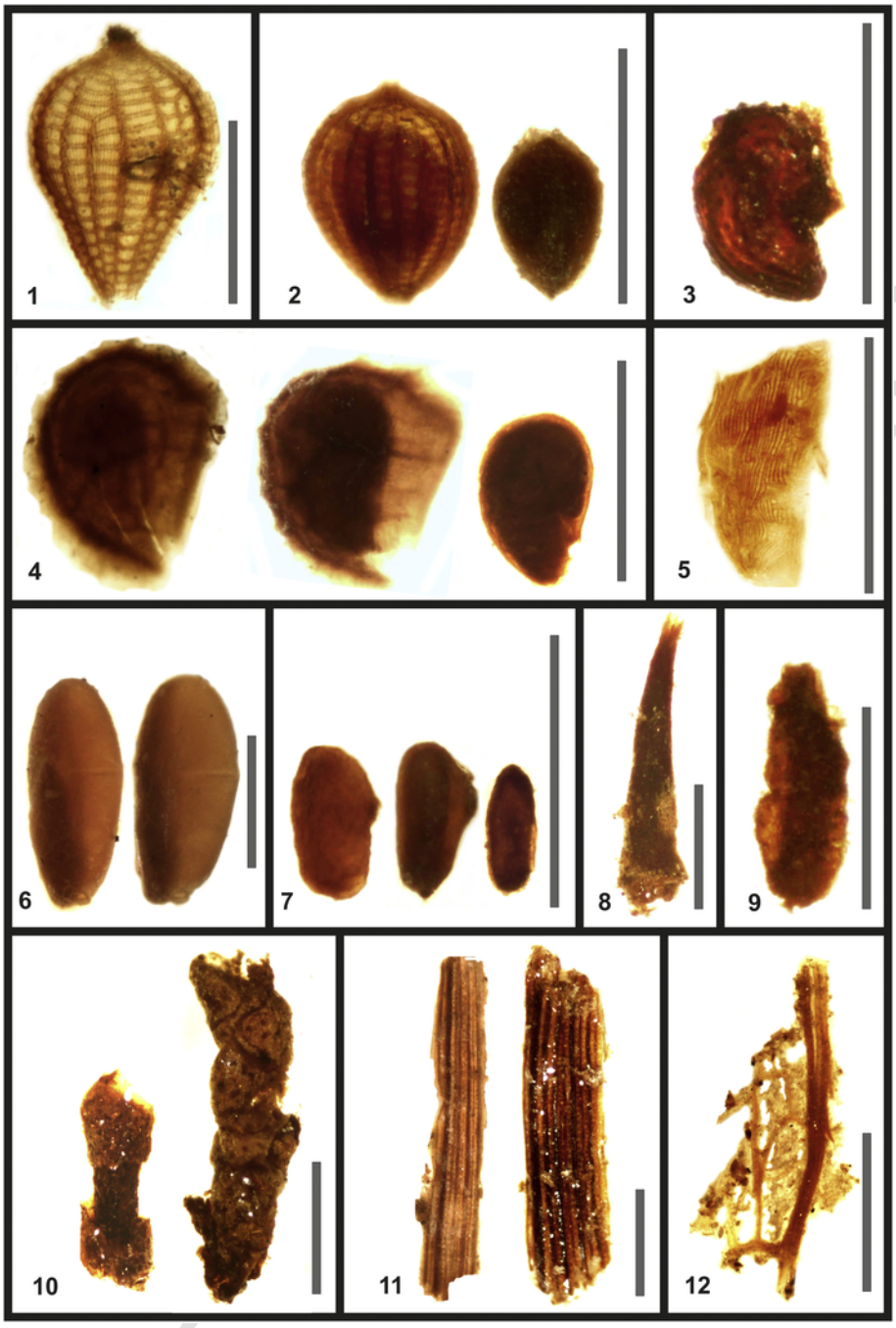

Plate III. Some of most typical plant macrofossils found in pellets from the faecal pellet accumulation (QII.4.2) (scale 1 mm). (1) *Fimbristylis bisumbellata*, nutlet; (2) *Isolepis setacea*, nutlet and seed; (3) *Glinus* cf. *lotoides*, fruit; (4) *Coronopus* cf. *niloticus*, fruits and seed; (5) *Ranunculus* subg. *Batrachium*, seed fragment; (6) cf. *Pennisetum*, grain; (7) seed indet. Type 1; (8) *Acacia s. l.*, thorn; (9) *Acacia s. l.*, leaf; (10) *Tamarix*, twigs; (11) Asteraceae, stem fragment; (12) leaf fragment indet.

3.5.1. Short- and medium-chain aliphatic lipids

Various short- (C_2–C_6) and medium- (C_6–C_{12}) chain acids – both unsubstituted and hydroxylated – were detected in both samples (cf. Table S1 polar compounds). Examples are malonic acid, succinic acid, suberic acid, lactic acid, malic acid and tricarballyic acid. Most of these acids are not source-specific. They may, for instance, represent metabolites of biochemical pathways in aerobic organisms. Hence, they are commonly found in faeces or urine (Tuchman et al. 1984; Verner 1988). Furthermore, they may also derive from plant tissues or be synthesised or transformed by intestinal microorganisms (Table 2). Tricarballylic acid, for instance, is known to be synthesised from *trans*-aconitic acid, a constituent of various grasses, by intestinal micro-organisms in ruminants and rats (Russell and Forsberg 1986; McDevitt and Goldman 1991). Striking is the relatively high concentration of these acids, as they are highly soluble in water and hence prone to leaching. Perhaps their preservation was facilitated by xeric conditions or by the formation of calcium salts, which are generally less soluble in water.

Table 1
Results of the macrobotanical analysis of selected pellets from the faecal pellet accumulation. Each column represents one faecal pellet. An asterisk indicates the largest specimens, which probably were generated by Barbary sheep. Numbers are absolute counts of remains, + stands for present in cases where the remains were difficult to quantify.

Main vegetation type	Wetland vegetation											(Dry) herbaceous vegetation											Acacia stands		
Dung pellet number	D1	D2	D6*	D10	D11	D12	D13	D15	D20	D23	D25	D3	D4*	D5	D7	D8	D9	D17	D18	D19	D21	D24	D14	D16	D22
Vachellia/Senagalia (thorn)																							5	3	2
Vachellia/Senagalia (leaf)																							2		1
Tamarix (leaf)	7	2	11	3		5	9		7					3							1			5	6
Fimbristyllis bisumbellata (nutlet)	24	4	13			6	2		12	17													8	3	
Isolepsis setacea (nutlet)				5	4			19			4			1			1								
Isolepsis setacea (seed)				9	7			15						2			3								
Batrachium sp. (nutlet)	1		2	1			3	6	1															1	
Rumex sp. (seed)	1	2	5		2		1			2							4								
Coronopus cf. *niloticus* (fruit)	9	4	1					5	2	3															
Coronopus cf. *niloticus* (seed)	2							1	8	9	1														
Glinus cf. *lotoides* (fruit)	2	1	4	2	1		4		2	1													2		
Cyperaceae (nutlet)	6		7		4	1			3	1	1			5									14		
Cyperaceae (stem)	4	2	5					3	5								4								
cf. *Pennisetum* (caryopsis)					1							1		2			1					1			
Poaceae indet (caryopsis)									1												2				
Poaceae (stem)	6								11		2	16	3	9	3			4	7	5	8	7			12
Aizoon sp. (seed)				1			2							2		3					3				
cf. *Zygophyllum* (fruit)												5	8			7	2								
cf. *Pulicaria* sp. (fruit)						1							5		1		1				2				
cf. Asteraceae (stem)				2	8	6	13					16	24	11	6	8	15	12	18	23	14	15		6	14
Asteraceae (stem + inflorescence)						1						4	7		4			2	1	2					2
cf. leaf nerves									1					1		4									
wood fragment indet	2		1					4										1					2	4	
seed indet type 1						1		1					2	3		1	1		1						
charcoal fragments	+	+		+				+							+						+	+	+	+	+

A significant difference between the two samples is the overall lower concentration of short- and medium-chain organic acids in the pellets (Table S1, e.g., malonic acid, succinic acid, lactic acid, malic acid, etc.) and the presence of xyloisosaccharinic and glucoisosaccharinic acids in the base-treated pellets only (Table S1, e.g., 3-deoxypentonic acids, 3-deoxypentonolactones, etc.). The latter are major degradation products of 4-O-substituted polysaccharides treated with strong bases (Hänninen and Niemelä, 1992; Knill and Kennedy 2003). Xyloisosaccharinic acids derive from 1 → 4 linked pentosans, such as xylan, whereas glucoisosaccharinic acids originate from hexosans, such as cellulose or starch (Table 2). Xylan and cellulose are structural polymers in the cell walls of higher plants, while starch serves as a form of energy storage in plants. The latter is probably a rather unlikely source, because starch is more rapidly digested than other polysaccharides by most animals (Hespell 1988; Pedersen et al. 2015).

3.5.2. Long-chain aliphatic lipids

Long- (C_{12}–C_{20}) and very long- (C_{21}–C_{30}) chain compounds such as alkanes, alkanols and alkanoic acids, were present in both samples (Fig. 5.1, 5.3–5.4). These are ubiquitous components in vascular plant and animal tissues. In addition, a small amount of branched and odd-chain acids – including mainly phytanic acid and *iso* and *anteiso* isomers C_{15} and C_{17} fatty acids – indicate a contribution of microbial fats (Harwood and Russell 1984; Ratledge and Wilkinson 1988).

A series of linear C_{12}–C_{23} methyl ketones and the isoprenoid 6,10,14-trimethylpentadecan-2-one are only observed in the pellets (Fig. 5.2). The carbon number range of the linear ketones indicates that they are derived from fatty acids (Ten Haven et al., 1989; Rogge et al. 2007; Ogihara 2008). The isoprenoid ketone, on the other hand, most likely derives from the microbial reworking of phytol (Table 2) (Ten Haven et al., 1989; Rontani and Volkman 2003).

Very long-chain alkanoic acids, alkanols and alkanes were also present in both samples (Fig. 5). These acids and alkanols derive from plant leaf waxes, as evidenced by their high carbon preference index and high average chain length (Table 2) (Kolattukudy 1980; Bush and McInerney 2013). A series of C_{16}–C_{26} α-hydroxyalkanoic acids, only present in the pellets, also derive from leaf waxes (Table 2) (de Leeuw et al. 1995).

3.5.3. Cutin and suberin acids

Cutin and suberin acids, which constitute a special category of C_{12}–C_{26} hydroxylated alkanoic and alkanedioic acids, were present in both samples (Table S1, Fig. S1). They were 2–3 times more abundant in the pellets than in the massive deposit. Cutin and suberin are both structural plant polymers, but they are found in different plant tissues: cutin in roots, barks and underground storage organs and suberin in non-woody leaf cuticles, flowers and fruits (Kolattukudy 1980; Walton 1990). The suberin/cutin ratio, determined following Otto and Simpson (2006) (Table 3), is 0.13 for the massive deposit and 0.09 for the pellets, indicating that in both cases the animal that produced the excrement had a clear dietary preference for plant leaves instead of woody plant parts. Even if they are more prone to digestion, leaf remains (Plate III, 9 and 12) were also recognised in the macrobotanical record in small amounts (Table 1).

While suberin acids exhibit a fairly uniform composition among botanical classes, cutin acids are less uniform and hence more informative about the plant source. The absence of ω-hydroxy C_{14} acid in both samples, for instance, indicates that the percentage of lower plants and gymnosperms (except Araucariales and Taxaceae) is very low or zero (Goñi and Hedges 1990; Otto and Simpson 2006). A plot of *x*,ω-dihydroxy C_{16} acid vs. 9,10,ω-trihydroxy C_{18} acid provides a fairly good distinction between tissues from gymnosperms, monocotyledonous and dicotyledonous angiosperms (Goñi and Hedges 1990). Comparison of the two coprolite deposits with reference plants from the literature (Fig. 7a) shows that both samples fall within the category of the dicots. This conclusion is further substantiated by a plot of the 10,ω vs. 9,ω-dihydroxy C_{16} acid isomers (Fig. 7b) (Goñi and Hedges 1990), showing that the cutin acids derive from dicots or from species from the Araucariales or Cupressales order. (See Fig. 8.)

3.5.4. Steroids and terpenoids

Among the steroids, 5β-stanols, such as coprostanol and 5β-stigmastanol and their corresponding 3α-epimers, dominate in samples from both deposits (Fig. 5a, b). These stanols are products of the microbial sterol reduction in the intestines of animals and are considered to be faecal biomarkers (Bull et al. 2002; Linseele et al. 2013). The predominance among these 5β-stanols of C_{29} 5β-stanols indicates that the excrement derives from a herbivorous animal (Bull et al. 2002; Linseele et al. 2013). In the pellets, the additional presence of a series of Δ^7-sterols, including lathosterol, spinasterol and stigmast-7-enol, is indicative for another type of dietary sterol input, because the Δ^7 double bond is not reduced in the intestines (MacDonald et al., 1983). These Δ^7-sterols are dominant in plants from the Caryophyllales order (Moreau et al. 2002), which contains many xero- and halotolerant species, as well as highly adaptable weeds found in waste deposits (Salt et al. 1991). In our macrobotanical record there are several taxa (e.g., *Tamarix, Aizoon* sp.) from this order (Table 1).

Terpenoid compounds were also retrieved from both samples. These reflect a dietary input of higher plants. The massive deposit contained only germanicol and lupanol, while the pellets also contained sclareoloxide, germacrene and β-amyrin.

3.5.5. Aromatic compounds

Aromatic compounds make up a considerable portion of the lipid extracts (see Table S1 for full details). The majority of these aromatic compounds were detected in the saponified residue only, indicating that they were chemically bound via ester linkages.

Substituted benzoic acids (e.g., vanillic acid and syringic acid) and cinnamic acids (e.g., coumaric acid and ferulic acid) were present in both deposits, along with their benzaldehyde and acetophenone derivatives. All of these aromatic compounds are likely derived from such plant tissues as lignin (e.g., after microbial lignolytic activity; Rabinovich et al. 2004), suberin (Kolattukudy 1980) or arabinoxylans (Belitz et al. 2009).

The massive deposit contained very high amounts of benzoic acid, hippuric acid and their corresponding *m*- and *p*-hydroxy derivatives, as well as a number of hydroxylated C_8 and C_9 phenylalkanoic acids (e.g., hydroxyphenylacetic acid) (Fig. 6, Table 2, Table S1). These compounds most likely derive from dietary polyphenols, such as flavanols or proanthocyanidins (Rechner et al. 2002; Manach et al. 2005; van Dorsten et al. 2010). Upon ingestion, polyphenols are transformed by colonic microbiota into simpler phenolic acids; after absorption, they are further metabolised to hippuric acid and its hydroxylated derivatives, which are excreted via the urine (van Dorsten et al. 2010). Polyphenols and hippuric acid are the major end-products of quinic acid breakdown (Adamson et al. 1970) and amino acid metabolism (viz. phenylalanine and tyrosine; Roowi et al. 2010). But unlike polyphenols, hippuric acid is not found in any dietary or bacterial source; it only occurs in urine from humans and animals (Bristow et al., 1992). Therefore, its presence provides clear evidence for urine in the massive deposit. Hippuric acid was not detected in the pellets.

The massive deposit also contained metabolites of another amino acid, namely tryptophane. These include nitrogen-containing aromates, such as quinolinic acid, kynurenic acid, picolinic acid and

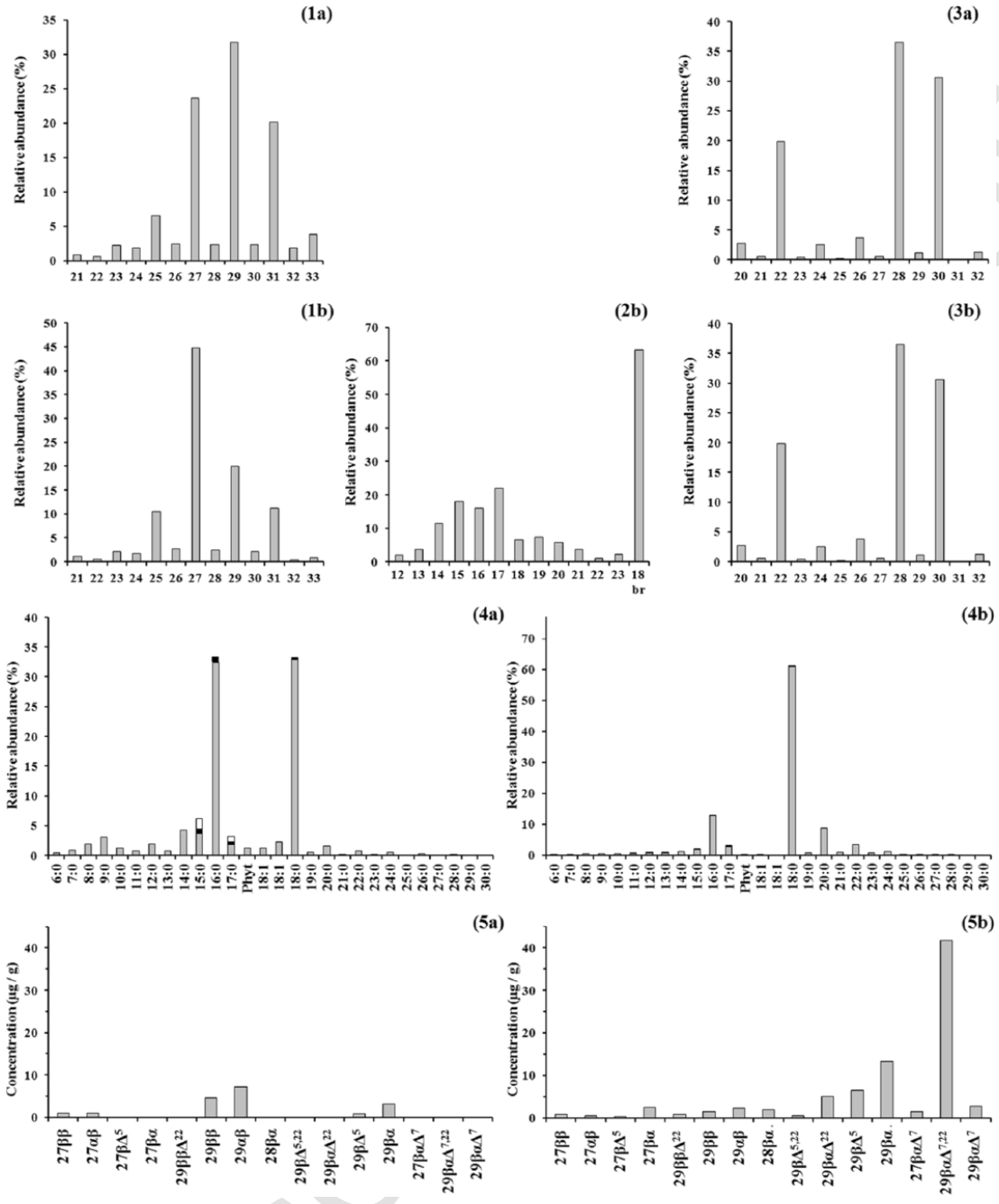

Fig. 5. Profiles of aliphatic and steroid lipids, viz. (1) *n*-alkanes, (2) methyl ketones, (3) *n*-alkanols, (4) fatty acids and (5) steroids of (a) the massive organic deposit (QII.4.3) and (b) the faecal pellet accumulation (QII.4.2). Alkanes, methyl ketones and alkanols are abbreviated by their carbon numbers (18br = 6,10,14-trimethylpentadecan-2-one). Fatty acids are abbreviated by carbon number: number of unsaturations. *Anteiso*, *iso* and straight-chain isomers are depicted in white, black and grey stacked columns, respectively (phyt = phytanic acid). Sterols are abbreviated by their carbon number, their steric configuration at C3 and C5 and an indication of double bond positions: 27αβ = coprostanol; 27αβ = epicoprostanol; $27\beta\Delta^5$ = cholesterol; 27βα = 5α-cholestanol; $29\beta\beta\Delta^{22}$ = 5β-stigmast-22-enol; 29ββ = 5β-stigmastanol; 29αβ = epi-5β-stigmastanol; 28βα = 5α-campestanol; $29\beta\Delta^{5,22}$ = stigmasterol; $29\beta\alpha\Delta^{22}$ = 5α-stigmast-22-enol; $29\beta\Delta^5$ = sitosterol; $27\beta\alpha\Delta^7$ = lathosterol; $29\beta\alpha\Delta^{7,22}$ = spinasterol; $29\beta\alpha\Delta^7$ = stigmast-7-enol. Absolute concentrations of aliphatic compounds are given in Table S1.

nicotinic acid, which are all metabolites of the kynurenin pathway (Stone and Darlington 2002). Both samples contained dipicolinic acid. This N-containing compound is unique to bacterial endospores, which are usually formed by Gram-positive bacteria (Lomstein et al. 2012), and is thus indicative of the action of gastrointestinal bacteria. In the massive deposit, there is further evidence for these bacteria in the form of enterolactone, which is formed from plant lignans by a consortium of intestinal bacteria (Lampe et al. 2006).

3.6. Integration of all lines of evidence

In this study, we adopted multiple approaches to gain detailed insight into the content of two coprolite deposits at the rock art site of Qurta, a massive latrine deposit (QII.4.3) and a faecal pellet accumulation (QII.4.2). Radiocarbon dating demonstrates that both deposits are older than 45 ka and thus in all probability are from the Upper

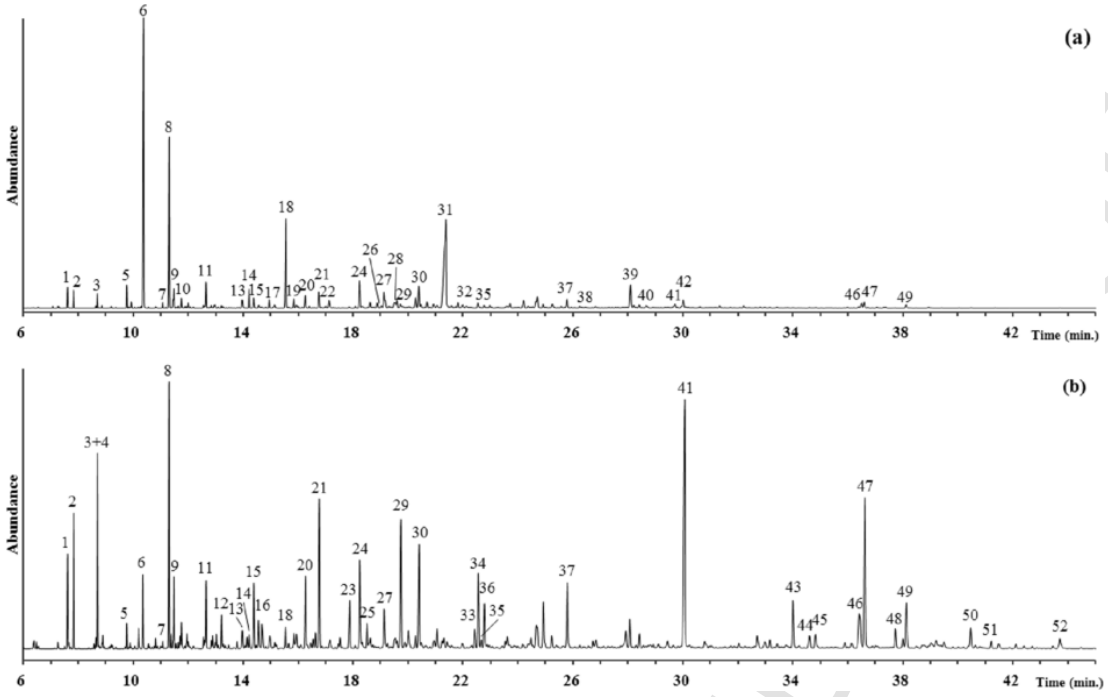

Fig. 6. GC–MS chromatograms (total ion counts) of the polar fraction of (a) the massive organic deposit (QII.4.3) and (b) the faecal pellet accumulation (QII.4.2). A list of all detected compounds along with corresponding retention indices and quantitative data can be found in the Supplementary Table S1. Legend to peak labels: 1 lactic acid, 2 glycolic acid, 3 oxalic acid, 4 furan-2-carboxylic acid, 5 malonic acid, 6 benzoic acid, 7 phenylacetic acid, 8 succinic acid, 9 2-methylsuccinic acid, 10 fumaric acid, 11 glutaric acid, 12 dihydroxybutyric acid, 13 mandelic acid, 14 malic acid, 15 adipic acid, 16 *o*-hydroxybenzoic acid, 17 methyladipic acid, 18 *m*-hydroxybenzoic acid, 19 2-hydroxyglutaric acid, 20 pimelic acid, 21 *p*-hydroxybenzoic acid, 22 2,5-furandicarboxylic acid, 23 3-deoxy-D-*threo*-pentonic acid, 24 suberic acid, 25 α-glucosaccharinic acid, lactone, 26 quinolinic acid, 27 tricarballylic acid, 28 orotic acid, 29 vanillic acid, 30 azelaic acid, 31 hippuric acid, 32 *m*-coumaric acid, 33 β-glucoisosaccharinic acid, 34 sebacic acid, 35 α-glucoisosaccharinic acid, 36 syringic acid, 37 C16:0 acid, 38 kynurenic acid, 39 *m*-hydroxyhippuric acid, 40 vanilpyruvic acid, 41 *p*-hydroxyhippuric acid, 42 C18:0 acid, 43 C20:0 acid, 44 hydroxy-C14:0 diacid, 45 dihydroxy-C15:0 acid, 46 hydroxy-C15:0 diacid, 47 dihydroxy-C16:0 acid, 48 C22:0 acid, 49 hydroxy-C16:0-diacid, 50 hydroxy-C22:0 acid, 51 C24:0 acid, 52 hydroxy-C24:0 acid.

Pleistocene, predating the Late Pleistocene rock art (Huyge et al. 2011). Despite their old date, the coprolites exhibit extraordinary preservation of the organic content. The results of all single lines of evidence (micromorphological features, plant remains and lipid biomarkers), together with contextual information, will now be integrated to address our first two research aims, (1) to verify the biogenic source of the massive organic deposit and the faecal pellet accumulation; (2) to examine the dietary components of both materials and relate this information to the palaeoenvironment through the palaeovegetation record preserved in the deposits.

3.6.1. Differences between the two coprolite finds

Whereas micromorphological features (viz. phytholiths and spherulites) and lipid biomarkers (viz. 5β-stanols) provide clear evidence that both deposits are composed of animal excrements (Shahack-Gross 2011; Baeten et al. 2012), the deposits are, overall, strongly dissimilar in macromorphological, micromorphological and molecular composition. The pellet deposit contains a larger relative amount of plant remains, with a notably better state of preservation, whereas it contains a much lower concentration of fine organic material. The massive deposit, on the other hand, contains hardly any pellet structures (and none at all in the petrographical samples) and showed a rather high degree of mineralisation, which prevented further identification of the plant macrofossils. The massive deposit contained a large quantity of an unidentified mineral in the basal part, where it had most likely accumulated through downward percolation not long after its initial deposition. Another significant difference is that only the massive deposit contains calcite spherulites. Their absence in the pellet deposit may be explained by the fact that spherulites are produced in different quantities by different herbivores (e.g., cows produce low amounts or none; Canti 1997). In view of the presence of whewellite and urinary acids, the absence of spherulites cannot be explained by post-depositional dissolution. Oxalate and opal phytoliths occur in both deposits, which suggests some similarity in food sources. The oxalate phytoliths are more scattered in the pellet deposit than in the massive deposit, where they also are more closely associated with the plant remains. This difference probably relates not only to post-depositional processes but also to a different mode of mastication and digestion.

Differences between the two coprolite deposits are also observed in their molecular composition. The most pronounced difference is the presence of urinary biomarkers in the massive deposit only, of which hippuric acid is the most diagnostic. Other animal metabolites found in the massive organic deposit are also compatible with urine. These include breakdown products of polyphenols and amino acids. Another significant difference is the presence of methyl ketones in the pellet deposit only. As these compounds are most likely formed from fatty acids by intestinal microorganisms, they are indicative of either the gut flora or the digestive system. The presence of enterolactone, a product of lignan metabolism, present in the massive deposit only, can be explained in the same way.

The two deposits thus clearly stem from different species but the radiocarbon dates of both are very similar. Most likely this is merely coincidental.

3.6.2. Species identification

Our initial, macroscopic assessment of the well-preserved pellets in the pellet accumulation left little doubt that they had been pro-

Table 2
Summary of lipid biomarkers and their diagnostics for samples of the massive organic deposit and the faecal pellet accumulation.

Compound category	Compounds/parameters	Diagnostics/possible origins	QII.4.3[b] org. deposit	QII.4.2[b] pellets
1. Short and medium chain aliphatic lipids	Unsubtituted/hydroxylated acids (lactic acid, malic acid, fumaric acid, ...)	Animal metabolites, plant tissues, microbial transformation products	+++	++
	C_5 and C_6 isosaccharinic acids	Polysaccharide plant polymers (e.g., xylan, cellulose)		++
2. Long-chain aliphatic lipids	$C_{12–20}$ even-numbered acids	Animal or plant tissues	++	+++
	Phytanic acid, $C_{15–17}$ odd-numbered acids	Microbial source	++	+
	Linear (branched) methyl ketones	Microbial degradation of fatty acids (phytol)		+
	$C_{20–32}$ alkanols, $C_{21–33}$ alkanes (high CPI, ACL)[a], $C_{21–30}$ acids and $C_{16–26}$ α-hydroxy acids[c]	Leaf wax from higher plants	+	+
3. Cutin and suberin acids	Low cutin/suberin ratio (see Table 3)	Dietary preference for plant leaves instead of woody plant parts	++	++
	Cutin source parameters (see Fig. 7)	Dicotyledonous angiosperms, Cupressales, Araucariales	++	++
4. Steroids and terpenoids	5β-stanols (predominance of C_{29} stanols)	Faecal biomarkers (herbivores)	+	+
	Δ^7-sterols	Caryophyllales (xero- and halotolerant plant species)		+
	Di- and triterpenoids (β-amyrin, lupeol, …)	Tissues from higher plants	+	+
5 Aromatic compounds	Benzoic acids (e.g., vanillic, syringic acid) and hydroxycinnamic acids (coumaric, ferulic acid)	Degraded plant tissues, fruits/vegetables, arabinoxylans	++++	+++
	Hippuric acid	Urine biomarker (end-product of polyphenol and amino acid metabolism)	++++	
	C_8 and C_9 hydroxyphenylalkanoic acids	Polyphenol metabolism	++	
	quinolinic, kynurenic and picolinic acid	Tryptophane metabolism	++	
	dipicolinic acid	Bacterial endospores	++	+
	Enterolactone	Gastrointestinal bacteria (lignan metabolism)	+	

[a] CPI = carbon preference index (mass. deposit: 9.3; pellets: 10.7), ACL = average chain length (mass. deposit: 28.6; pellets: 27.7).

[b] The presence/absence is indicated as follows: + (0–100 μg g^{-1}), ++ (100–1000 μg g^{-1}), +++ (1000–10,000 μg g^{-1}), ++++ (> 10,000 μg g^{-1}).

[c] α-hydroxy fatty acids were only present in the pellets (QII.4.2).

Table 3
Sum of suberin and cutin monomers (in μg g^{-1}) and suberin/cutin ratio.

Suberin and cutin parameters[a]	QII.4.3 org. deposit	QII.4.2 pellets
Suberin (ΣS)	0	74
Cutin (ΣC)	1648	4745
Suberin or cutin (ΣS^{v}C)	244	409
Suberin/cutin ratio	0.13	0.09

[a] Sum parameters – based on Otto and Simpson (2006) – were as follows: ΣS = C_{20}–C_{26} α,ω-alkanedioic acids + C_{20}–C_{24} ω-hydroxyalkanoic acids; ΣC = C_{12}–C_{17} x,ω-dihydroxyalkanoic acids + C_{11}–C_{18} x-hydroxy-α,ω-alkanedioic acids; Σ(S^{v}C) = C_{18} x,ω-dihydroxyalkanoic acid + C_{16}, C_{18} α,ω-alkanedioic acids + C_{16}, C_{18} ω-hydroxyalkanoic acids; suberin / cutin ratio = (ΣS + ΣS^{v}C)/(ΣC + ΣS^{v}C).

duced by small bovids. The attribution was subsequently supported by micromorphological features present, such as phytoliths, and a predominance of plant-derived faecal biomarkers (viz. C_{29} 5β-stanols), which are both indicative of herbivores. The plant macrofossil content exhibited a low frequency of woody plant remains and an overall dominance of dicots (flowering plants). Furthermore, the presence of cutin and suberin biomarkers confirms that the source animal had consumed almost exclusively non-woody plant parts (e.g., leaves, flowers and fruits). The cutin appeared to derive from dicots rather than from monocots (grasses) or gymnosperms (non-flowering plants). Therefore, we may assume that the source animal is most likely a browser, such as Dorcas gazelle, ibex or Barbary sheep (Osborn and Helmy 1980). It is not excluded that more than one of these species is represented.

Our initial, macroscopic assessment of the massive organic deposit was less conclusive and suggested that it consisted of faeces left by rock hyrax, which is also an herbivorous animal. This attribution was subsequently confirmed by micromorphological features (e.g., phytoliths and spherulites) and faecal 5β-stanols with a clear predominance of C_{29} over C_{27}. Only mammals are able to reduce dietary sterols to 5β-stanols, thus excluding excrement from birds or cold-blooded animals (Leeming et al. 1996). Bile acids or bile alcohols, which might provide additional clues (Linseele et al. 2013), were not detected. Cutin and suberin biomarkers show that the source animal had preferentially consumed non-woody plant parts and was most likely a browser. The co-occurrence of urinary and faecal biomarkers in the massive deposit suggests that this sample constitutes a communal latrine or midden, narrowing the options to antelope and hyrax, both of which are known to use latrines (Fiorelli et al. 2013). But because only hyraxes are known to defecate on rock faces (Scott 1996), we could rule out antelope. Rock hyraxes form middens in places where colonies of these animals urinate and defecate over a long period of time (Osborn and Helmy 1980, p. 465; Chase et al. 2012). Poor preservation of plant macrofossils, such as observed in the massive organic deposit at Qurta, is typical for hyrax middens (Chase et al. 2012). Furthermore, the absence of urea in this sample is consistent with recycling of urea by herbivores in arid zones, a feature that has also been observed for hyraxes (Hume et al. 1980; Hume, 1999). The presence of urine may explain the unidentified Mg- and Na-bearing crystalline compound that is present in the lower part of the massive deposit. New minerals may form in faecal deposits depending on ion availability and pH (Shahack-Gross, 2011). In this case, magnesium could possibly derive from animal urine (Shahack-Gross et al. 2003). The few pellets found in the deposit are smaller than those of current hyraxes and we suspect that degradation processes affected their size.

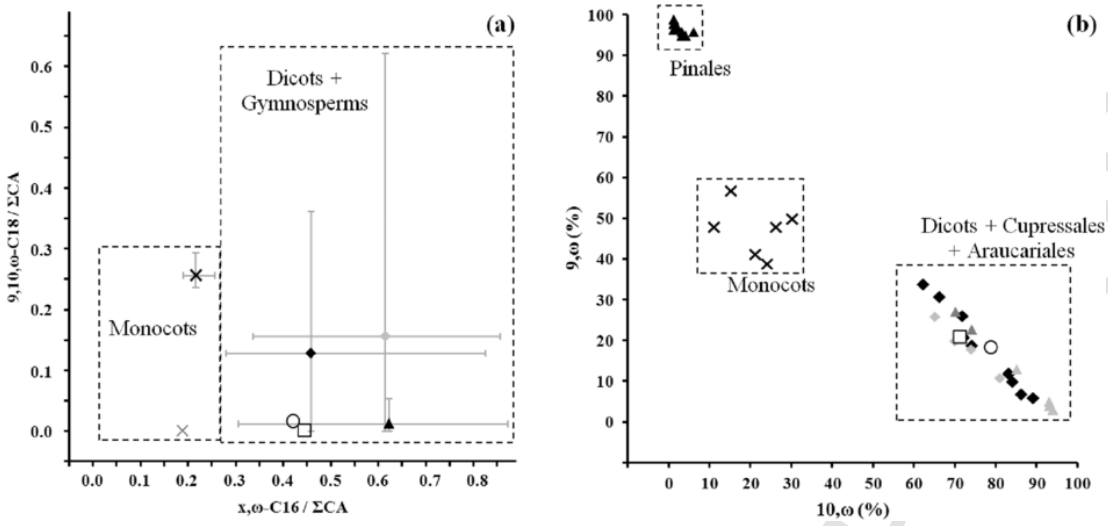

Fig. 7. Plots of cutin source parameters: (a) ratio of 9,10,ω-trihydroxy C_{18} acid/ΣCA over x,ω-dihydroxy-C_{16} acid/ΣCA with ΣCA the sum of all cutin acids (i.e., C_{14}-C_{18} ω-hydroxy acids, α,ω-dioic acids, mid-chain hydroxy acids and diacids), (b) ratio of 9,ω-dihydroxy-$C_{16:0}$ vs. 10,ω-dihydroxy-$C_{16:0}$. Included in these plots are the samples QII.4.3 (○) and QII.4.2 (□), together with references values for gymnosperms from the order of the Pinales (▲), gymnosperms from the order of the Araucariales (▲), gymnosperms from the order of the Cupressales (▲), temperate monocots (×), tropical monocots (×), temperate dicots (◆), tropical dicots (◆) (references values were taken from Goñi and Hedges 1990, and Otto and Simpson 2006).

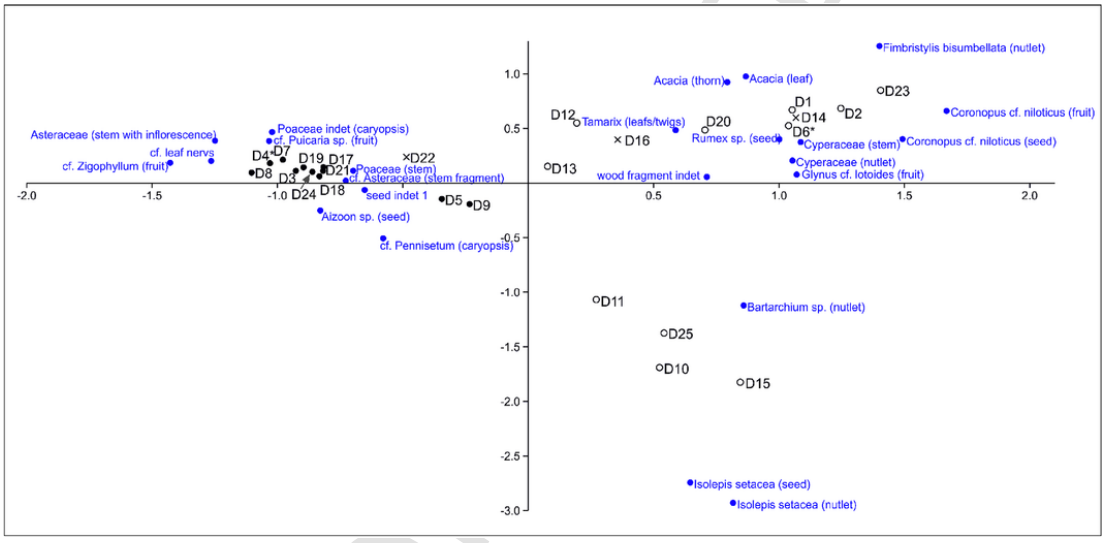

Fig. 8. Results of the correspondence analysis of the quantified palaeobotanical data of the pellets from the faecal pellet accumulation in Qurta (see Table 1) (PAST, Hammer et al. 2001). (black dots = samples containing open vegetation indicators; white dots = samples containing wetland indicators; crosses = samples containing acacia remains).

3.6.3. Palaeodietary and palaeoenvironmental significance

Plant macrofossils and biomolecular evidence preserved in faecal remains serve not only as evidence of the animals' diet, but also as indicators of the major vegetation types. However, the diet of a given animal is not a direct reflection of the environment. Specific dietary preferences, nutritional needs or seasonality affect what is ingested (cf. Carrión et al. 2005; Ghosh et al. 2008; Lewis, 2011), and the passage of plant foods through the intestines is associated with digestive alterations or losses. Studies on the endozoochorous dispersal of plants have pointed out that seeds of a wide variety of taxa can survive passage through the gut of livestock animals and that most of these seeds emerge around 1.5 to 3 days after ingestion (Janzen 1982; Manzano et al. 2005). However, in an experimental study on sheep (Wallace and Charles 2013), significant biases in the composition of plant remains in excrements were observed: plant parts larger than 2 mm are unlikely to remain intact unless they are protected by a highly resistant coat. This may explain why remains of seeds and fruits in the faecal pellets from Qurta are generally smaller than 2 mm, since other small bovids should have similar digestion systems to sheep. Vegetative plant remains in the pellets also tend to be smaller than 2 mm and are generally associated with a lesser taxonomic resolution than the fruit and seed remains (Table 1). These size-related biases in the macrofossils have to be taken into account during interpretation.

The macrobotanical content of the pellets could be grouped according to the predominant habitat. Correspondence analysis revealed two main groups, one with species indicative for wetland vegetation, the vicinity of a water basin or a shore, and the other with species typical for a dry, open, herbaceous vegetation (Fig. 11). These were

the most common environments accessible to the small wild bovids. The dominance of wetland vegetation in about half of the studied pellets indicates that this habitat constituted an important feeding area. The occurrence of *Tamarix* leaves, some still attached to twigs (Fig. 7j), together with the wetland indicators, suggests that *Tamarix* shrubs preferentially grew in a wet environment. This area could well correspond to the fertile banks of the Nile, which may have been attractive as a feeding area for the small bovids because they could find shelter in the rocky hinterland. The overall vegetation during the period, however, was probably open and rather arid. This argument is substantiated by the rarity of finds of wood tissue fragments in the pellets (most probably originating from twigs), suggesting a scarcity of woodland or wooded shrubs suitable for browsing. Nevertheless, woody vegetation was not totally absent, as shown by the presence of leaves and thorns of *Acacia s .l*. Indications for aridity were also detected by lipid biomarker analysis, namely through the presence of Δ^7-sterols in the pellet deposit. These steroids are biomarkers for plant species from the Caryophyllales order, comprising xero- and halotolerant species (Moreau et al. 2002). Plant species from this order were also represented in the macrofossil content, namely *Tamarix* sp., *Rumex* sp. and *Aizoon* sp., making up roughly 20% of all identified plant species. The macrobotanical and biomarker evidence, combined with the radiocarbon dates, thus indicates that the pellets are likely from MIS 3 or 4.

4. General implications and concluding remarks

4.1. Determining the origins of ancient coprolites

Ancient coprolites provide a rich source of information with regard to diverse issues, such as palaeodiet, palaeoenvironment and palaeoclimate. However, a prerequisite for understanding their palaeoecological significance is to know their biological source, because the botanical content of coprolites – whether characterised by visual inspection or by biomarker analysis – may be biased by the animal's selective feeding, seasonal behaviour or digestive losses.

Species identification of the animal that produced the coprolites is not always straightforward. Coprolites can be described according to a standard system based on external macroscopic features and their content (Jouy-Avantin et al. 2003). Clearly, morphometric analyses constitute a starting point for identification (e.g., Chame 2003; Linseele et al. 2010; Riemer, 2011). These were helpful in identifying the faecal pellet deposit from Qurta (viz. gazelle, ibex, Barbary sheep) but failed to establish the source of the massive latrine deposit. Our results demonstrate that, in such cases, analysis of the organic fraction can yield additional evidence (see also Linseele et al. 2013). For instance, biomarkers for the plant polymers cutin and suberin in both deposits show a dietary preference for leafy parts of plants other than grasses. These results correspond well with the macrobotanical data for the pellets. Unfortunately, it was not possible to assess the correspondence between biomarkers and macrofossils for the massive latrine deposit, because plant remains were poorly preserved in that deposit. This illustrates that botanical studies, despite their ability to identify dietary or vegetation components, are limited to coprolites in which macrofossils are well preserved. Biomolecular analyses, on the other hand, may circumvent this problem by targeting the entire organic fraction, including the amorphous organic groundmass. In this way, it is possible to identify dietary components that are not represented in the botanical remains or that are not recognisable due to intense mastication or digestion processes. A disadvantage of lipid biomarkers, however, is their rather low specificity. Continued identification of novel compounds, analysis of reference substances and degradation experiments (e.g., Gill et al. 2010; Correa-Ascencio et al. 2014) may improve their diagnostic utility in the future.

4.2. Paleoenvironmental indications from the coprolites

Plant remains and biochemical residues preserved in the studied coprolites suggest that the animals that produced them, browsed and grazed in a rather arid environment dominated by sparse herbs and occasionally single trees. Further also wetland and water plants provide information on the diversity of habitats accessible for those animals.

4.3. A novel biomarker for urine

The present study identified hippuric acid as a novel biomarker. This compound is not found in any dietary or bacterial source; it occurs only in urine from humans and animals (Bristow et al. 1992). Indications for this compound, viz. abundant benzamide signals, were already reported by Carr et al. (2010) for ancient hyrax middens. In our study, the urine metabolite was detected as the original glycine-benzoate conjugate. Furthermore, its particular co-occurrence with faecal 5β-stanols provides unambiguous evidence for an animal latrine. Such middens have the ability to preserve well in hot and dry environments, where the urine rapidly evaporates and thereby seals in the majority of the associated organic matter (Chase et al. 2009).

4.4. The preservation of lipid biomarkers and analytical protocols

Another noteworthy finding of this study is the fact that the lipid biomarkers in the coprolites exhibit an excellent state of preservation given their pre-Holocene age. Lipid preservation in coprolites is highly variable, and is related to age, mode of preservation and diagenetic history. It was already known that desiccated faeces contain lipid concentrations one order of magnitude greater than those recovered from lithified coprolites (Gill 2011). Our research confirms that arid climates indeed provide suitable conditions for lipid biomarker preservation (see also Romanus et al. 2008). Differences may also be expected depending on the analytical protocols. In our study, we adopted two sample preparation techniques which are common to coprolite research but to our knowledge have never been applied together, namely classical Soxhlet extraction and extraction after base treatment (also known as saponification). With the saponification treatment, the extraction yield was twice that of the Soxhlet treatment, demonstrating that a significant portion of the lipids were chemically bound. Base treatment thus releases compounds which would otherwise not be identified, such as the constituents of structural plant polymers (e.g., cutin) or polysaccharides. The downside of base treatment is that the harsh conditions of the base treatment alter the original chemical composition (cf. Correa-Ascencio and Evershed 2014). Therefore, a classical solvent extraction provides a basis of comparison to identify artefacts of the saponification analytical methodology (e.g., hydrolysis of wax esters). Our results thus demonstrate that it is advisable to perform both preparation techniques.

4.5. Molecular composition of the organic fraction of coprolites

This study also highlights an underexplored source of information in coprolite research, namely the molecular composition of the organic fraction. To our knowledge, too few researchers have investigated the detailed characterisation of organic compounds. Notable attempts include studies by Hollocher et al. (2001), who discovered bacterial signatures in Cretaceous dinosaur coprolites, and Carr et al.

(2010), who characterised the organic fraction of Holocene hyrax middens. Also noteworthy are studies by Gill and co-workers that focused on specific plant sapogenins (Gill et al. 2009) and on biomarkers for foregut fermentation (Gill et al. 2010). Other coprolite studies are mainly limited to faecal steroid biomarkers (e.g., Sistiaga et al. 2014a, 2014b). Instead of focusing on specific compounds, we extracted all solvent-extractable compounds and explored their possible diagnostic values. This led to the following three discoveries: hippuric acid is a urine biomarker, cutin and suberin acids allow us to distinguish between woody and leafy diets and to assess the consumption of grasses, and Δ^7-sterols appear to be useful as biomarkers for xero- or halotolerant plant species.

4.6. The strength of multidisciplinary studies

Coprolite research is strengthened by the combination of multiple strands of evidence (e.g., Reinhard and Bryant, 1992). The Qurta coprolite study shows that molecular data can be better understood through a combination of micromorphological and botanical evidence. Together, these three strands yielded evidence that we would otherwise not have been able to access, leading to a more complete understanding of the research objects and provides the most informative conclusions. We have thus shown the efficiency of the particular multidisciplinary approach we have adopted (see research aim 3). We therefore encourage further interdisciplinary coprolite research.

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Uncited references

Huyge and Vandenberghe, 2011
Quick et al., 2011

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