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ORIGINAL ARTICLE

Multi-proxy analysis of waterlogged preserved Late Neolithic canine excrements

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Abstract

Multi-proxy analysis of the coprolites which were found during excavations at two Late Neolithic (fourth millennium BC) pile-dwelling sites (Črnelnik and Stare gmajne) in Slovenia yielded some new insights into human–dog relations and behaviour. The digested content is presented in a multidisciplinary approach, in which palynological, palaeoparasitological, archaeobotanical and archaeozoological features are studied and genetic signs are tested. Beside the origin of the coprolites, the size of an animal and the diet, the faeces provided some additional information, such as health, status, nutrition habits, environment and season.

Keywords Dog coprolites · Multi-proxy analysis · Late Neolithic · Pile-dwellings · Slovenia

Introduction

Recent excavations at two Late Neolithic pile-dwelling sites (Črnelnik and Stare gmajne) in Slovenia yielded sub-fossil excrements, i.e. coprolites. They were from dog (*Canis familiaris*) or humans and are excellently preserved (waterlogged). Entirely preserved coprolites are known from exceptional circumstances like mummy intestinal remains, or from pelvic areas (Brönnimann et al. 2017). Desiccated coprolites e.g. from Egypt (Marinova et al. 2013; Wood and Wilmshurst 2016; Baeten et al. 2018) and carbonised as dung pellets, e.g. from Israel and Syria (Smith et al. 2019; Zachary et al. 2019; Landau et al. 2020) are frequently encountered, while completely preserved waterlogged canine or human excrements are to our knowledge, only rarely found (Macphail 2000; Ismail-Meyer and Rentzel 2004; Wood et al. 2016; Brönnimann et al. 2017).

Coprolites are a unique source of diverse information, they offer the potential to gain a wide range of insights into certain aspects of biology, ecology and archaeology (Wood and Wilmshurst 2016). They constitute palaeodietary records and information on the behaviour of prehistoric humans or animals (Reinhard and Bryant 1992; Wood and Wilmshurst 2016). The origin of a coprolite is initially determined by morphology (outer structure and shape) and the size of the excrement. Dog excrement appears very similar to that of humans or pigs, but the inner composition differs considerably. It was found that numerous digested bone fragments are commonly preserved in dog coprolites (Brönnimann et al. 2017), mainly fish bone fragments, while human coprolites mainly contain plant remains, especially cereals (Byrne 1973; Tolar and Galik 2019). The coprolite from the site Črnelnik (Figs. 1 and 2a; Velušček et al. 2018) had already been analysed and

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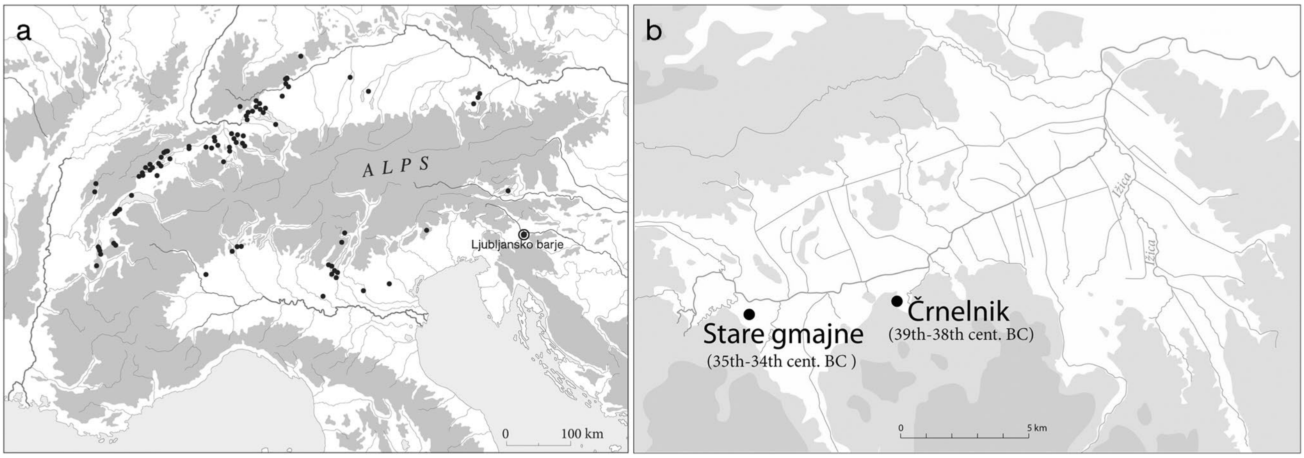

Fig. 1 **a** Circum-Alpine pile-dwellings with the most SE region—Ljubljansko barje in Slovenia. **b** Ljubljansko barje region with two Late Neolithic sites: Črnelnik and Stare gmajne

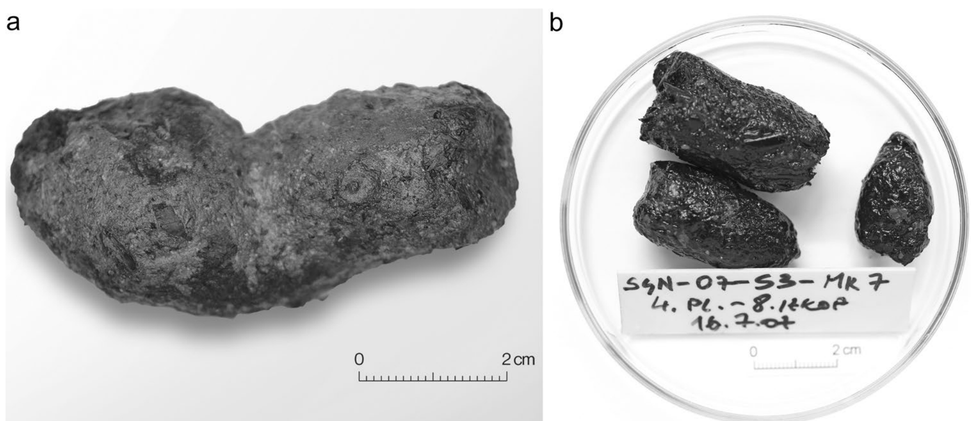

Fig. 2 **a** Coprolite from Črnelnik site and **b** examples of coprolites from Stare gmajne site

gave interesting results. It contained many flat fish skull bones and isolated pharyngeal teeth and scales of Cyprinidae, which permitted the determination of its origin, i.e. from a dog, and the conclusion that the individual had eaten at least one or two meals which consisted only of fish heads (Tolar and Galik 2019). Macro- and microremains preserved in coprolites can give additional information, especially if they are preserved in a waterlogged state (Akeret and Jacomet 1997; Akeret et al. 1999; Kühn et al. 2013; Tolar and Galik 2019). Preserved seeds and pollen can suggest ecological conditions as well as the deposit period (and consequently the settling period); organic refuse management and the culture of fish-eating may be indicated as well. The micro-remains, for example parasites, can give additional information about the state of health (Reinhard et al. 2013; Le Bailly and Araújo 2016), while successful isolation and sequencing of DNA from fossilized faeces may yield information on the genome as well as the diet of the individual (Bon et al. 2012) and consequently its kinship with others.

As the analysis of a single dog coprolite from the Črnelnik site (Fig. 2a) gave encouraging results (Tolar and Galik 2019), the extended analyses (with original results) of another six items (Fig. 2b) of a similar shape, structure and preservation state from another fourth millennium BC pile dwelling-site in Slovenia (Stare gmajne, Fig. 1), are presented in this study. A further aim of this study is to combine and compare the main findings from both sites. The specific research questions are: 1. the coprolites' canine/human identity (i.e. origin); 2. the size of the animal/s; 3. the coprolite content (plant and animal macroremains, pollen, parasites and DNA); 4. similarities/dissimilarities between the coprolites from different sites; and 5. possible other information on health, status, nutrition habits, environment, season and kinship.

Materials and methods

Sixteen dog or human coprolites were found at the Late Neolithic Stare gmajne site at Ljubljansko barje, Slovenia, in the year 2007 (Figs. 1 and 2b). They were recovered during small excavations, where a trench of 15 m^2 was excavated (Velušček 2009). The detailed analyses of six of them (samples 1–6, Table 1) is presented in this article. Each coprolite had been preserved through waterlogging and before analysis was stored at 4 °C (Fig. 2b).

The coprolite from the Črnelnik site (Fig. 2a) had been macro-analysed previously. Only plant and animal macro-remains were analysed, the results being already published in Tolar and Galik (2019). Five possible means of data extraction were employed when analysing coprolites from Stare gmajne site, i.e. plant and animal macro-remains (MR), pollen, parasites and DNA extraction. Before analysing and destroying the coprolites, all were photographed, described and measured (Table 1).

Macro-remains

Each coprolite sample (nos. 1, 2, 4, 5; Table 1) was separately gently disaggregated and washed through a 0.056 mm mesh sieve. The macro-remains caught on the sieve were sorted using a Leica MZ75 stereomicroscope at up to ×50 magnification and were identified with the aid of the reference collection of plant and animal macro-remains of the Institute of Archaeology ZRC SAZU, Austrian Archaeological Institute ÖAW, and Faculty of Natural Sciences and Mathematics, University of Maribor. Identification atlases and keys were also used: Schmid 1972; Vigne 1995; Cohen and Serjeantson 1996; Granadeiro and Silva 2000; Cappers et al. 2006.

Palynology

Pollen analysis was carried out on coprolite sample 1. One cm^3 subsample from the interior of the coprolite was extracted for standard pollen analysis procedure (7% HCl, 10% NaOH, 40% HF, acetolysis, staining with safranine, mounting in silicone oil; Bennett and Willis 2002). To determine the pollen concentration one *Lycopodium* tablet (with 20,848 spores) was added (Stockmarr 1971). A Nikon Eclipse E400 light microscope at ×400 magnification was used for pollen determination. Identification was performed using the reference collection of the Institute of Archaeology ZRC SAZU, as well as keys and atlases (Moore et al. 1991; Reille 1992, 1995).

Palaeoparasitology

Parasite remains were extracted from coprolite samples 1, 5 and 6. This followed the three step RHM method, including rehydration in aqueous solution of 0.5% Tri-Sodium Phosphate (TSP) and 5% glycerinated water, homogenization and microsieving stages (Dufour and Le Bailly 2013). This method allows the extraction of the dissemination forms of digestive parasites, mainly the eggs of worms, such as roundworms, flatworms or acanthocephalans. Parasite egg size varies between 30 and 160 µm in length, and between 15 and 90 µm in width (Ash and Orihel 2007). Prepared samples were studied using a light microscope at the Chrono-environment laboratory in Besançon, France. Eggs were identified through their morphological and morphometrical traits, as well as the possible presence of ornamentation on the eggshell.

Table 1 Descriptions of the researched coprolites from Stare gmajne site and analyses performed

Sample	Material	Weight (g)	Measurements (cm)	Fraction for MR (ml)	Analyses
1	1 whole	n.d	n.d	10	Zoology, botany, palynology, parasitology
2	2 smaller whole	n.d	n.d	20	Zoology, botany
3	1 whole and 1 half	n.d	4.7–2.5–1.5 2.6–1.7–1.5	n.d.	DNA
4	2 halves	10.63	2.5–2–1.5 3–2.3–1.8	5	Zoology, botany
5	1 whole	15.73	4.5–2.5–1.5	3	Zoology, botany, parasitology
6	1 whole	10.78	4–2–1.2	n.d.	Parasitology

n.d. no data. *Measurements* length – max. width – min. width

aDNA extraction

aDNA isolation testing was carried out on coprolite sample no. 3. To avoid possible cross-contamination, laboratory work was conducted in an isolated room in a dedicated aDNA laboratory. All surfaces in the lab were routinely double wiped with bleach and rinsed with absolute ethanol. All consumables, disposables, tools and instruments were externally bleached and UV irradiated before entering the lab and then subjected to routine cleaning before, during and after use.

After removing the surface of the sample, the central part of the coprolite was used for aDNA extraction with the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) and with the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions, with some slight modifications. The subsample was extracted in triplicate.

DNeasy PowerSoil Kit

First we added 35 mg of the coprolite subsample to the provided PowerBead Tubes and vortexed it gently. The additional steps followed the manufacturer's protocol. At the end we re-suspended the samples in 50 µl of TE buffer with 0.05% Tween 20 and stored them at -20 °C until amplification.

QIAamp fast DNA Stool Mini Kit

We weighed 200 mg of the coprolite subsample into a 2 ml microcentrifuge tube and added 1 ml InhibitEX buffer to the sample. We vortexed the sample until it was thoroughly homogenized and then performed additional steps following the protocol. We re-suspended the sample in 100 µl of buffer ATE and stored it at -20 °C until amplification. Measurement of aDNA concentration and amplification is described in ESM1.

Results

The coprolites from Stare gmajne (Table 1) were whole or fragmented (mostly halves; Fig. 2b, Table 1), very similar in shape, structure and morphology, 10–15 g in weight, 2.5–4.5 cm in length and around 2 cm maximum width. Therefore, it would appear that they belonged to a same-sized group of individuals, maybe even to the same animal/person, since they were found within 3 m^2 in the 15 m^2 excavated trench (Velušček 2009). DNA isolation testing was performed to try and solve that question.

Plant and animal macro-remains

Plant and animal macro-remains in four of the analysed coprolites indicate minor consumption of plant species (cereals, flax; Table 2) and major consumption of small vertebrate species (mostly fishes; fewer birds and small mammals; Table 3). The preservation of macroremains was mostly non-carbonised (i.e. waterlogged) except for cereals and charcoal fragments.

Fish remains

The coprolite samples (1, 2, 4 and 5) from Stare gmajne yielded 150 identifiable fish remains in total. The remains were scattered amongst the samples (i.e. coprolites) and vary from 5 remains in sample no. 4 up to 121 fish remains in sample no. 2 (Table 4).

About half of the fragmented material is only recognisable as fish remains. The species distribution of fishes clearly shows cyprinids with only a few identifiable species (see Table 4). A small common carp (*Cyprinus carpio*) is represented by a second vertebra. Other cyprinids are identified by more or less fragmented pharyngeal bones of chub/dace (*Leuciscus* sp.) and roach (*Rutilus rutilus*). Besides a mid-sized roach all the others represent small specimens (Fig. 3). European perch (*Perca fluviatilis*) is represented by typical ctenoid scales and a caudal vertebra. All the bones indicate rather small individuals (Fig. 3). The more abundant Northern pike (*Esox lucius*) remains consist of 7 isolated teeth, a single vertebra and several other cranial elements such as an epihyale, a palatinum and a hyomandibulare (Table 4).

In general the fish remains from Stare gmajne coprolites stem from mainly small and some very small individuals. Few Northern pike and a roach represent larger but only medium-sized individuals (Fig. 3). Most of the *corpus vertebrae* depth measurements are less than 3.5 mm and indicate fishes, which were certainly smaller than 10 cm (Fig. 4). The

Table 2 Plant macroremains (charcoal, fruits, seeds) in four coprolites (sample nos. 1, 2, 4 and 5) from Stare gmajne

Sample	1	2	4	5
Fraction volume (ml)	10	20	5	3
Cerealia		1 frg. C		
Linum usitatissimum			2 frg. NC	
Chenopodium album	2 NC	5 NC		
Schoenoplectus lacustris		5 NC		
Charcoal	3 frg	3 frg	6 frg	3 frg

C carbonised, *NC* non carbonised, *frg.* fragment

Table 3 Animal macroremains (bones, scales, teeth) in four coprolites (sample nos. 1, 2, 4 and 5) from Stare gmajne

Sample	1	2	4	5
Fraction volume (ml)	10	20	5	3
Fish pharyngeal teeth (Cyprinid)	3	10		
Pharyngeal bones (Cyprinid)		2		
Fish scales		x NI		
Fin rays		x NI		1 NI
Fish vertebrae	Cyprinid	x NI	1 NI	3 (Cyprinid), 1 (perch), 3 NI
Fish ribs		3 NI		
Fish flat bones, cranial elements	Cyprinid	Cyprinid	1 (pike)	2 (Cyprinid)
Postcranial elements (Cyprinid)		22		
Mammal bones	x (WVS)	x (WVS)	x (WVS)	
Coprolite small mammals		1 C		
Bird bones	x (D/CS)	x	x	
Other bone frg		x (?frog)		

NI not identified, *C* carbonised, *WVS* water vole sized, *D/CS* duck/crow sized, *x* numerous fragments

carp vertebra definitely indicates a very young and small fish, while another very small cyprinid vertebra indicates a perennial small sized species.

In general, the skeletal element distribution contains more postcranial remains and fewer cranial remains, besides a few fin rays and scales. Within the cyprinids cranial and serial anatomical elements appear to be more or less balanced. Northern pike presents more cranial elements including isolated teeth while the evidence of European perch is identifiable fish scales and a few postcranial elements (Fig. 5).

Bird and mammal remains

In three of the four analysed coprolite samples from Stare gmajne (nos. 1, 2 and 4) bird bone fragments were identified (Tables 3 and 5). Taxonomic affiliation could not be ascertain. According to the sizes of the bone fragments (mainly the diameters of long tubular bones; Fig. 6) it can be concluded that the body size of the birds is comparable to ducks or crows. In all three samples comparable numbers of fragments were recognized, i.e. from 54 to 66 (Table 5), on average 60 bone fragments that were taxonomically assigned to birds. Anatomically, bone fragments belonged to three groups of bones: skeleton of the thorax (Fig. 6a), long tubular bones (cf. radius or femur) (Fig. 6b, c), and furcula (Fig. 6d).

As to the number of fragments, remnants of appendicular skeleton were predominant (79.9%). Cranial (3.3%) and pelvic bones (1.7%) were also present. 13.8% of the bone fragments could not be anatomically determined (Table 5).

Fragments were also measured. The average length of the fragments was 6.5 ± 3.1 mm and the average width was 2.3 ± 1.3 mm (details in Table 6).

The bone fragments (identified skeletal elements and the estimated body sizes of the birds) recognized from the analysed coprolites are completely in line with available archaeozoological data from the cultural layer of e.g. the Hočevarica site (Janžekovič and Malez 2004).

Due to the pronounced fragmentation of mammal remains a more precise taxonomic identification of these finds was not possible (see Table 3). The size of the fragments indicates water vole sized individuals.

Palynological results

Analysis of the coprolite sample no. 1 reveals something of the vegetation around Stare gmajne Late Neolithic settlement. Altogether 305 pollen grains were counted in the 1 cm^3 subsample (Table 7), the pollen concentration was low (8,893 pollen grains/cm^3). Palynological analysis shows a high number of water-dependent taxa (*Drosera*, *Alnus*, *Salix* and especially a high percentage of Cyperaceae). Furthermore, anthropogenic indicators, like cereals (Cerealia-type) and potentially cultivated plants (*Linum*, *Vitis*) were found. Trees and shrubs comprise around 40% of the pollen counts and herbs and spores represent 60%.

Palaeoparasitological results

Analyses conducted on three coprolites (nos. 1, 5 and 6) from Stare gmajne gave positive results (Table 8). Tapeworm eggs were identified in samples nos. 1 and 5. Eggs measured 55–57 µm in length and 37–41 µm in width. They are ovoid, operculated, and present a small knob on the abopercular side. Egg surfaces present a punctuated

Table 4 Fish taxa and elements recognized in four coprolites (sample nos. 1, 2, 4 and 5) from Stare gmajne

Fish taxa	Element	1	2	4	5
Cyprinidae	Articulare	1			
Cyprinidae	Basioccipitale		2		1
Cyprinidae	Ceratohyale				1
Cyprinidae	Dens	3	8		
Cyprinidae	Hyomandibulare		1		
Cyprinidae	Os pharyng.		2		
Cyprinidae	Pleurale costa		3		
Cyprinidae	Praemaxilla		1		
Cyprinidae	Quadratum		1		
Cyprinidae	Scapula		1		
Cyprinidae	Tripus		3		
Cyprinidae	V.caud.med		1		
Cyprinidae	V.thor		7		1
Cyprinidae	V.thor.1		4		
Cyprinidae	V.thor.2	1	1		
Cyprinidae	V.thor.3		1		1
Cyprinidae	V.ultimat				1
Cyprinus carpio	V.thor.2		1		
Esox lucius	Dens	1	6		
Esox lucius	Epihyale			1	
Esox lucius	Hyomandibulare		1		
Esox lucius	Palatinum		2		
Esox lucius	V.thor	1			
Leuciscus sp.	Os pharyng.		1		
Perca fluviatilis	Scale	5			
Perca fluviatilis	V.caud				1
Pisces	Finray	2	3		1
Pisces	Ind		32		
Pisces	Pleurale costa		6		
Pisces	Scale		1		
Pisces	V.caud.post		1		
Pisces	Vertebra		28	4	3
Rutilus rutilus	Os pharyng.		3		
Sum		14	121	5	10

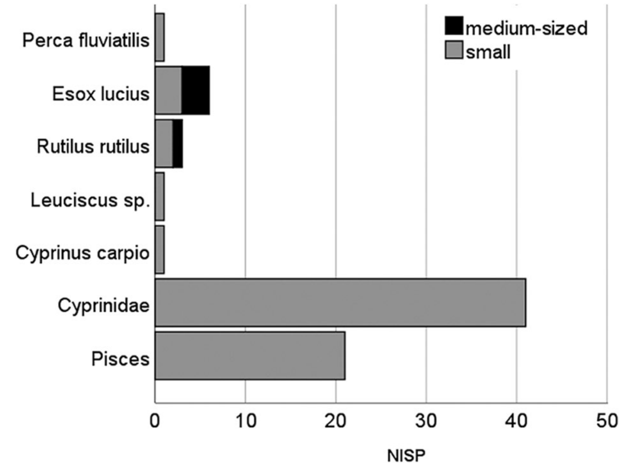

Fig. 3 Qualitative size distribution of fishes in the analysed samples nos. 1, 2, 4 and 5 from Stare gmajne. *NISP* number of identified specimens

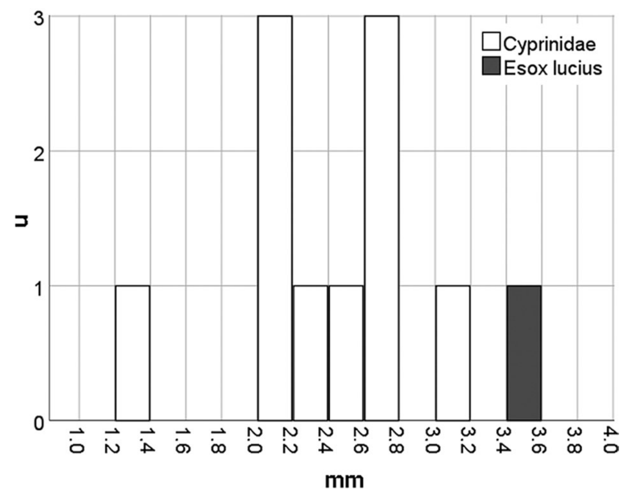

Fig. 4 Size distribution of *corpus vertebrae* depths of fishes, in the analysed samples nos. 1, 2, 4 and 5 from Stare gmajne

ornamentation with a more or less dense pattern (Le Bailly et al. 2005). They could belong to different species of the fish tapeworm, genus *Diphyllobothrium*, or belong to the genus *Spirometra* (Fig. 7a). They are transmitted to mammals through consumption of raw or undercooked fishes for the fish tapeworm, and for *Spirometra* through the meat of various animals, such as rodents, reptiles or mammals, that are intermediate hosts of the parasite larvae (Ash and Orihel 2007; Taylor et al. 2007). Eggs of another tapeworm belonging to the genus *Alaria* were also retrieved from sample no. 5 (Fig. 7b). The eggs are ovoid and operculated, and have a size between 76–84 µm long and 50–54 µm wide. The parasite is particularly present in wild or domestic canids after the ingestion of frogs or toads. Finally, eggs of whipworms, genus *Trichuris*, were identified in samples nos. 5 and 6 (Fig. 7c). The eggs are lemon-shaped with two polar plugs, and vary in size between 55–56 µm long and 26–28 µm wide. Infestation with whipworm occurs when the host ingests food or water polluted with the eggs. All retrieved parasites, particularly the presence of *Alaria* sp., seem to confirm a canid origin for the coprolites.

aDNA results

The DNeasy PowerSoil Kit revealed the best quality and highest quantity of DNA and also fragment amplification in qPCR and PCR. Unfortunately, the sequencing of

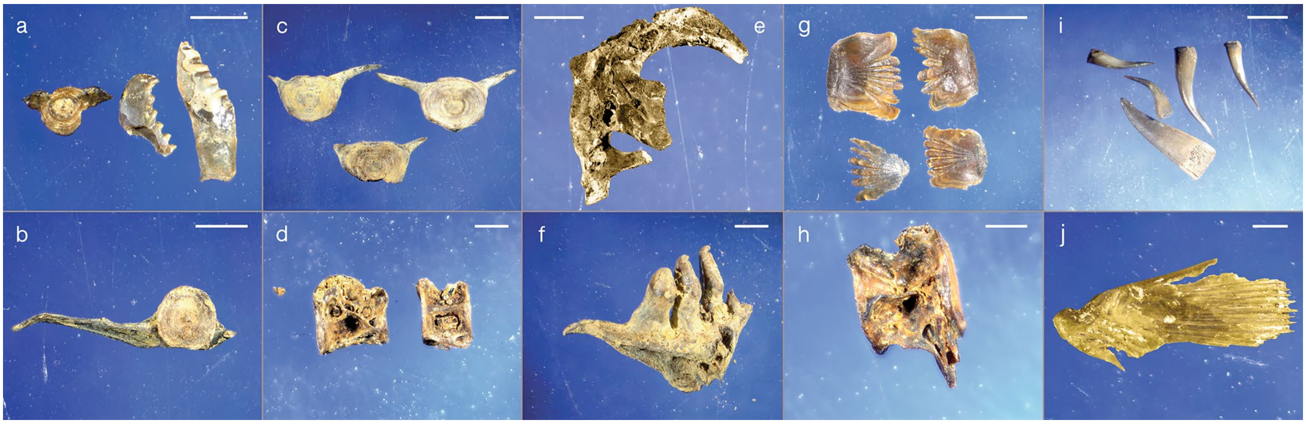

Fig. 5 Some of the fish remains in the investigated coprolites from Stare gmajne: **a–d** Cyprinidae vertebrae and pharyngeal teeth; **e**, **f** *Rutilus rutilus* Os pharyngeale; **g**, **h** *Perca fluviatilis* scales and vertebrae; **i**, **j** *Esox lucius* teeth and epihyale. Scale bars: 2 mm

Table 5 Anatomical regions and abundance of bird bone fragments in the analysed coprolites nos. 1, 2 and 4 from Stare gmajne

Sample	1	2	4	Sum
Appendicular skeleton	64	32	45	141
Thorax skeleton	2	3	1	6
Cranial bones		4	2	6
Pelvic bones		3		3
Not determined		12	13	25
All GRPS	66	54	61	181

Fig. 6 Fragments of bird bones from the coprolites nos. 1, 2 and 4: **a** sternum; **b** epiphysis of long tubular bone; **c** diaphysis of long tubular bone; **d** furcula

amplified fragments in the classical PCR reaction were unsuccessful. The sequencing of fragments revealed by qPCR was successful but due to short fragment length (< 80 bp), we cannot yet form any conclusion about the origin of the coprolite. We need additional fragment amplifications to get a longer consensus sequence.

Discussion

Analyses of coprolites from two Late Neolithic sites confirm that we are dealing with original more than 5,000 years old dog (*C. familiaris*) excrement. Beside mainly bone content (which is fragmented due to chewing), the absence of a larger quantity of vegetable food (characteristic of humans; Byrne 1973) as well as fur or animal hair (characteristic of e.g. wolf; https://www.volkovi.si; Skrbinšek 2010), suggest that we are dealing with dog excrement in both the Črnelnik as well as the Stare gmajne examples. The presence of eggs of two tapeworm and one whipworm genus in all three analysed coprolites from Stare gmajne additionally confirms the canid origin. The sizes (especially the widths of approx. 2 cm; Table 9) of the analysed coprolites from both sites suggest that the pile-dwellers' dogs tended to be medium sized; wolves for example have excrement 2 to 4 cm in width (Skrbinšek 2010). This suggestion is completely in line with available archaeozoological data (e.g. Bartosiewicz 2002).

Beside the question of the origin of the coprolites and the size of dog, one of the research questions of this study was also the content of the coprolites and the similarities/dissimilarities between the two pile-dwelling sites. Details of the Črnelnik site coprolite are published in Tolar and Galik (2019).

At both sites fish remains in the coprolites predominate (Table 9; Tolar and Galik 2019). It is obvious that dogs were fed mainly with small to mid-sized Cyprinids, northern pike and European perch. While the analysis of the coprolite from the Črnelnik site led to the interesting conclusion that the dog was fed only with fish heads (Tolar and Galik 2019), the analyses from the Stare gmajne site do not indicate the same. The skeletal element distribution, especially in coprolite sample no. 2, does not suggest feeding with selected body parts of fish. The skeletal element distribution in the Stare gmajne coprolites numbers more

Table 6 Anatomical region and dimensions of bird bone fragments (mm) in the analysed coprolites nos. 1, 2 and 4 from Stare gmajne (mean ± standard deviation; minimum–maximum)

	Sample 1		Sample 2		Sample 4	
	Length	Width	Length	Width	Length	Width
Appendicular skeleton	5.9 ± 3.4 0.9–16.2	2.3 ± 1.2 0.6–7.7	7.4 ± 2.8 2.7–16.5	2.2 ± 0.9 1.0–5-4	5.9 ± 3.4 3.0–21.3	2.1 ± 1.0 0.6–5.1
Thorax skeleton	11.0 ± 4.8 7.6–14.3	3 ± 0.8 2.4–3.6	9.1 ± 2.5 6.5–11.5	3.6 ± 1.7 2.5–5-5	8.6	2.7
Cranial bones			7.0 ± 1.3 5.5–8.2	3.2 ± 0.6 2.3–3.6	7.0 ± 2 5.6–8.4	3.5 ± 1.2 2.6–4.3
Pelvic bones			10.6 ± 1.0 9.5–11.3	7.4 ± 2.8 4.7–10.2		
Not determined			6.9 ± 1.3 5.1–10.0	1.7 ± 1.4 0.5–4.8	5.5 ± 1.6 3.1–7.9	2.3 ± 1.0 1.1–4.2

Table 7 Identified pollen grains in coprolite sample no. 1 (in 1 cm^3 subsample)

Plant taxa	No. pollen grains
Pinus	4
Picea	1
Betula	3
Fagus	23
Tilia	1
Quercus	26
Carpinus betulus	2
Alnus	21
Ulmus	1
Salix	1
Corylus	38
Cyperaceae	155
Poaceae	2
Cereals	2
Linum	1
Chenopodiaceae	2
Ranunculus sp.	17
Vitis	1
Apiaceae	1
Filicales	2
Drosera	1
Sum	305
Lycopodium (marker)	715

Table 8 Intestinal parasites identified (n) in three coprolite samples (nos. 1, 5 and 6), subsamples of 5 g each

Sample	1	5	6
Volume, weight (g)	5	5	5
Diphyllobothrium/Spirometra sp. eggs	8	4	
Alaria sp. eggs		11	
Trichuris sp. eggs		2	1

postcranial remains and less cranial remains, besides a few fin rays and scales, which is in contrast to the Črnelnik coprolite. These new and however unusual Neolithic fish remains from the coprolites complement the already available data about prehistoric fishing and fish consumption at Slovenian pile-dwellings. Several sites with prehistoric fish remains have been documented at Ljubljansko barje; i.e. at Ig, Hočevarica (Govedič 2004; Križnar and Kovalchuk 2016) and Založnica (Velušček et al. 2011) pile-dwellings. The identified exploited fishes from the cultural layers of these sites are common carp, common rudd (*Scardinius erythrophthalmus*), European perch, northern pike, common roach and the sheatfish (*Silurus glanis*); this is similar to the species distribution in the coprolites from Stare gmajne and Črnelnik sites recorded here. One new fish species was documented in coprolite sample no. 2 from Stare gmajne for the first time at Slovenian pile-dwellings, i.e. chub/dace (*Leuciscus* sp.), which, in contrast to the others, is a typical freshwater fish. There is an obvious size discrepancy between large fishes, recovered from the cultural layers and representing human food debris (e.g. Govedič 2004), and the small to middle-sized fishes of the same species yielded from the dog coprolites. This can be explained in three ways: 1. methodological: by the fact that all fish remains were carefully recovered from the coprolites using fine 0.056 mm mesh sieves (vs. fish remains recovered from the cultural layers at the Hočevarica site, where 1 and 2 mm mesh sieves were used); 2. taphonomical: larger fishes, birds and mammals are more thoroughly chewed by dogs and therefore these bone fragments are less recognizable (vs. small fishes that could just be swallowed whole and the bones preserved more or less completely); and 3. behavioural or habitual: the dogs were fed only with small fishes and leftovers (i.e. fish heads in the case of Črnelnik; Tolar and Galik 2019). In relation to the second, i.e. taphonomical statement, the claim that dogs from Stare gmajne were fed only on small fishes cannot be completely denied. That is

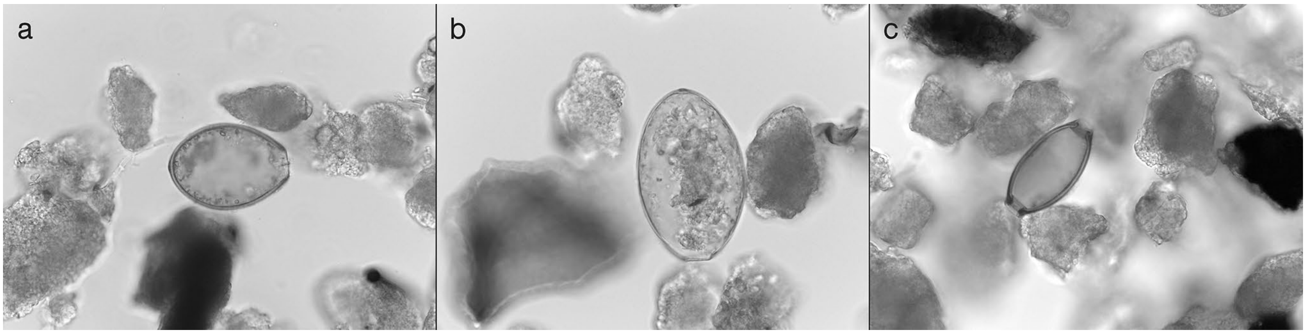

Fig. 7 **a** *Diphyllobothrium/Spirometra* sp. (tapeworm), **b** *Alaria* sp. (tapeworm), **c** *Trichuris* sp. (whipworm)

Table 9 Comparison of the analysed coprolites from the two sites Črnelnik and Stare gmajne. The Stare gmajne column summarizes the results of all six analysed coprolites (see Table 1)

	Črnelnik coprolite	Stare gmajne coprolites
Size (cm)	6–3–2	4.5–2.5–1.2
Deposit period	Late summer–autumn	Early spring
Fish parts	Cranial bones	Trunk and cranial bones
Fish species	Small Cyprinidae (rudd or roach)	Small-medium sized Cyprinidae (roach, chub/dace, carp), Percidae (perch), Esocidae (pike)
Other animal species	n.a	Birds (Passeriformes), smal mammals, frog?
Plant macroremains	Charcoal, flax, fruits/nuts	Charcoal, flax, cereal
Parasites	n.a	Tapeworms and whipworm

Size length – max. width – min. width.; *n.a.* not analysed

to say, although it is known that digested bone fragments constituted only a small portion of the bones consumed, at least some teeth, vertebrae and/or scales (i.e. the most resistant parts) of the larger fishes would have been also preserved and found in the examined coprolites (e.g. Jones 1986; Butler and Schroeder 1998; Russell and Twiss 2017 and references therein). The methodological explanation may have much greater impact on the results. Besides the inappropriate sieve mesh sizes used at the Hočevarica site (when sieving sediments from the cultural layer), there is another very important fact, i.e. the Črnelnik site coprolite was preserved in much better condition when analysed than the Stare gmajne site coprolites, since the latter were found in 2007, while those from the Črnelnik site were recovered seven years later, in 2014. All of them were stored in a refrigerator till the year 2017 when the analyses began (see Fig. 2). Lastly, but also important, is the size (length) of individual faeces, which also varies. In addition to their preservation state, this surely affects the results obtained. For example there is sample no. 2 from Stare gmajne with the largest fraction of organic residues that were obtained on a sieve (20 ml) and therefore the most representative. The fraction from the Črnelnik site was of exactly the same volume (Tolar and Galik 2019). It is necessary therefore to take into account the fact that this study is based on the analyses of 6 coprolite samples from Stare gmajne site and only one, albeit much better preserved, from the Črnelnik site (Table 9, Fig. 8).

Fig. 8 Rare, but well preserved plant macroremains in the coprolite from the Črnelnik site: blackberry, flax, goosefoot, turnip, birch and water chestnut (see Tolar and Galik 2019)

Going back to the content, beside fish skeletal element distribution (i.e. body vs. cranial), there are some other dissimilarities between the coprolites from the two considered sites. At the Črnelnik site only fish remains are present in the coprolite, while in the Stare gmajne coprolites, although in a minority, birds and small mammal remains could also be recognized. In comparison with bird remains excavated within different settlement areas, where amongst the human food (poultry) debris, elements of extremities of different species of duck/crow sized birds also prevailed (e.g. Ericsson 1987; also Janžekovič and Malez 2004), it can be concluded that the dogs were also fed, besides small fishes, with discarded remains of poultry used as human food at the Stare gmajne site. Due to pronounced fragmentation we could not determine the importance of other vertebrates (i.e. small mammals and frogs?) for the dog's nutrition.

Plant macroremains in all the analysed coprolites also confirm diverse vegetable food, but in a lesser extent. Plant macroremains were, with the exception of one charred cereal fragment and charcoal, preserved by waterlogging (Table 2, Fig. 8) and in good condition, suitable for identification.

Beside possible nutrition resources, plant remains in coprolites confirm typical ecological conditions at both sites (Tolar et al. 2011; Velušček et al. 2018). Small charcoal fragments, a few seeds/fruits of cultural as well as weed taxa (flax, cereals, turnip, white goosefoot) and gathered plants (blackberry, water chestnut), confirm an anthropogenic area, while seeds/fruits of lakeshore bulrush and birch demonstrate water—marshy terrain at both sites (Table 2, Fig. 8). Pollen analysis of coprolite sample no. 1 from Stare gmajne suggests similar conclusions. Vegetation around the settlement mostly consisted of wetland taxa (*Drosera*, *Alnus*, *Salix* and especially a high percentage of Cyperaceae). High percentages of early succession taxa (*Betula*, *Alnus*, *Corylus*) and a low percentage of a late successional taxon (*Fagus*) suggests forest clearance/anthropogenically altered vegetation. Moreover, pollen from cereal (Cerealia-type) and potentially cultivated plants (*Linum*, *Vitis*) shows anthropogenic activities in the area.

Plant studies of the coprolites highlight different seasons of human presence at the two sites. The coprolite from the Črnelnik site contained fruits/seeds that ripen in the late summer–autumn (water chestnut, blackberry, birch), while sample no. 1 from the Stare gmajne site has high percentages of pollen of early successional taxa (birch, hazel, alder), indicating an early spring deposit period. Low pollen abundance in this coprolite examined probably reflects indirect ingestion, e.g. through drinking the surrounding water (Wood et al 2016).

Conclusions

Well-preserved excrement remains seem rare in archaeological contexts, but are very useful in environmental studies (Bryant and Dean 2006). Such studies can provide unique insights into the biology and ecological interactions of past species (Wood and Wilmshurst 2016). The present study therefore adds to the growing body of coprolite research, including a growing focus on multi-proxy analysis (Delhon et al. 2008; Marinova et al. 2013; Wood and Wilmshurst 2016; Baeten et al. 2018; Dunseth et al. 2019; Smith et al. 2019; Landau et al. 2020). Most studies of coprolites seem to focus on phytoliths, pollen (Horrocks and Irwin 2003; Delhon et al. 2008; Marinova et al. 2013; Wood et al. 2016; Smith et al. 2019; Zachary et al. 2019; Landau et al. 2020) and parasites (Le Bailly et al. 2003; Kühn et al. 2013; Maicher et al. 2017); the incorporation of archaeobotanical and archaeozoological identifications in this manuscript is an important contribution.

The identification of the coprolites from Stare gmajne as canine is convincing, especially considering the high quantity of fish bones, presence of some plant remains, tapeworm and whipworm, and the consideration of size.

Vertebrate remains from the analysed coprolites show a predominant representation of either the least meaty body parts (i.e. fish heads, small fish) or skeletal elements from which the meat is believed to have been routinely separated in advance of cooking/roasting it (i.e. bird body bones and extremities; cf. Ericsson 1987). When skeletal elements of virtually all anatomical regions of the animal are represented, they belong to relatively small specimens, which may have been occasionally discarded by man (e.g. small fish). Such a picture confirms the thesis of small to medium-sized prehistoric 'turbary' dogs having been tolerated around the settlements almost as mere scavengers, making their relationship to the human population reminiscent of that of free-roaming pariah dogs (Bartosiewicz 2002). Given the high importance of hunting for local prehistoric pile-dwelling communities (see e.g. Drobne 1973; Toškan et al. 2019), the basic instincts of these animals may have been exploited without the need of proper breeding in the form of conscious, target oriented selection. Stray dogs breeding freely in panmixia—which favoured the emergence of the already mentioned small to medium sized specimens—can perform this basic task just as well. The same holds true for passive guarding of the flocks and people, since the latter just had to take advantage of dogs being strongly territorial and spontaneously alarm-raising animals. As a matter of fact, an upper size limit may has been subconsciously reinforced by humans, many of whom would not tolerate the presence of too large, "wolf-like" beasts around the settlement (Bartosiewicz 2002).

Beside meat nutrition and the possible care (or non-care) of the dogs as well as organic refuse management and the culture of eating fish and birds, the research gives environmental information as well. Natural water and marshy as well as anthropogenic opened landscape was again confirmed (e.g. Andrič et al. 2008; Tolar et al. 2011), as well as the importance of the lake for the Late Neolithic economy.

Thus, it was established that the coprolites from Stare gmajne were excreted in spring, while the one from the Črnelnik site in autumn.

When analysing such important and small samples, it is very important to plan the research precisely and to use appropriate methods (photography, measurement, cleansing of the outer surface of possible contamination, keeping the remains waterlogged, the performing of many analyses on the same coprolite, using the smallest possible sieve for wet sieving). It is also important to begin analyses as soon as possible after the discovery (i.e. removal from the sediment). Two coprolites from the Stare gmajne site are still untouched, preserved in a waterlogged state and stored in a refrigerator. The intention is to upgrade the investigation with additional DNA and biomarker analyses.

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