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– ARTICLE –

Microfossil analysis of sediments from Carpenters Gap 1 and 3 archaeological sites, Kimberley, Western Australia, provides evidence for processing cf. *Dioscorea transversa* and cf. *Colocasia esculenta* since c. 30,000-25,000 cal BP

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Abstract

Previous palaeo-starch studies in Australia, almost all using stone tool analysis, have yielded a considerable body of information on Indigenous plant processing. Here we build on this work with starch analysis of 58 sedimentary samples from Carpenters Gap 1 and 3 rockshelters, Kimberley, Western Australia. Starch of two species native to the region, supported by calcium oxalate crystal and xylem evidence, was identified at both sites: cf. *Dioscorea transversa* (pencil yam) tuber, dated from 30,372-27,846 cal BP; and cf. *Colocasia esculenta* (taro) corm, dated from 26,202-25,143 cal BP. The taro material has not previously been reported in Australian archaeological contexts. We discuss other possible sources of this starch type that have similar, relatively tiny sized grains, showing several differentiating features of taro starch. Results show promise for isolating starch and associated material from archaeological sedimentary records in Australia, which can provide a complimentary line of evidence alongside stone tools. The presence of another microfossil type, euglenoid cysts, provides evidence for gathering wetland/aquatic resources.

***Keywords*:** rockshelter; starch; *Dioscorea transversa*; *Colocasia esculenta*; euglenoid cysts

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1. Introduction

The Carpenters Gap (CG) archaeological sites CG1 and CG3 in the Kimberley, Western Australia have received considerable scientific attention over the last 30 years or so, which has enabled long-term historical research into the nature and timing of cultural activity and environmental transformation in the region (Figure 1). O'Connor (1995) first excavated these sites during 1992-1993. Since then, further research has included chronological, stone artefact, and zooarchaeological (faunal remains) studies (Maloney *et al.* 2018; O'Connor *et al.* 2014). There have also been archaeobotanical investigations, including macrobotanical analyses of wood charcoal (i.e., anthracological) remains (Frawley and O'Connor 2010) and non-woody (i.e., carpological) macrobotanical remains comprising seeds, fruits, nuts, and other floristic elements (Dilkes-Hall *et al.* 2019; McConnell 1998; McConnell and O'Connor 1997), alongside microbotanical analyses using pollen (Rowe *et al.* 2020) and phytoliths (Wallis 2001).

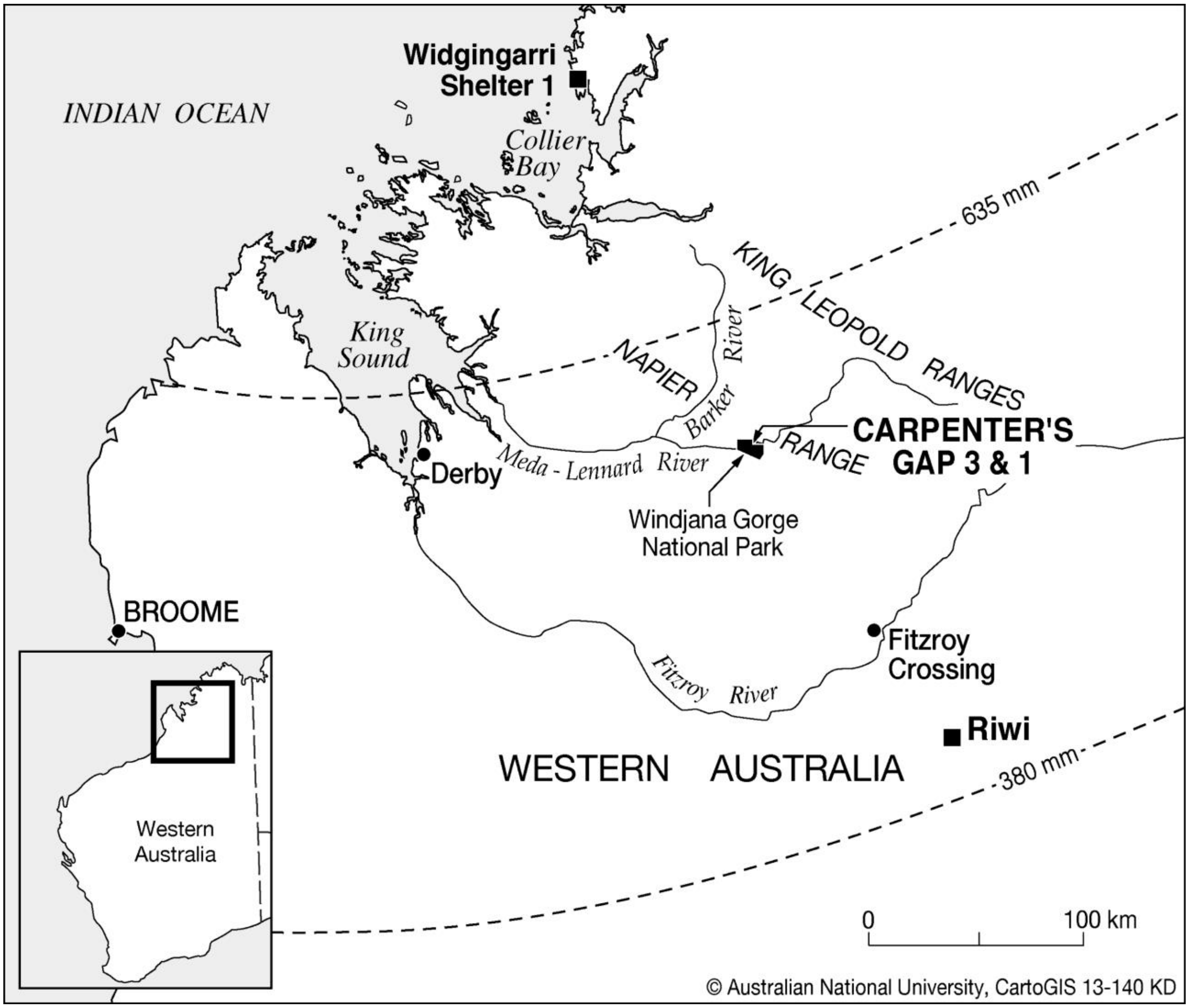

Figure 1: Map showing study area and site locations.

Starch grain analysis has been conducted at a number of Australian archaeological sites (e.g., Atchison and Fullagar 1998; Cosgrove *et al.* 2007; Field and Fullagar 1998; Field *et al.* 2009, 2016; Fullagar *et al.* 2015; Hayes *et al.* 2018, 2021; Owen *et al.* 2019; Slack *et al.* 2024; Wallis *et al.* 2020) yet has not been carried out at the Carpenters Gap sites to date. The focus of starch grain analysis has been almost exclusively on stone artefacts rather than sedimentary records. This is with the exception of Atchison and Fullagar (1998), who observed unidentified starch grains on stone artefacts and in sediments from Jinmium rock shelter, northwest Northern Territory. Turnbull *et al.* (2023) considered that there was a general lack of archaeobotanical studies in Australia, which was related to reliance on highly localised collection efforts of modern plant specimens for comparison. The aim of this study is to expand the Carpenters Gap findings using another line of evidence to investigate plant utilisation further. We present here results of recovering and analysing microfossils from previously sampled sedimentary records from the two Carpenters Gap sites.

2. Study area and sites

The two sites are located 3 km apart on the northern margin of the Windjana Gorge National Park in the Napier Range, south central Kimberley, Western Australia (Figure 1). The range is a 350-million-year-old remnant Devonian limestone reef complex (Playford *et al.* 2009). Other notable Pleistocene-aged regional archaeological sites which preserve archaeobotanical material are Riwi Cave (Balme *et al.* 2019; Dilkes-Hall *et al.* 2020a; Whitau *et al.* 2017) and Widgingarri Shelters 1 and 2 (Dilkes-Hall *et al.* 2020b) (Figure 1.)

2.1. Carpenters Gap 1 (CG1)

CG1 is a large north to north-east facing limestone rockshelter situated 25 m above the savannah plain, and approximately 4 km from a permanent water pool formed by the Lennard River at Windjana Gorge to the west (Figure 1). The entrance height is approximately 20 m, with a 50 m^2 floor area incorporating a boulder rockfall towards the opening. The weather protection of the boulders and alkaline sediments provide good preservation conditions for organic remains (O'Connor 1995). The rockshelter is devoid of vegetation, a result of limited sunlight and water.

CG1 contains a variety of archaeological evidence spanning a period of c. 50,000 years. Occupation at the site commenced between 51,000-45,000 cal BP (Maloney *et al.* 2018) and continued into the Last Glacial Maximum (LGM), and throughout the Holocene. Owing to the excellent preservation of faunal and macrobotanical remains in the deposit it is one of the most intensively studied archaeological sites in Australia. Maloney *et al.* (2018) presented analyses of the stone artefact and faunal assemblages bolstered by numerous ^{14}C dates to reconstruct changes in technology, mobility, and diet spanning the last c. 47,000 years. McConnell and O'Connor (1997) carried out a study of non-woody macrobotanical remains recovered from Square A covering the last c. 40,000 years. While predominantly examining seeds, the study also included other types of plant material such as bamboo fibre, tubers, uncharred wood shavings, wood fragments, grass stems, leaves, bark, and fibres. These remains comprised a variety of economic taxa which were interpreted as reflecting changing environments around the site, and thus availability of different foods. For example, Chenopodiaceae and other grass seeds and parts were recovered only during the LGM through until the beginning of the Holocene and were argued to have been used as a drought food when conditions became more arid, while vine thicket taxa such as *Terminalia* and *Vitex glabrata* were most abundant in the Holocene and were correlated with wetter conditions (McConnell 1998).

Dilkes-Hall *et al.* (2019) built from McConnell's earlier work (McConnell 1998; McConnell and O'Connor 1997), with analysis of non-woody macrobotanical remains from Square A2, the deepest square with the longest chronology, covering the last c. 47,000 years. Results showed site occupation intensity and subsistence were closely associated with plant resources derived from ecologically productive, monsoon rainforest ecosystems (e.g., *Terminalia* spp. and *V. glabrata*). Consistent use of these plants across periods of aridity indicates ecological knowledge and management of important resources ensuring availability despite changes in regional climate observed by McConnell (1998) and in stone and faunal records (Maloney *et al.* 2018). Human-plant relationships, as evidenced from macrobotanical records, highlight the importance of monsoon rainforests in the lifeways of Aboriginal groups from the earliest peopling of the south-central Kimberley region.

Wallis (2001) studied phytoliths from the sedimentary records, which were identified as being derived from cultural vegetal inputs introduced by people to the site, as well as animal vegetal inputs introduced by faecal pellets. This record demonstrated changes in the abundance and proportions of taxa over time, which inferred changes in palaeo-vegetation and climate in the region. For example, Cyperaceae (sedges) phytoliths increased during the LGM, which was interpreted as related to increased human mobility within drier landscapes. Rowe *et al.*'s (2020) palynological study provided broad detail to the vegetation patterns identified in the previous studies. Results revealed initial, pre-glacial, mixed wooded vegetation. Tree decline occurred from the start of the Holocene, with a transition from monsoon forest to thicket and eucalypt forest to woodland. Mid-Holocene vegetation changes suggested a climatic change to drier and more variable conditions towards and into the late Holocene.

Frawley analysed the wood charcoal from a single assemblage/context from Square A2, providing a complimentary but differing perspective on the vegetation history at CG1 (Frawley and O'Connor 2010). The analysis identified species which were not detected in the non-woody macrobotanical, phytolith, or pollen analyses, presumably because the wood entering the site was predominantly gathered for fuel (additionally, some taxa do not produce phytoliths). *Terminalia*, an important food taxon found in the other macrobotanical analyses, also appears in the wood charcoal record in all phases. This indicates that monsoon rainforest/vine thickets persisted within proximity to the site even during the Last Glacial phase when the wood charcoal more generally reflects a change to dry woodland species. The Holocene wood charcoal record, in keeping with other botanical records, shows an increase in diversity and number of identified taxa as well as an increase in monsoon rainforest/vine thicket taxa.

2.2. *Carpenters Gap 3 (CG3)*

CG3 is an extensive limestone shelter approximately 30 m above the savannah plain with a lower cave extending at least 30 m into the range. The main deposit is within the cave and has extensive surface cracking, reflecting seasonal or periodic wetting and drying. CG3 was first excavated by O'Connor in 1993. ^{14}C estimates obtained on charcoal, seeds, and freshwater shell showed a c. 30,000-year occupation. Although not all the excavation details were originally published, some of these findings have been since incorporated into more recent work (e.g., O'Connor and Veth 2006:36). New excavations were carried out in 2012, and the findings were reported by O'Connor *et al.* (2014), who also provided further details on the original 1993 excavations.

O'Connor *et al.*'s (2014) study offered some preliminary evidence for low level occupation following first peopling, with an occupational hiatus during the LGM. As with most Australian sites, evidence for occupation increased sharply from the mid-Holocene. Faunal remains, interpreted as the likely remnants of food, suggested foraging by people in the immediate surroundings of the site, throughout the entire period of occupation. The faunal remains from the 1993 excavation were accidentally damaged during pre-treatment, which precluded any further analyses. The faunal remains from the 2012 excavation have not yet been quantified.

3. Methods

Analysis was carried out on sediment samples from 58 spits (excavation units) from the original excavations: 22 from CG1 and 36 from CG3 (Table 1). Spit depths were approximately 3 cm. Spit sub-samples were prepared for analysis with 5% Calgon followed by heavy liquid separation using sodium polytungstate (1.8 s.g., for 3 min. at 1500 rpm) (Horrocks 2020; Piperno 2006). Other types of microfossil material with ≤1.8 s.g. are included in this separation. Separated material was mounted on slides with glycerol jelly. Precautions were taken to avoid starch grain contamination by modern plants and adjacent sediments (Crowther *et al.* 2014; Ma *et al.* 2017).

Other material of interest, in this case calcium oxalate crystals, xylem cells, and euglenoid cysts, can be separated along with starch in this method of preparation. Although calcium oxalate is heavier than the 1.8 s.g. of the heavy liquid solution used in the starch extraction procedure, if present the crystals are generally recovered in reasonable, often abundant amounts with this method, caught up or embedded in other detritus. This can result in relative under-representation of crystals but in this study the cost of the analysis and limited size of some of the samples precluded a separate extraction. Starch was assessed qualitatively by presence/absence, with some samples deemed to have noticeably higher concentrations

Photomicrography was carried out using a Canon EOS 600D camera mounted on a Nikon 400E microscope (with measurements made using a calibration slide) and a Zeiss Axio Imager.Z2m (which takes automated measurements). Modern plant reference material was obtained from Australian and New Zealand herbaria. A modern reference collection of specimens of plant species utilised by people of Oceania, housed at Microfossil Research Ltd, Auckland, was also used.

Table 1: Microfossils at Carpenters Gap 1 (sq. B) and 3 (sq. A). CG1-18a = baked, 27a = hearth, CG3-25a = baked; *from Sample 3a (Maloney *et al.* 2018); + = present, ++ = relatively very high concentration.

Spit (EU)	cf. *Dioscorea transversa* starch	cf. *Colocasia esculenta* starch	Euglenoid cysts	Cal yr BP (94.5% prob.)
CG1				
3		+		*4526-4156
5	+			
6		+		
7				
11	+	+		
12	+	+		
13		+		4821-4423
14	+	+	+	
15		+		
9		++		6183-5761
16				22,284-21,438
18		+		
18a	+	+		
19	++			
21	++	+		
24				22,193-21,057
26	++			
27	++			
27a	++	+		
28	++	+		
29	++			
30	++			23,465-20,021
CG3				
10			+	441-755 5589-5326
11		+		
12		+	+	6436-6298
13		+	+	
14		+	+	
15		+	+	
16		+		11,590-10,876
17		+		
18		+		15,540-14,140
20				
22				
24		+		13,447-13,201
25		++		
25a		++		
26	+	+		
27				
28	+	+		
29				17,014-16,442 17,870-16,249
30				12,715-11,709
32	+			16,101-15,762
34		+		
37			+	17,208-16,658
38				17,429-16,928
39		+		17,429-16,928
41	+	+		
44				
46		++	+	
49	+			26,202-25,143
51		+		
53	+	+		
55		+		
58				46,660-23,955 30,372-27,846
60	+			
61				30,484-29,624 31,467-31,010
64				33,992-33,147
67				33,847-32,970

4. Results

4.1. Starch and associated material

4.1.1. Type 1

This starch type comprised individual ovoid to tri-ovoid grains, with eccentric hila, found in 13 samples from CG1, dated from 23,465-20,021 cal BP; and seven samples from CG3, dated from 30,372-27,846 cal BP (Table 1, Figure 2). Grains mostly measured up to 55 µm in longest diameter, with occasional grains up to 75 µm. Eight samples from CG1 noticeably had relatively high grain concentrations (100+, 22x40 mm coverslip). The grains were degraded, with most showing discolouration, and all had lost the extinction or so-called "Maltese" cross which shows in cross-polarised light in non-decayed grains (fresh starch grains generally are birefringent). Many grains however showed high or partially high visibility in cross-polarised light (Figure 2O-S).

In addition, styloids (longitudinally elongated calcium oxalate crystals, forming rectangular columns) with straight or occasionally bevelled ends, measuring up to 6 µm wide and up to 109 µm in length, were found in a few of the samples containing the starch grains (Figure 3A-D). Calcium oxalate crystals generally are birefringent. The styloids showed some decay, such as fragmentation and pitting, although all showed high visibility in cross-polarised light. A small amount of xylem (a type of tracheary tissue often observed in bundles) with small slit-like pits in the cell walls was also observed (Figure 3E).

These archaeological microfossils are very similar to those of *Dioscorea transversa* (pencil yam) tubers (Figure 4-6). The combined correlates in this case thus include 1) large ovoid to tri-ovoid starch grains with eccentric hila, 2) styloids with straight and occasionally bevelled ends, and 3) xylem cells with small slit-like pits in the walls. Unlike the starch grains and calcium oxalate crystals, xylem of the *Dioscorea* spp. of Oceania appears not to be species-specific (M. Horrocks, unpubl. data). *Dioscorea* spp. "produce no to few phytoliths of little to no taxonomic significance" (Piperno 2006: Table 3.2).

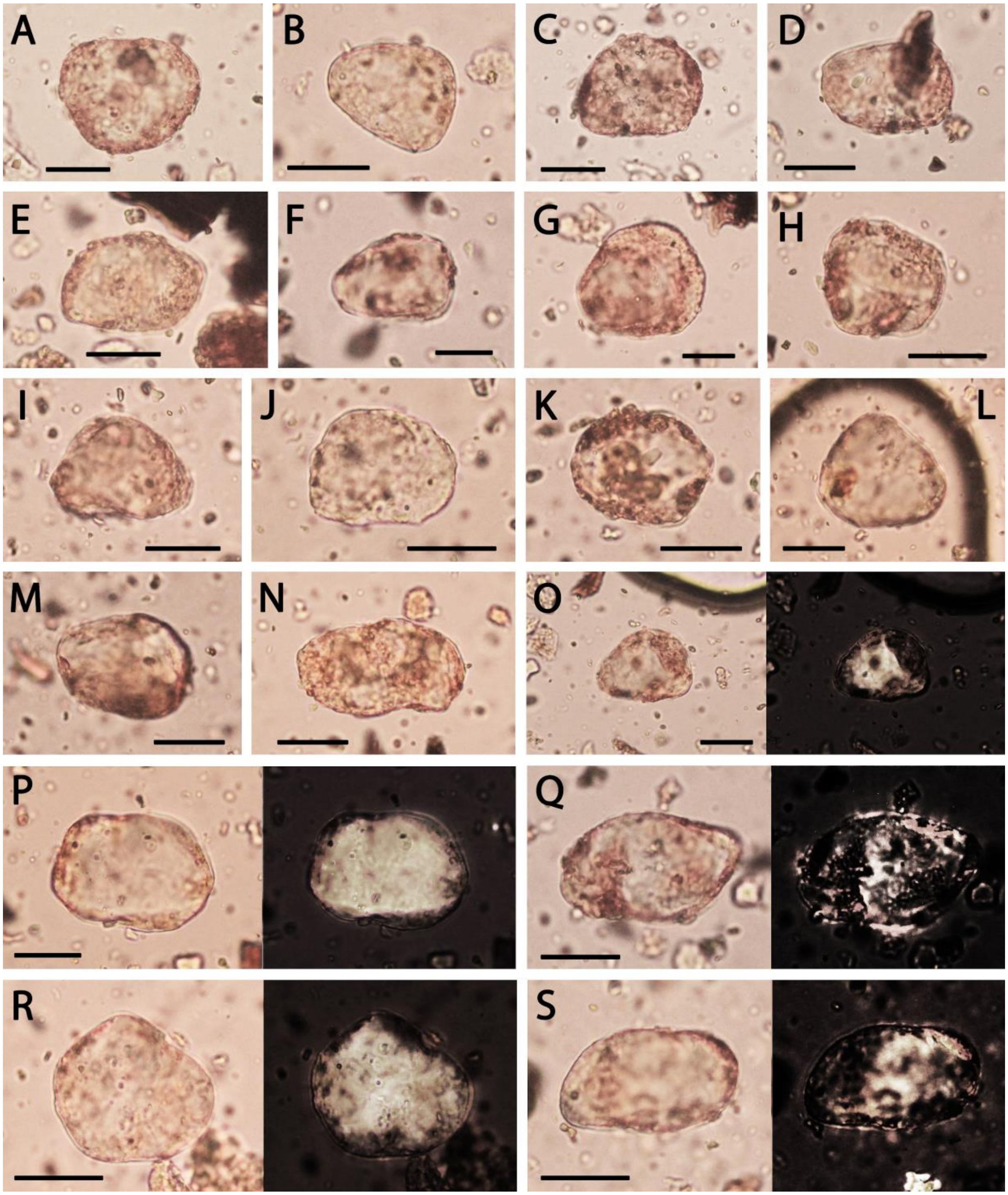

Figure 2: Type 1 starch from Carpenters Gap. 600x; black background = cross-polarised light (CPL); scale bars = 20 µm; spit numbers (in brackets) shown in Table 1. A-H, N-Q (CG1-26); I-M, R, S (CG1-29). A-S: individual ovoid to tri-ovoid to occasionally irregularly shaped grains, showing discolouration and variable stages of preservation (cf. *Dioscorea transversa* starch grains, Figure 4, 5, upper images). O-S: grains also show high visibility in cross-polarised light, possibly due to permineralisation.

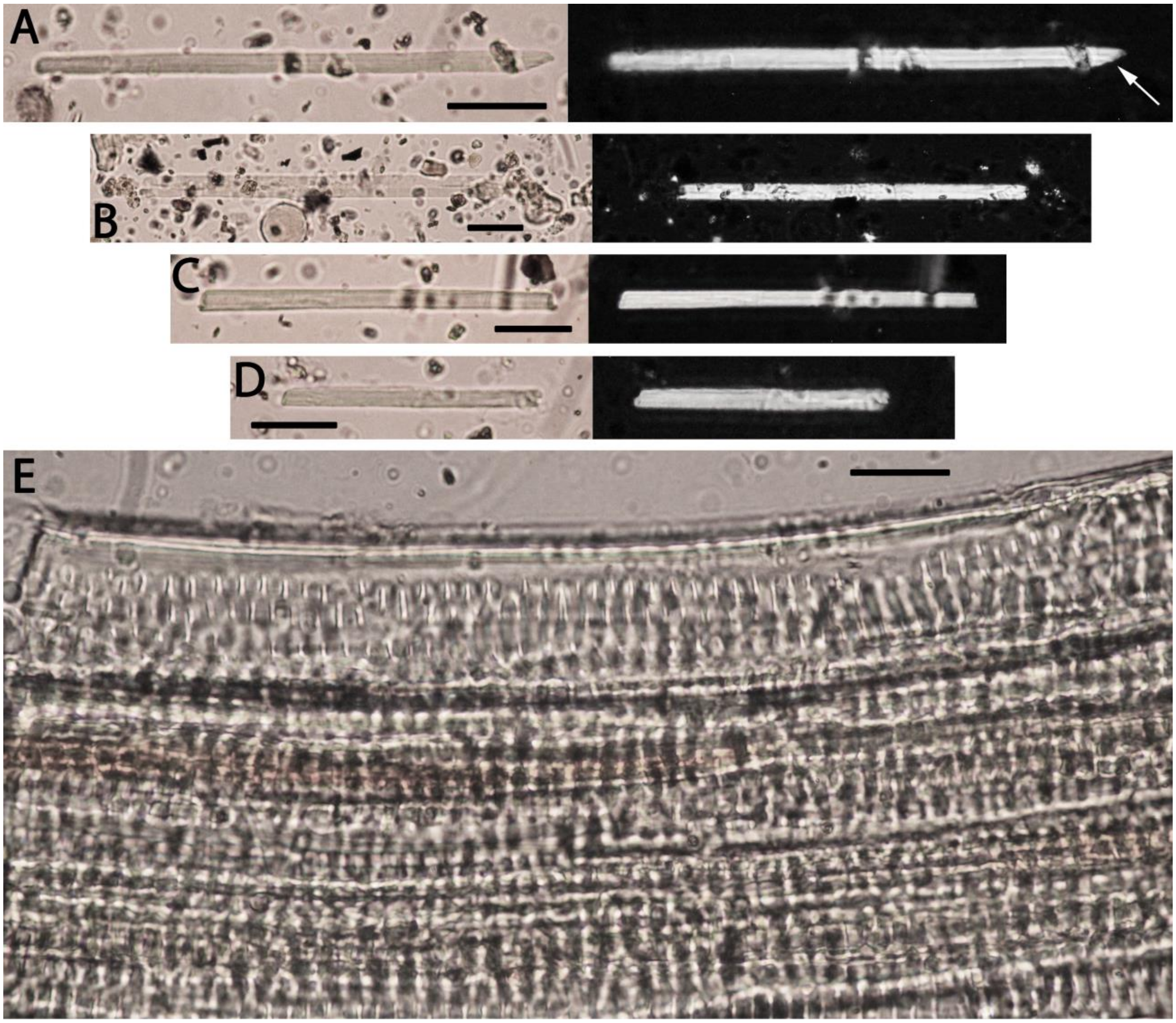

Figure 3: Type 1 styloids and xylem from Carpenters Gap. A-D, 400x, E, 600x; black background = cross-polarised light (CPL); scale bars = 20 µm; spit numbers (in brackets) shown in Table 1. A (CG1-5), B-D (CG-18a): styloids showing straight ends except for one bevelled end (arrow), and high visibility in cross-polarised light (cf. *Dioscorea transversa* styloids, Figure 6A). E (CG3-49): xylem bundle with small slit-like pits in the cell walls (cf. *D. transversa xylem*, Figure 6B).

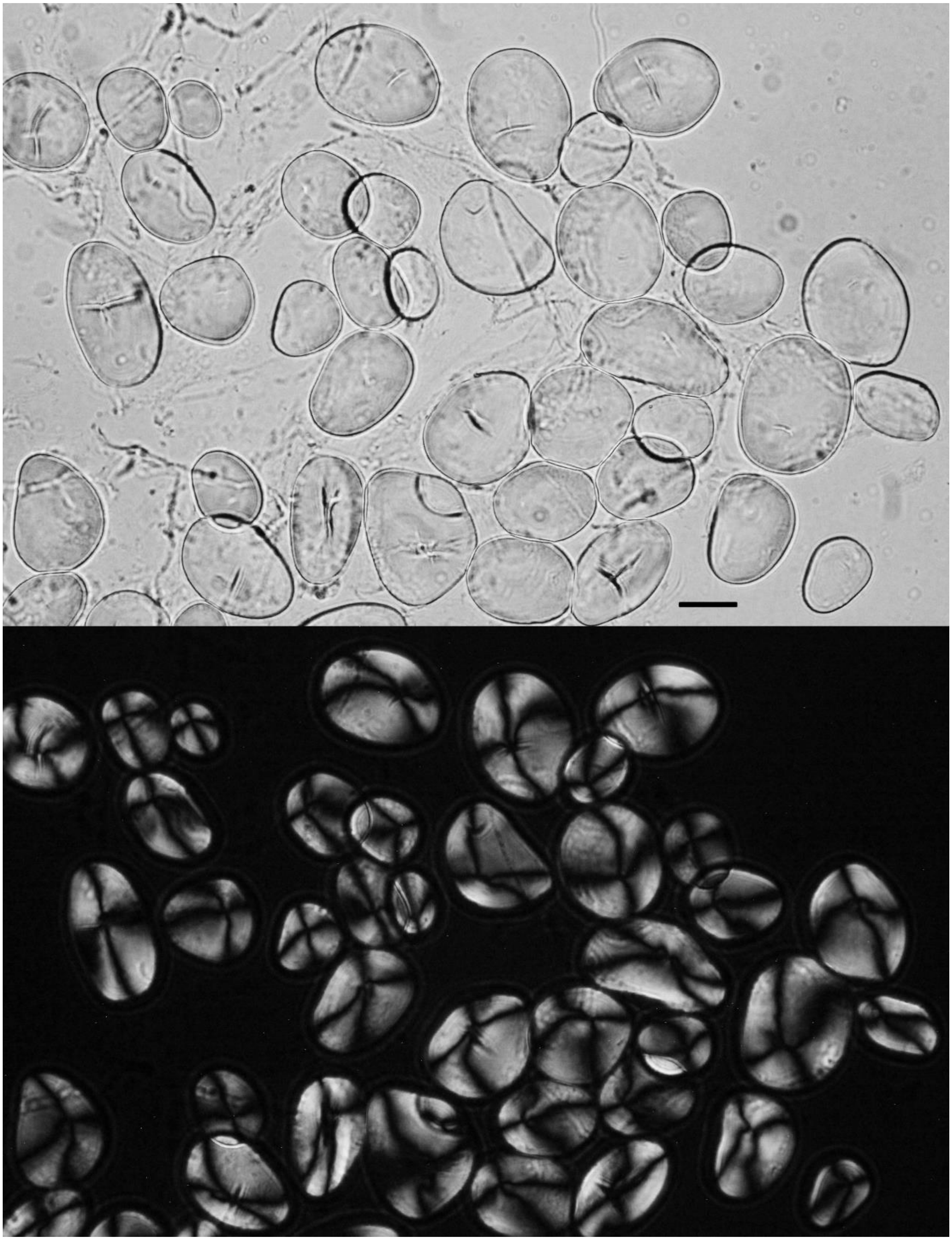

Figure 4: Modern reference *Dioscorea transversa* starch grains, showing characteristic ovoid to tri-ovoid shape. 400x; black background = cross-polarised light (CPL); scale bar = 20 µm.

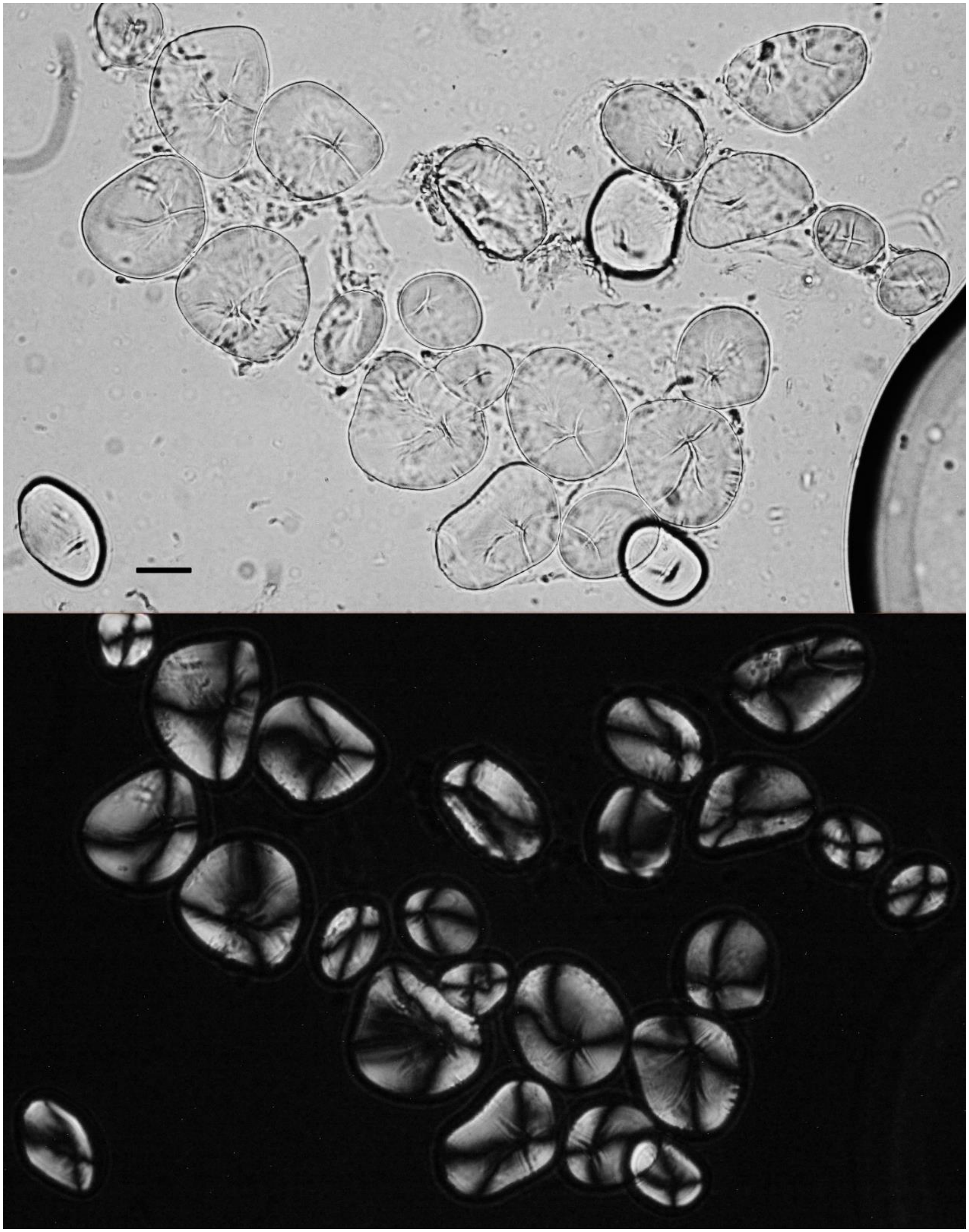

Figure 5: Modern reference *Dioscorea transversa* starch grains, showing some larger shapes than those in Figure 4. 400x; black background = cross-polarised light (CPL); scale bar = 20 µm. These grains show increased hilum fissuring due to slide coverslip compression.

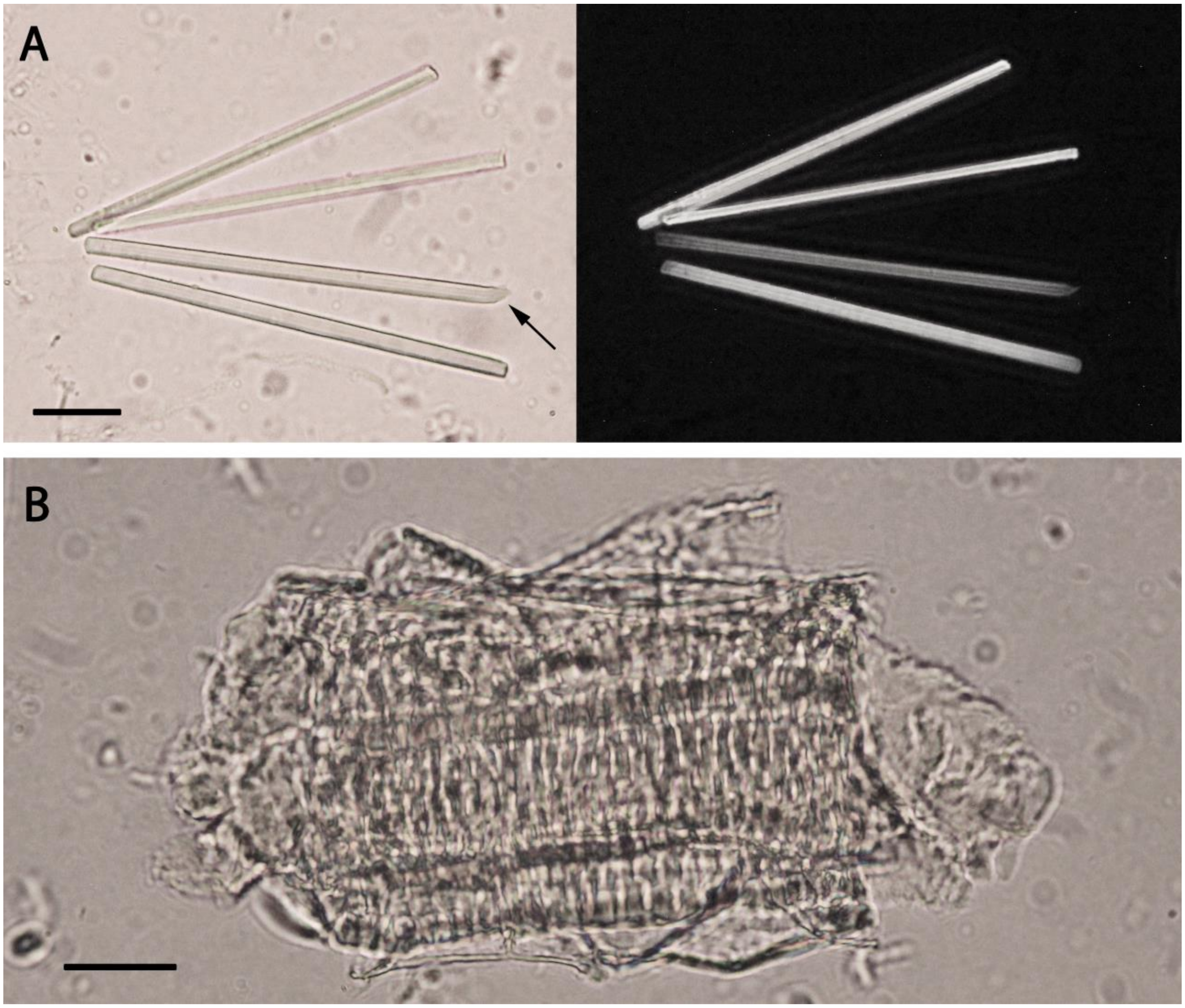

Figure 6: Modern reference *Dioscorea transversa* styloids (400x) and xylem (600x). Black background = cross-polarised light (CPL); scale bars = 20 µm. A: four styloids, showing characteristic straight and occasional bevelled (arrow) ends. B: part of xylem bundle with characteristic small slit-like pits in the cell walls.

4.1.2. Type 2

This starch type comprised masses of amyloplasts (sub-cellular units specialised in synthesising and storing starch grains) and fragments thereof found in 13 samples from CG1, dated from 22,193-21,057 cal BP; and in 20 samples from CG3, dated from 26,202-25,143 cal BP (Table 1, Figure 7-9). These contained numerous, densely packed, sharply angular starch grains, the vast majority ≤4 µm, with relatively rare grains up to 9 µm. One sample from CG1 and three samples from CG3 had very high concentrations (100+ masses, 22x40 mm coverslip) of this starch, and the remainder had very low concentrations (<10 amyloplast fragments, 22x40 mm coverslip). Some amyloplasts contained aggregations of starch grains (Figure 9C, D).

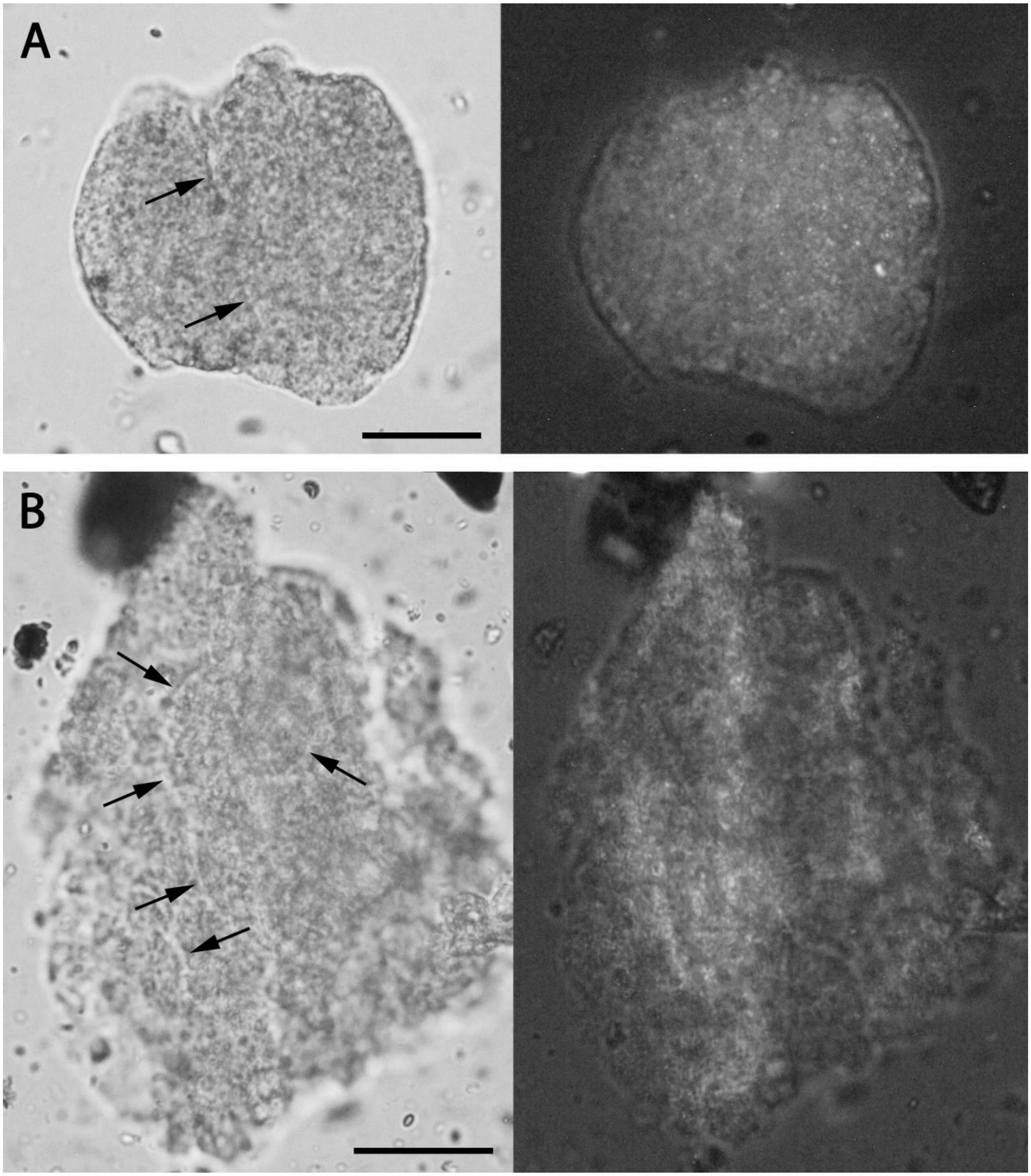

Figure 7: Type 2 starch from Carpenters Gap. 600x; black background = cross-polarised light (CPL); scale bars = 20 µm; spit numbers (in brackets) shown in Table 1. A (CG1-27a), B (CG3-46): clumps of amyloplasts (left shows some visibility in CPL, possibly a result of permineralisation) containing high concentrations (up to 1000s) of densely packed, tiny angular starch grains (cf. *Colocasia esculenta* amyloplasts, Figure 11). Spherical to sub-spherical amyloplast shapes visible (arrows). Hila appear as dark grey/black dots, imparting a grainy appearance to the mass.

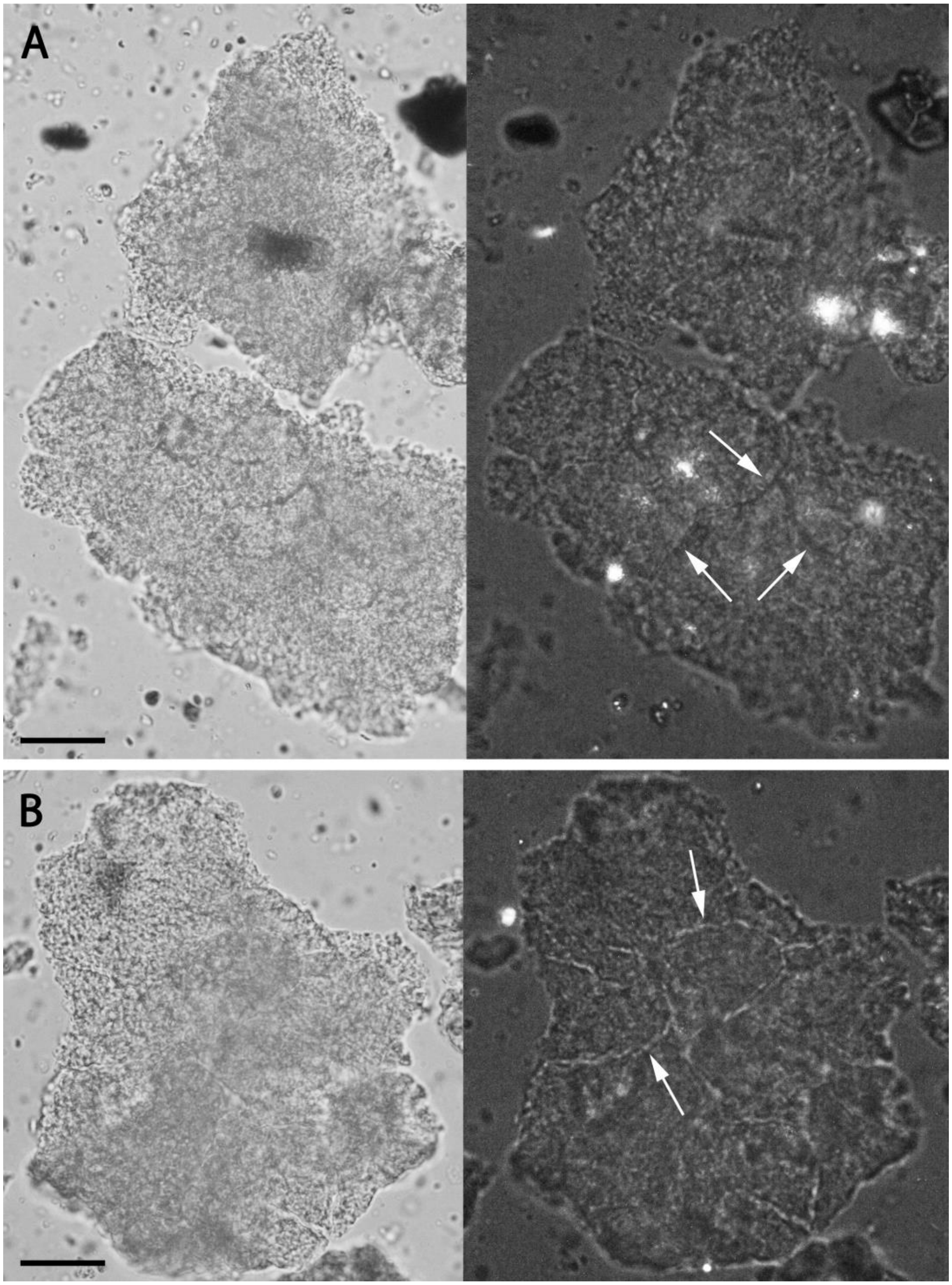

Figure 8: Type 2 starch from Carpenters Gap. 600x; black background = cross-polarised light (CPL); scale bars = 20 µm; spit numbers (in brackets) shown in Table 1. A, B (CG3-46): clumps of amyloplasts (left shows some visibility in CPL) containing high concentrations (up to 1000s) of densely packed, tiny angular starch grains (cf. *Colocasia esculenta* amyloplasts, Figure 11). Spherical to sub-spherical amyloplast shapes visible (arrows). Hila appear as dark grey/black dots, imparting a grainy appearance to the mass.

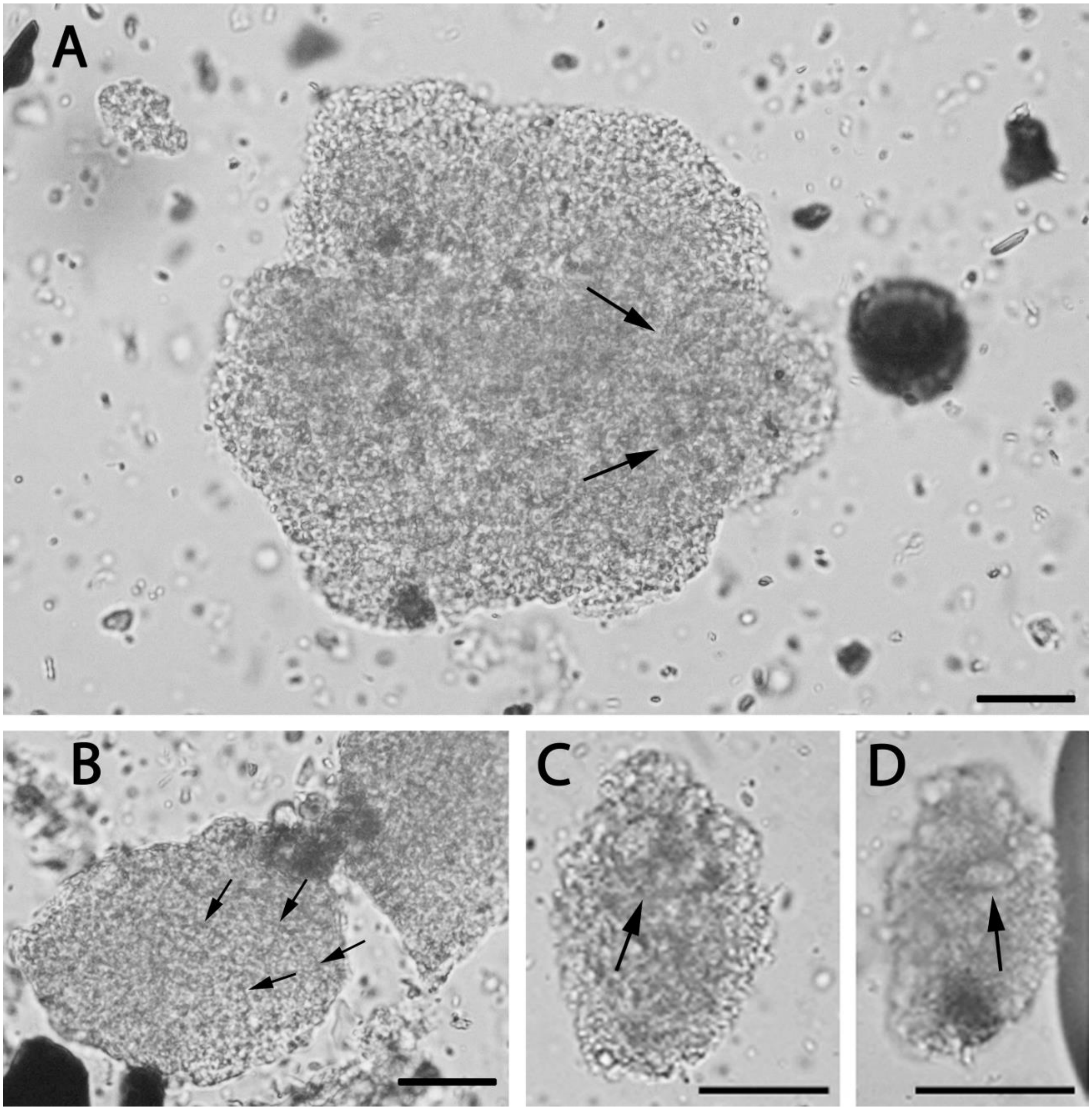

Figure 9: Type 2 starch from Carpenters Gap. 600x; scale bars = 20 µm; spit numbers (in brackets) shown in Table 1. A (CG3-46): mass of starch, with several curved amyloplast projections. Complete amyloplast shape visible, right (arrows) (cf. *Colocasia esculenta* amyloplasts, Figure 11). B (CG3-46): spherical shaped clump of amyloplasts, left, pulling away from starch mass, right, with curved amyloplast shapes faintly visible (arrows) (cf. *C. esculenta* amyloplasts, Figure 11). C, D (CG3-46): degraded amyloplasts with starch grain aggregations visible (arrows) (cf. *C. esculenta* amyloplasts with starch grain aggregations, Figure 11).

In addition, raphides and druses (needle-like and composite calcium oxalate crystals, respectively) and fragments thereof were found in some of the samples containing the starch grains (Figure 10A-D). Raphides measured approximately 1.25-1.50 µm wide and up to 48.0 µm in length, while druses measured up to 38 µm in diameter. The crystals ranged from occasionally reasonably well preserved to mostly fragmented and highly degraded, although almost all showed birefringence (high visibility) and pleochroism (different colours) in cross-polarised light. A small amount of xylem (the second of only two xylem types found in this study) with helical cell wall thickening was also observed (Figure 10E).

These archaeological microfossils are very similar to those of *Colocasia esculenta* (taro) corms (Figure 11, 12). The combined correlates in this case thus include 1) amyloplasts or fragments thereof containing relatively tiny, densely packed, sharply angular starch grains and occasional starch grain aggregations; 2) calcium oxalate crystals comprising druses and raphides; and 3) xylem cells with helical cell wall thickening.

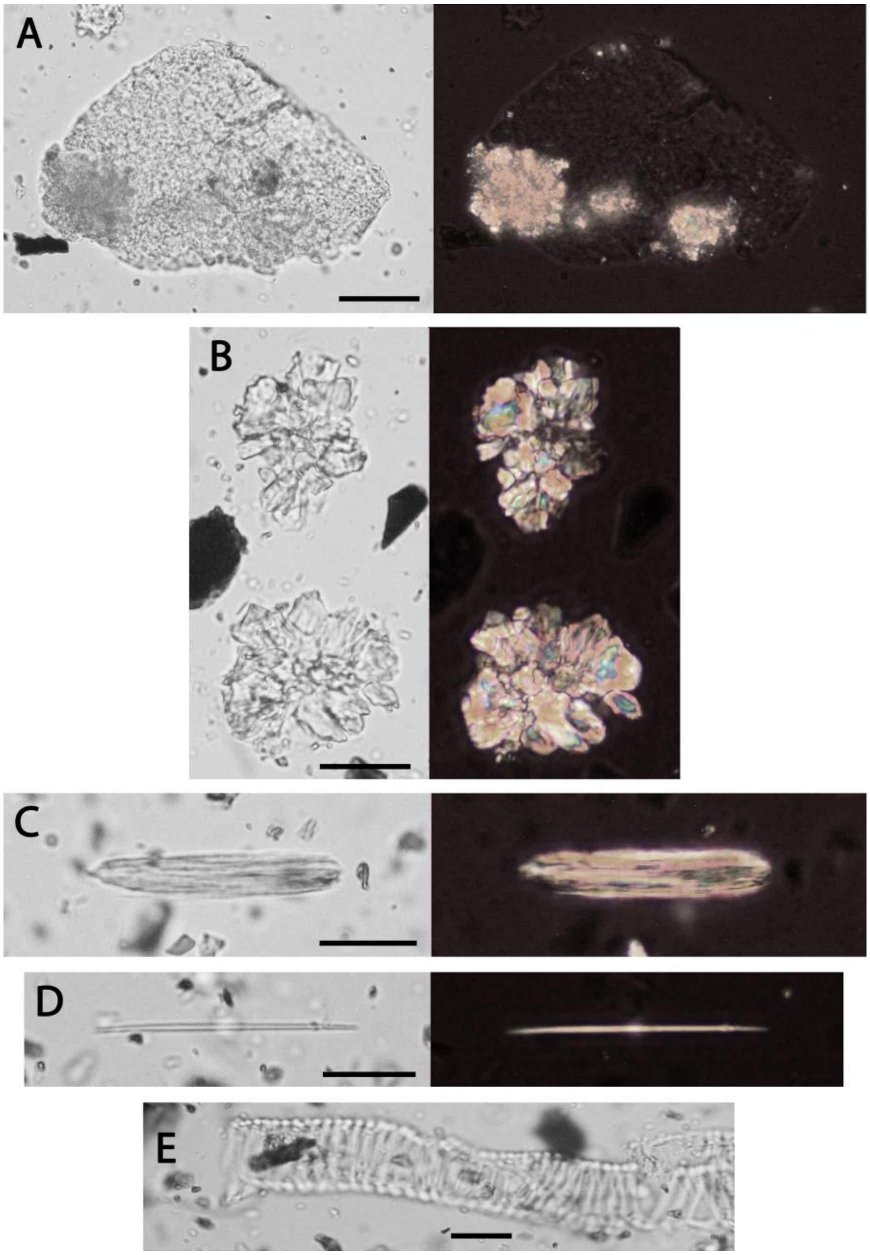

Figure 10: Calcium oxalate crystals from Carpenters Gap. 600x; black background = cross-polarised light (CPL); scale bars = 20 µm; spit numbers (in brackets) shown in Table 1. A (CG1-11): amyloplast clump with druse inclusions, which show high visibility and pleochroism in CPL (cf. *Colocasia esculenta* amyloplasts with druse inclusions, Figure 12A). B (CG3-11): two druses, which show high visibility and pleochroism in CPL (cf. *C. esculenta* druses, Figure 12B). C (CG1-11): idioblast remnant as a raphide clump, showing high visibility and pleochroism in CPL (cf. *C. esculenta* raphide clump, Figure 12C). D (CG1-11): individual raphide, showing high visibility and pleochroism in CPL (cf. *C. esculenta* raphide, Figure 12D). E: xylem showing helical cell wall thickening (cf. *C. esculenta* xylem, Figure 12E.)

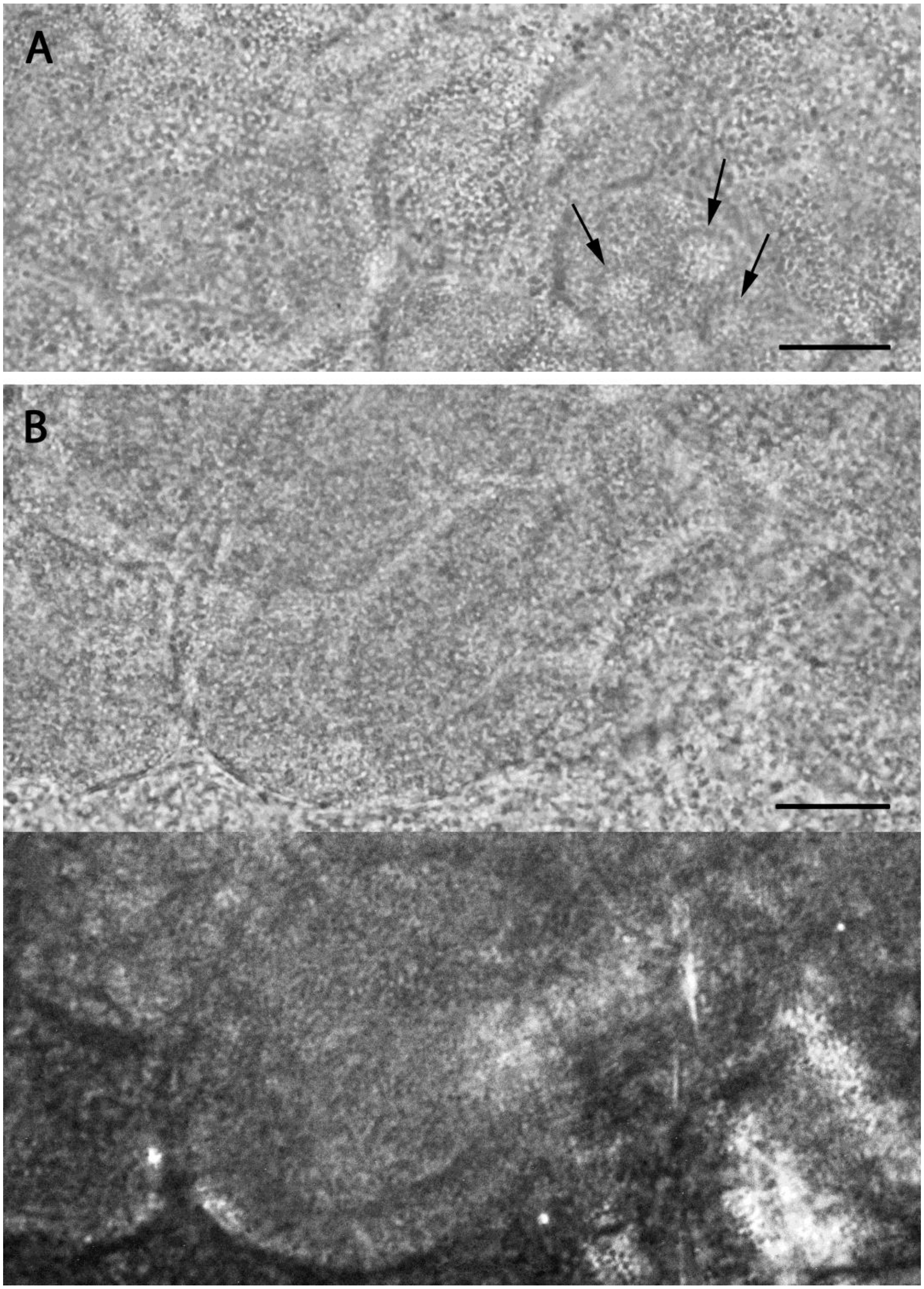

Figure 11: Modern reference *Colocasia esculenta* starch. 600x; black background = cross-polarised light (CPL); scale bars = 20 µm. A-B: amyloplast material containing high concentration (up to 1000s) of densely packed, tiny angular starch grains. A shows starch grain aggregations (arrows). Spherical to sub-spherical amyloplast shapes clearly visible. Hila appear as dark grey/black dots, imparting a grainy appearance to the mass. High visibility shown in B in CPL.

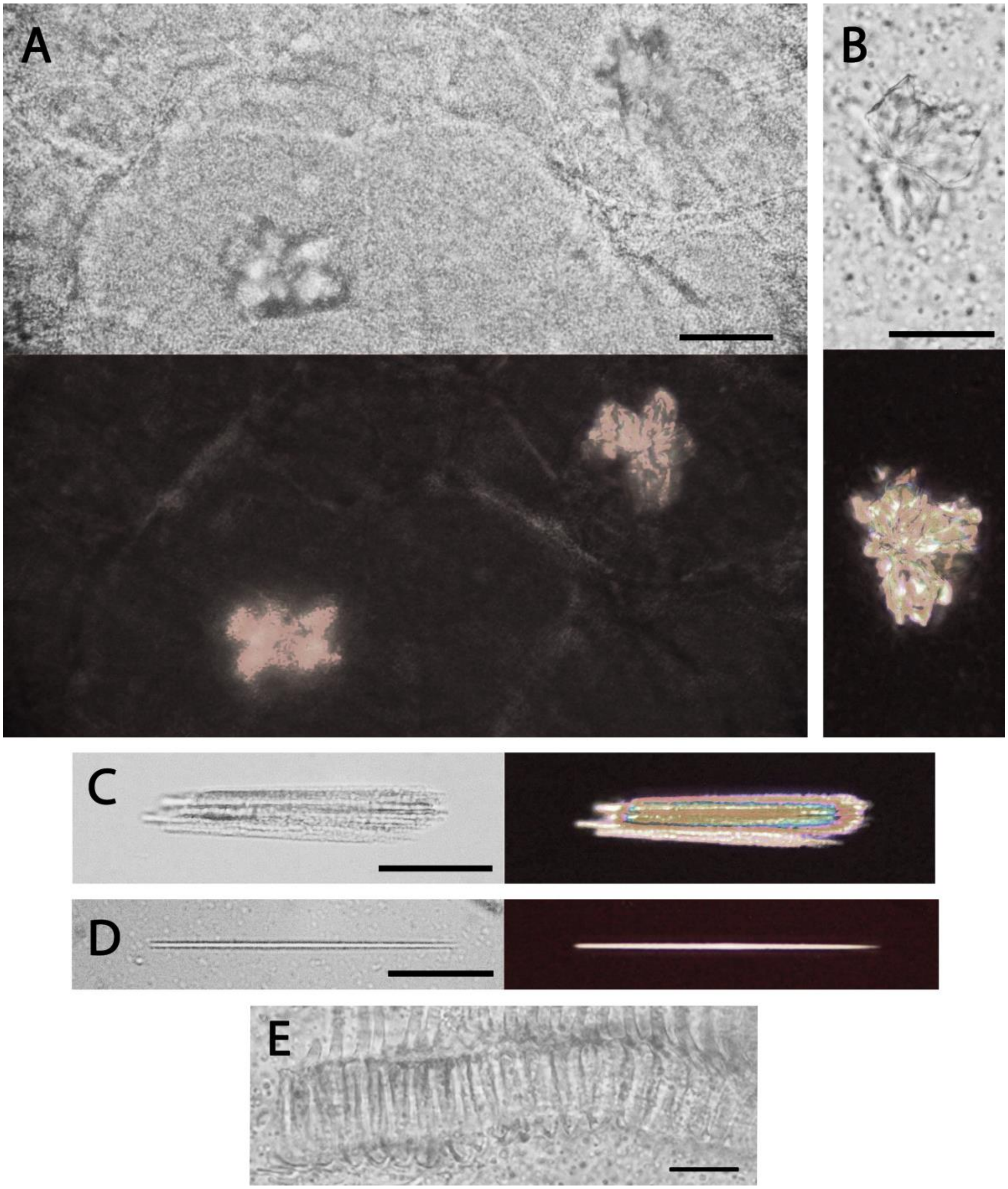

Figure 12: Modern reference *Colocasia esculenta* calcium oxalate crystals and xylem. 600x; black background = cross-polarised light (CPL); scale bars = 20 µm. A: amyloplasts showing two druse inclusions, with angular crystal projections, and characteristic pleochroism in CPL. B: individual druse among scattered individual starch grains, showing angular crystal projections, and characteristic high visibility and pleochroism in CPL. C: raphide clump, showing characteristic high visibility and pleochroism in CPL. D: individual raphide, showing characteristic high visibility and pleochroism in CPL. E: xylem showing characteristic helical cell wall thickening.

4.2. Euglenoid cysts

Another type of microfossil was recovered in the extractions. These microfossils were observed in one sample from CG1 and seven from CG3 (Table 1, Figure 13). The specimens are discoidal, circular, approximately 33x12 µm in diameter, with concentric, fingerprint-like surface ornamentation. These features are consistent with those of a species of *Chromotriletes* s.l. (van de Schootbrugge *et al.* 2024), which is a type of euglenoid (a single celled protist).

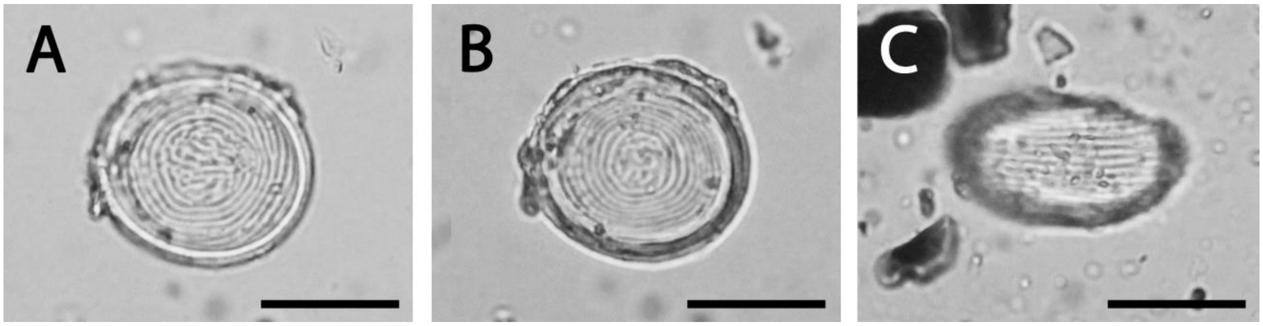

Figure 13: Euglenoid cysts from Carpenters Gap. 600x; scale bars = 20 µm; spit numbers (in brackets) shown in Table 1. A, B (CG3-13): both sides of one specimen, showing characteristic discoidal, circular shape, with concentric, fingerprint-like surface ornamentation. C (CG-14): side view.

5. Discussion

5.1. Taphonomy and preservation

The starch identifications in this study are based on the known Indigenous Australian economic plants and their biogeographical distribution, and descriptions of starch morphology of taxa that have so far been published. Some of the economic taxa have not yet been determined as to whether they produce significant starch. As the full list of descriptions is thus incomplete, we take a cautious approach to our results.

There is however a possible strengthening factor of the evidence to consider with our results. In one of the previous CG1 studies, McConnell and O'Connor (1997) found that the weights of the lithic artefacts in the sedimentary column were a good correlation between the presence of lithics and macrobotanical remains. They concluded that this implied that the bulk of the remains were related to human occupation of the site, either directly, as food, or useful materials brought to the rockshelter, or as material incidentally brought in with other items. We see no logical reason not to extend this line of taphonomic reasoning to microfossils in the same sediments. Support for this view is provided by Wallis'(2001) study aimed at assessing the different mechanisms by which plant microfossils may have entered the CG1 record. "These include introduction by humans and animals, wind or water action and from their growth on the site itself" (Wallis 2001:107). Release of microfossils from the weathering bedrock was discounted based on the nature and age of the parent material from which the shelter deposits are formed and good preservation of the phytoliths. Uncarbonized seeds and other fragile organics are found at depth in the CG1 sedimentary sequence (Maloney *et al.* 2018), ruling out water movement through the deposit which could transport microfossils or other materials. To test the contribution of wind and animals as agents of microfossil deposition, Wallis (2001) conducted a number of experiments. Microfossil traps covering an 18-month seasonal cycle were installed both inside the shelter, and outside at the base of the talus slope on the edge of the open savannah plain below the shelter (Wallis 2001:108). While the study did reveal transport of microfossils in the trap outside the shelter, these primarily comprised burnt grass phytoliths, carbonised particles, and pollen. Starch was rare in the trap outside the shelter. Significantly fewer phytoliths and pollen were found in the in-shelter traps and starch was not mentioned as recovered at all (Wallis 2001:109). Wallis (2001) also analysed the vegetal contents of faecal pellets deposited by animals within CG1 and concluded that they may have contributed significant quantities of phytoliths to the site, predominantly deriving from macropodids. This is in line with subsistence patterns of macropodids, which are predominantly based on grasses (Wallis 2001:110). As macropodids do not typically eat starches in the wild, it is unlikely that faecal pellets account for the starch grains and associated material in the CG1 sediments. We therefore think that it is unlikely that animals, wind, or water action account for the starch material in CG1. This is also the conclusion that Wallis (2001) reached for the diatoms and sponge spicules recovered from the CG1 sediments.

No similar taphonomic studies have been undertaken at CG3; however, the same factors should be considered when assessing potential agents of microfossil deposition into the archaeological deposit. The excavations at CG3 were carried out in the deep lower cave about 17 m inside the drip line (O'Connor *et al.* 2014). Due to the steep talus that has to be descended to enter the lower cave, we consider CG3 less likely than CG1 to be used for shelter by macropodids, and no faecal pellets were observed on the surface of the deposit at the time of the initial (1993) or subsequent (2012) excavation. Crevices in the lower cave could provide suitable lairs for the northern quoll (*Dasyurus hallucatus*), and dens for carnivores such as Tasmanian devils, thylacines, and dingoes. Carnivores can be ruled out and while the northern quoll is omnivorous, the vegetal component of its diet consists of fruit and nectar. It is unlikely that it would even be able to access the starch grains and associated material of *Dioscorea transversa* tubers or *Colocasia esculenta* corms. Due to the recessed and protected nature of the CG3 lower cave, the sediments are also unlikely to receive much wind-blown microfossil material, and while water seepage through fissures in the limestone saturates the deposit seasonally, microfossils derived from the limestone and sedimentary rock forming the range would more likely be from marine organisms and would not contain starch material.

5.2. Type 1 starch – cf. Dioscorea transversa starch and associated material

Dioscorea transversa (Dioscoreaceae, yams) is endemic to eastern and northern Australia (Govaerts *et al.* 2007). The starch grains of cf. *D. transversa* tubers separated from sediments in this study appear very similar in size and shape to those of this species in previous studies (Field *et al.* 2016; Hayes *et al.* 2021) (Figure 2, 4, 5). The styloids and xylem match also (Figure 3A-D, 6A). The reference material we examined showed grains measuring up to a maximum of 75 μm in longest diameter, with the majority up to approximately 55 μm. The styloids measured approximately 5 μm wide and up to 115 μm in length, with straight and occasionally bevelled ends. We did not observe starch grains from any other northern Australian *Dioscorea* spp., namely *D. bulbifera*, native to northern Australia, nor *D. alata*, thought to be a late Holocene introduction (Denham *et al.* 2009). Starch of these two species can be differentiated from that of *D. transversa* (Loy *et al.* 1992).

The high visibility in cross-polarised light shown by many of the starch grains in this study is possibly a result of permineralisation via silicification (Figure 2O-S). Studies of palaeo-macrobotanical material have shown this effect (Butts and Briggs 2011). This transformation has been carried out experimentally in the laboratory using silicifying solutions, including with modern reference *D. praehensilis* starch grains (Mercader *et al.* 2018:Figure 2).

Another yam that can be considered in our study is *D. hastifolia*, the second of Australia's two endemic species of this family. Although endemic to SW Western Australia and never reported in the Kimberley, we nonetheless describe its starch grains and calcium oxalate crystals here for the first time (Appendix: Figure A1, A2) and compare these with those of *D. transversa*. The *D. hastifolia* tuber material we examined showed elongated ovoid grains, with eccentric hila, measuring up to 80 μm in longest diameter, with the majority up to approximately 60 μm. Styloids were present, measuring up to 5 μm wide and up to 123 μm in length, and tapering to a point at both ends. Notwithstanding its logical geographic preclusion in our case, this species can thus be ruled out based on starch and styloid morphology. Its starch grains are notably more elongated and less ovoid than those of *D. transversa* (Figure 4, 5) and its styloids appear to always taper to a point at both ends, with no straight ends observed. We did not find any styloids like those of *D. hastifolia* in our study.

Although some other plant families produce calcium oxalate crystals, we consider the styloids (and xylem) in this study as supporting evidence for *D. transversa* starch. Starch of *D. transversa* has previously been identified on Holocene grinding stones from Arnhem Land (Hayes *et al.* 2021).

Used extensively by Indigenous groups across Northern Australia, *D. transversa* tubers are a dry-season staple food (Low 1988) more nutrient-dense than the modern introduced equivalent, the potato, being considerably higher in carbohydrate, protein, fibre, zinc, and iron (Brand *et al.* 1983). The small young tubers can be eaten without any preparation (Maiden 1889). On Kija and Jaru Country in East Kimberley, the long *D. transversa* tubers are documented as eaten raw or lightly roasted, cooked in ashes and hot coals, the flesh and taste similar to that of sweet potato (Wightman 2003). The top of the tuber was left attached to the tendril of the vine when the rest of the tuber was harvested, ensuring that the tuber would grow again the following year. There are reports of yams being planted on offshore islands (Karadada *et al.* 2011; O'Dea *et al.* 1991). Beyond food, the importance of *D. transversa* in Aboriginal lifeways is demonstrated visually as one of the most common motifs identified in Kimberley plant-depictive rock art (Grey and Balanggarra Aboriginal Corporation 2024).

5.3. Type 2 starch – cf. Colocasia esculenta starch and associated material

5.3.1. Features of Colocasia esculenta starch and associated material

Colocasia esculenta (Araceae, aroids) is native to tropical northern Australia (Hay 2011; Matthews *et al.* 2014). The starch grain masses and amyloplasts, and calcium oxalate crystals and xylem of cf. *C. esculenta* starch grains separated from sediments in this study (Figure 7-10) appear very similar to those viewed in previous studies (e.g., Burley *et al.* 2018; Crowther 2005; Flexner *et al.* 2024; Horrocks *et al.* 2007, 2008a, 2008b, 2011, 2012a, 2012b, 2015, 2020, 2022, 2023a, 2023b, 2024a, 2024b, 2025a 2025b, 2025c; Horrocks and Nunn 2007; Horrocks and Thomas 2022; Horrocks and Weisler 2006; Kirch *et al.* 2024; Loy *et al.* 1992, Peterson *et al.* 2020).

Of the described pre-contact economic starch taxa of Oceania, *C. esculenta* has among the tiniest grains, although there has been inconsistency in the reporting of grain size, as shown in the following examples. Starting at the smaller end, Pérez and Bertoft (2010:391) give a size of "Submicrons-2 μm". All the studies listed immediately above that involve our microbotanical analyst (MH) generally give a size range of ≤4 μm for almost all grains. Using their graph showing starch grain mean sizes (μm) and standard deviation of modern reference plant species, Loy *et al.* (1992:906) show a size of 2-6 μm. Zhang *et al.* (2024) compared two *C. esculenta* varieties under drought stress and found that during this 20-week trial, one variety (Longxiang) gave a size of 1.25-4.00 μm with no occasional larger grains, while the other (Xiangsha) gave a size of 1.0-3.5 μm with some grains showing >6 μm. Coster *et al.* (2025) give a size of 2.53-9.87 μm. Payne *et al.* (1941) give an average size of 4.5 μm, with a minimum size of 2.5 μm and a maximum size of size of 9.3 μm. Amin (1955) gives a size of 3-10 μm (from Seideman 1966). Seidemann's (1966) starch atlas gives a size of 3-15 μm, increasing in large grains up to 21 μm. There appears to be little information regarding *C. esculenta* starch grain size in Reichert's (1913) work. Further research on this variation is obviously required, especially given that Seidemann's (1966) maximum grain size is twice that of the next largest several reports. This does not discredit the innovative starch work in these studies; compared with pollen and spores, we are in some respects still at the pioneering stage of palaeo-starch research. Thus, at this stage of the research, consideration should be given to grain size variation as a result of variable environmental conditions (especially drought), age of corm, and possible inherent differences between varieties (Zhang *et al.* 2024), and associated lines of evidence such as calcium oxalate crystals and xylem, and biogeographic contexts. Studies involving other starch-rich crop species have also shown variation in starch grain size due to drought stress. For example, Li *et al.* (2015) compared endosperm starch grains of two wheat (*Triticum aestivum*) varieties under drought stress and found a significant reduction in grain sizes in both varieties, with the reduction in wheat cv. Xindong 23 six times more pronounced than that for wheat cv. Xindong 20.

Colocasia esculenta starch has several distinctive features but can be challenging to identify because of its relatively tiny size (Horrocks *et al.* 2023). The corm amyloplasts contain hundreds or thousands of densely packed starch grains, are ovate, up to approximately 185 μm in diameter, and have very thin enclosing double membranes. The individual starch grains appear sharply angular, while the central vacuole often appears as a dark gray/black dot, giving a grainy appearance. The tiny grain size is near the limits of light microscopy, so grains are often best looked for in archaeological samples as groups, that is, amyloplasts or fragments thereof. In archaeological sediments, *C. esculenta* amyloplasts are commonly fragmented and without visible membrane tissue. Often though, cell wall and/or membrane outlines can be seen in starch masses as thin curved lines (Figure 7-9, 11). Very well-preserved examples of this can be seen in the previous microfossil studies of Crowther (2006:Plate 58), Horrocks *et al.* (2024a:Figure 12A, B), and Horrocks *et al.* (2024b:Figure 11J.) Distinctly visible aggregations of starch grains, perhaps better described as compound grains, can also occur within *C. esculenta* amyloplasts (Figure 9C, D, 11). When comparing the very small starch grains of cf. *C. esculenta* with taxa with similar sized grains, it is important to note that these distinctive features can thus often only be seen in masses of starch, rather than with a few grains.

Although some other plant families produce calcium oxalate crystals, we consider the raphides and druses in this study as supporting evidence for *C. esculenta* starch. Aroids generally have very high concentrations of calcium oxalate crystals in both their shoots and subterranean tissues (Sunell and Healey 1979) compared to many other plant families. Xylem with helical wall thickening is also produced by some other taxa, including other aroids and the Poaceae family. Nonetheless, when this xylem type is found in association with cf. *C. esculenta* starch and crystals and is only one of two different xylem types found as in this study, it could be considered supporting evidence.

Indigenous groups across the Kimberley region eat the large "elephant-ear" shaped herbaceous leaves and starchy corms of *C. esculenta* after cooking/roasting in hot coals (Karadada *et al.* 2011; Wightman 2003), a process that dissolves the soluble, sharp acrid crystals that are caustic when raw (King and Horsfall 2023). Early ethnographic observations of this species in northern Australia are summarised in the work of Denham *et al.* (2009).

5.3.2. Consideration of other possible Kimberley sources of starch Type 2

The seed record from CG1, ascertained from previous macrobotanical studies, showed peaks of seeds of several taxa that we consider in relation to our starch findings: Chenopodiaceae-Amaranthaceae (Cheno-Am) (McConnell and O'Connor 1997), *Terminalia* spp., *Vitex* cf. *glabrata*, and cf. *Grewia breviflora* (Dilkes-Hall *et al.* 2019). Modern reference seed material of *V. glabrata* and *G. breviflora* we examined did not reveal any starch grains (M. Horrocks, unpubl. data).

Regarding Cheno-Am, a survey of the literature strongly suggests that the vast majority, if not all, Chemo-Am taxa have starch grains virtually less than 3 μm, with many taxa less than 2 μm or 1 μm (e.g., Reichert 2013; Seidemann 1966). As a local Kimberley example of Cheno-Am, seed material of *Atriplex bunburyana* examined showed clumps of starch grains <5 μm (M. Horrocks, unpubl. data). The fossil starch of this study is unlikely to relate to this taxon because the latter lacks the distinctive large ovoid, thin walled amyloplast shape of *Colocasia esculenta*. Seed material of *Terminalia ferdinandiana* we examined showed a very similar starch result to that of the Cheno-Am (M. Horrocks, unpubl. data).

Several other taxa with starch grains similar or a little larger in average size to those of *C. esculenta* can likely be excluded on the basis of other morphological features. Starch grains of the tissues of the widespread native Australian grass (Poaceae) taxon, *Triodia* (spinifex) spp., can be ruled out based on their size, with a mean size of 8.65-9.01 µm and a maximum size up to 16.7 µm (Hayes *et al.* 2018). Starch we examined from the endosperms of another widespread native grass, *Dactyloctenium radulans* (button grass), lacks the distinctive large ovoid, thin walled amyloplast shape (M. Horrocks, unpubl. data). *Oryza australiensis* (Australian wild rice), a grass native to monsoonal northern Australia, can be ruled out because it also lacks the amyloplast shape, its largely angular starch grain morphology includes many spherical grains, its grains average larger size (around 7 µm at the centre of the endosperm), and its compound grains comprise far fewer individual grains (Kasem *et al.* 2011). In addition, almost all members of Poaceae do not produce significant amounts, if any, of calcium oxalate crystals (see Prychid and Rudall [1999] for a review of the crystals in Monocotyledons). Starch grains of two starch-rich Kimberley Indigenous economic taxa (McMahon 2006) that can be ruled out because they are much larger, are those from the tubers of *Amorphophallus galbra* and rhizomes of *Nymphaea violoceae* (Hayes *et al.* 2021).

5.3.3. The lack of Colocasia esculenta in Australian palaeo-records

Only one record of *Colocasia esculenta* pollen appears to exist for Australia, which comes from a sediment core collected from Lake Euramoo, northeast Queensland, dated to around 10,000 BP (Haberle 2005). This pollen paucity is not surprising as this species is entomophilous and produces little pollen, which has particularly poor preservation and is prone to damage during the acetolysis process, and thus rarely recovered (Nusaifa Beevi 2009). It is worth noting also that *C. esculenta* produces “no to few phytoliths of little to no taxonomic significance” (Piperno 2006:Table 3.2), which can limit its visibility in phytolith records.

5.3.4. The distribution of Colocasia esculenta

The antecedent of *Colocasia esculenta* evolved in Southeast Asia (Matthews 1991, 2014) and is thought to have expanded to Sahul during the late Miocene to late Pliocene (Ahmed *et al.* 2020, Matthews 1991). A recent study of DNA diversity in cultivated and wild *C. esculenta* specimens did not support the hypothesis of domestication in New Guinea (Ahmed *et al.* 2020). However, it is possible that this species underwent recurrent localised domestications across the Indo-Pacific in the past, with recurrent subsequent marginalisation and loss of many local varieties following sequential introductions of cultivars, initially diploids and then triploids, from a few source regions on mainland and Island Southeast Asia (Ahmed *et al.* 2020; Denham 2018; Denham *et al.* 2009). As with most cultivars of starch-rich economic taxa, *C. esculenta* cultivars tend to produce larger starch yields.

The natural distribution of *C. esculenta* includes the northern tropical regions of Western Australia, Northern Territory, and Queensland (Hay 2011; Matthews *et al.* 2014). Several inland populations have been identified in the Kimberley region (see herbaria specimen map in Denham [2009:638]). It grows in open areas with permanent fresh water: creeks, billabongs, swamps and splash-zone of waterfalls, at low to moderate elevation. In the Kimberley it occurs partially submerged in, or at the edge of, watercourses (Rye

1992). The corms are an Indigenous food in the region (Rye 1992:982), and in the Top End of the Northern Territory the tubers are harvested during the wet season (Cowie 2000:215).

5.4. Euglenoid cysts

The euglenoids that the Carpenters Gap 1 and 3 cysts are from inhabit shallow freshwater ponds and freshwater environments with abundant organic matter (Gojdics 1953; Hindák *et al.* 2000; Leander *et al.* 2017). These aquatic markers, presumably reflecting processing of wetland/aquatic resources at the sites, e.g., freshwater mussels (see O'Connor *et al.* 2014: Table 1), Cyperaceae, and presumably *Colocasia esculenta*, were first recorded archaeologically in the New Guinea Highlands (Horrocks *et al.* 2025c). Wallis' (2001) microfossil research previously revealed other aquatic markers at CG1, in the form of diatoms and sponge spicules.

6. Conclusion

The identification of starch, calcium oxalate crystals, and xylem throughout much of both the CG1 and 3 rockshelter profiles strongly suggests Indigenous gathering and on-site processing of starch-rich economic plants since c. 30,000-25,000 cal BP. The results show promise for separating starch material from sediments in Australian archaeological contexts, providing an additional or alternative line of evidence to starch analysis on stone tools. The results also broaden our understanding of Indigenous plant use at CG1 and 3 as yams, while recorded as an important food resource in the Kimberley ethnobotanic literature, were not identified in previous archaeobotanical analyses. Euglenoid cysts provide further supporting evidence for gathering wetland/aquatic resources.

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Data Availability Statement

Data is available on request from the authors.

Partnerships

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

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualisation, M.H.; investigation, M.H., I.D-H.; resources, M.H., S.O., I.D-H., writing–original draft preparation, M.H.; writing–review and editing, M.H., S.O., I.D-H.; visualization, M.H.; funding acquisition, M.H.

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Appendix A

Photomicography of *Dioscorea hastifolia* starch grains, styloids, and xylem.

Figure A1: Modern reference *Dioscorea hastifolia* starch grains, showing typical elongated ovoid shape. 400x; black background = cross-polarised light (CPL); scale bar = 20 μm.

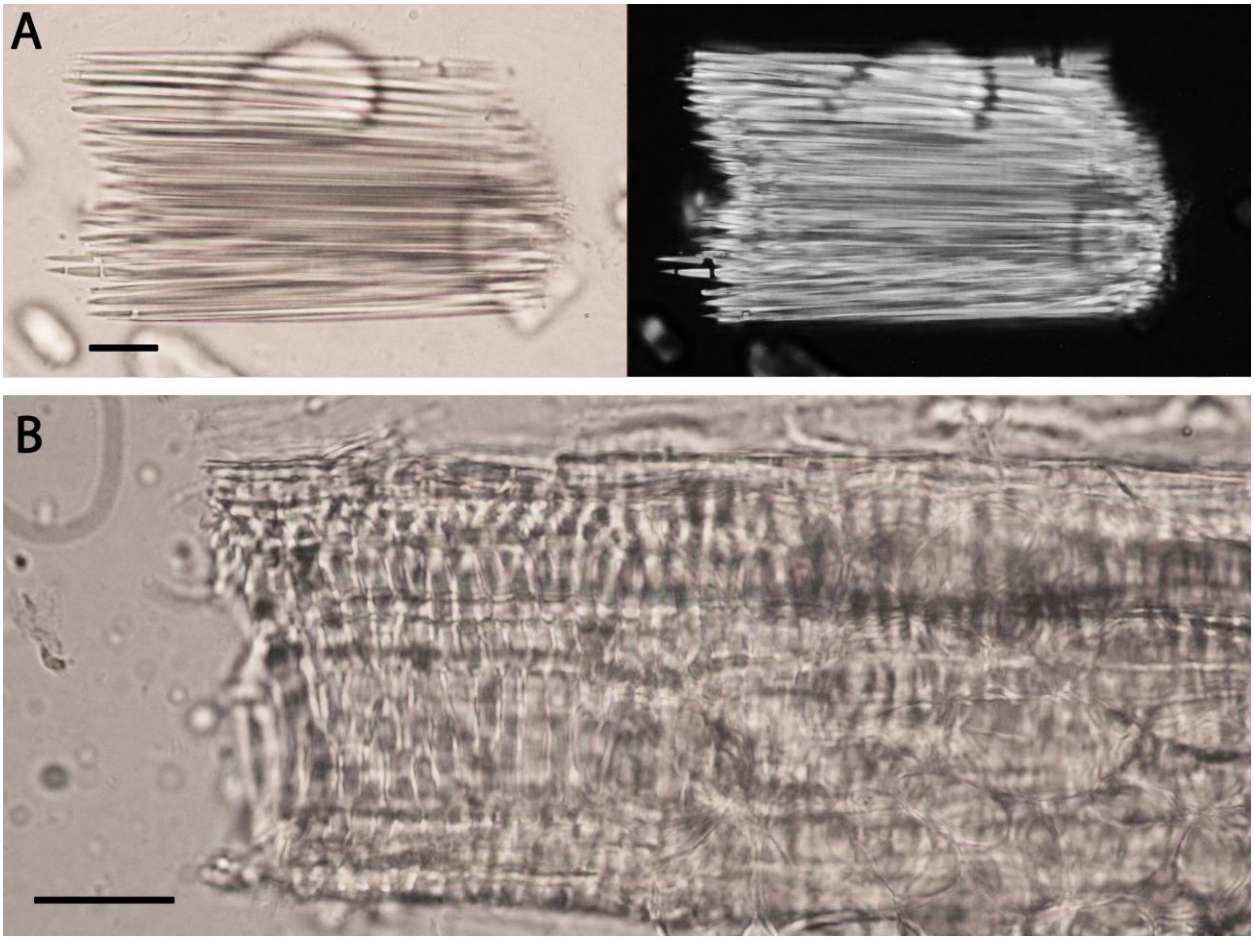

Figure A2: Modern reference *Dioscorea hastifolia* styloids (400x) and xylem (600x). Black background = cross-polarised light (CPL); scale bars = 20 µm. A: bundle of styloids, showing characteristic ends tapering to a point. B: part of xylem bundle with characteristic small slit-like pits in the cell walls.

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