



Starchy food residue on a potsherd from a late Holocene hunter-gatherer site in Argentine Patagonia: towards the visibility of wild underground storage organs

Maria Laura Ciampagna¹ · Soledad Molares² · Ana Haydeé Ladio³ · Aylen Capparelli¹

Received: 30 November 2019 / Accepted: 29 November 2020 / Published online: 22 January 2021
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Abstract

The aim of this paper is to present recent advances in the microbotanical analyses of an organic residue on a potsherd from a late Holocene hunter-gatherer site in Argentine Patagonia, which is the first evidence of this kind for the processing of starchy food. Standard methods were carried out for starch grain recovery and morphometric analysis, care being taken in the prevention of cross-contamination and evaluation of post-depositional factors. Diagnostic features of the ancient starch grains recovered were compared against those from a list, available in the bibliography, of plants potentially processed in pottery, but no match was found. An anatomical and starch grain reference collection was established with three of the most frequently consumed traditional wild underground storage organs (USOs) of Patagonia, *Alstroemeria aurea* Graham, *Tropaeolum porifolium* Cav. and *Diposis patagonica* Skotts., in order to compare these against the archaeobotanical record. We suggest that *T. aff. porifolium* (and probably also *A. aff. aurea*) were processed in the pot from which the sherd came, and discuss these results in terms of a better understanding of the role of wild USOs in subsistence and the possible cooking methods used in hunter-gatherer societies in Argentine Patagonia.

Keywords Patagonian archaeobotany · Pottery · Microbotanical studies · Tropaeolum · Diposis · Alstroemeria · Starch grains

Introduction

The purpose of this paper is to present recent advances in the microbotanical analyses of an organic charred residue on a potsherd from a late Holocene hunter-gatherer shell midden

(site 3) at the Monte Loayza locality, on the coast of northern Santa Cruz Province, our study area, in Argentine Patagonia. This kind of analysis is very scarce there, and this work in particular constitutes the first evidence from residues on pottery of the processing of starchy food. Diagnostic features of the ancient starch grains recovered were compared against those from a list of plants potentially processed by local hunter-gatherers in the past, if starch grain descriptions were available in the bibliography. However, no match was found there with the ancient samples. As no available data existed for three of the traditionally most frequently-consumed wild underground storage organs (USOs) of Patagonia, a reference collection was established in order to compare them against the archaeobotanical record, which allowed us to suggest a taxonomic identification and to evaluate possible cooking methods used on the archaeologically recovered sample, as presented below.

Communicated by G. Fiorentino.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00334-020-00818-7>.

✉ Aylen Capparelli
aylencapparelli@fcnym.unlp.edu.ar

¹ División Arqueología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata-CONICET, Av. 122 y 60 s/n, CP 1900 La Plata, Buenos Aires, Argentina

² CIEMEP, Universidad Nacional de La Patagonia-CONICET, Gral. Roca 780, CP 9200 Esquel, Chubut, Argentina

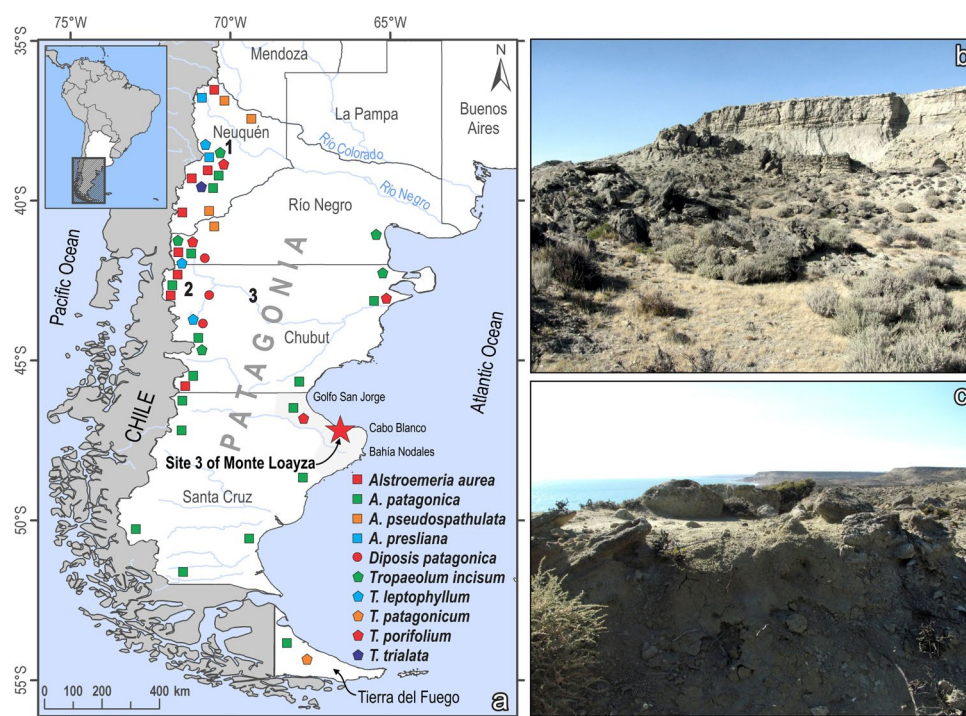
³ INIBIOMA, Universidad Nacional del Comahue-CONICET, Quintral 1250, CP 8400 San Carlos de Bariloche, Río Negro, Argentina

The archaeological background and characteristics of the pottery record in Patagonia

Argentine Patagonia is a very rich archaeological region where hunter-gatherer subsistence persisted from the very beginning of settlement in America, 14,300/13,000 yrs cal BP, until the 19th century (Prates et al. 2016, 2020; Llano et al. 2020). The coast of northern Santa Cruz Province in particular, located in southeastern Patagonia (Fig. 1a), has provided evidence of its earliest occupations at ca. 8,000–7,000 BP. These were found in rock shelter sites just inland from the coast, as well as in shell midden sites distributed on the Atlantic coast (Zubimendi et al. 2015). The rock shelter sites had a low density of archaeological material, suggesting seasonal occupations of short duration, while the shell midden sites showed a higher density of archaeological material and repetitive occupation over time, especially during the late Holocene, when there was intensive use of marine resources for subsistence (Zubimendi et al. 2011). It is during this last period that there were wider mobility patterns and more intense social interactions took place between hunter-gatherer groups (Zubimendi and Ambrústolo 2016), and new technologies appeared, such as ceramics. The pottery record for Patagonia in general, and for the coast of northern Santa Cruz in particular, is very scarce, fragmentary, frequently associated with surface deposits, and of late appearance in prehistory. Only a few potsherds were recovered from stratigraphic excavation in the study area, which ranged chronologically between

1,420 ± 50 yrs BP and 370 ± 50 yrs BP (Roumec et al. 2020). The dates of the earliest pottery record in this area are similar to those of other areas of Patagonia, for example ca. 1,500–1,200 BP in the north, ca. 1,100 BP in the southwest, and ca. 900 BP on the coast of central Patagonia (Politis et al. 2001; Cassiodoro and Tessone 2014; Schuster 2014). In our area, the pottery record is associated with shell midden sites, as well as with *chenque*, burial structures under stone mounds. At Monte Loayza a large number of fragments of decorated pottery were recovered; while some belonged to edges, a very small number were recognized as base remains. These different proportions are thought to occur because of the probably relatively low visibility of bases in the predominantly globular or oval shape of the pots (Roumec et al. 2020). The technical and morphological features of this material are still under study, as are their origins, but for other coastal Patagonian areas the pottery was locally made (Schuster 2014). Several hypotheses have been proposed for the use of pottery by hunter-gatherers in Patagonia, all of which consider it multifunctional. The pots were thought to have served for the processing and storage of fats (Cassiodoro and Tessone 2014), increased use of plants for food (Gómez Otero 2006), preparing fermented drinks and cooking food, based on ethnohistorical written sources (Prates et al. 2016). It is interesting to note that while $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic analysis of organic residues from ceramics from the central plateau of Patagonia demonstrates the processing and cooking of animals from the arid steppe (Cassiodoro and Tessone 2014), fatty acid,

Fig. 1 Argentine Patagonia, **a**, location of Monte Loayza Site 3 on the coast of northern Santa Cruz (shaded in light grey), as well as of the archaeological sites with direct archaeobotanical evidence of USOs and the modern distributional data on *Alstroemeria* spp., *Tropaeolum* spp. and *Diposis patagonica* in Argentine Patagonia (based on Sanso 1996; Rapoport et al. 1999; Zuloaga and Morrone 1999; Andersson and Andersson 2000; SIB 2002; Garralla and Bulacio 2011; Muñoz-Schik and Moreira-Muñoz 2013; Bulacio et al. 2017); **b–c**, general view of Monte Loayza Site 3; **1**, cueva de Haichol; **2**, Sendero de Interpretación rockshelter; **3**, Campo Moncada 2 rockshelter



isotopic and chromatographic results from potsherd residues from the lower course of the Río Colorado, in northern Patagonia, and from the coasts, suggest mainly the processing and cooking of plant foodstuffs, and to a lesser extent, the cooking of meat of land animals such as *Lama guanicoe* (guanaco) or marine fish, as well as the extraction or storage of fats from seal-like pinnipeds or fish (Gómez Otero 2006; Schuster 2014; Stoessel et al. 2015).

Microbotanical analysis, starch grains and potsherds

Archaeobotanical research dealing with the recovery and identification of plant microremains from organic residues on potsherds contributes to the discussion of the use of these pots, and provides knowledge about the plant resources used and their processing methods. This leads to a better understanding of the role plants may have played for ancient human peoples (Samuel 2006; Zarrillo et al. 2008; Crowther 2012; Barton and Torrence 2015; among others). However, there is only one antecedent for this kind of study of Patagonian hunter-gatherer groups in Argentina, carried out by Pérez and Erra (2011) on potsherd fragments from the Meliquina site, Provincia Neuquén, northwest Patagonia, dated ca. 730–920 yrs BP, where processing of *Zea* (maize) was identified from diagnostic phytoliths. In contrast, the study presented here from the Monte Loayza Site 3 potsherd (Fig. 1a) is the first where starch grains have been identified. Starch grain research has been carried out throughout the world on different kinds of archaeological material, such as stone tools, residues on potsherds, dental calculus and the soil matrix. It has proved to be useful in answering questions about the ancient use of starchy food, as well as contributing to various insights on cultivation, domestication and past human diet (Loy 1994; Torrence and Barton 2006; Copeland and Hardy 2018; Kovárník and Benes 2018; among others). In recent years, particular emphasis has been placed on the need for this kind of study to give detailed descriptions of, firstly, the processes leading to formation of the archaeological context under study, as well as post-depositional processes that may have occurred and affected the starch deposits; secondly, actions undertaken to prevent contamination of archaeological samples with modern starch; and thirdly, the care that must be taken to consider all these factors when taxonomic identification is proposed (Mercader et al. 2017, 2018; Copeland and Hardy 2018). The formation processes of charred residual organic potsherd deposits are related, on the one hand, to the capacity of the porous material of ceramic bowls to facilitate the absorption of organic particles onto the vessel wall, by drawing moisture from the food (Skibo, in Crowther 2012). On the other hand, they are related to many factors; the method of cooking in a pot and the conditions of moisture and

temperature; the way of cooking such as roasting or boiling; the type of raw material, whole plant parts or flour; the physical and chemical properties of the starch grains and interaction with other organic compounds such as lipids, proteins, enzymes, acids and salt; the method of heat transfer (convection or conduction); environmental factors such as air pressure, which determines the temperature reached at high altitudes; and finally, the type of fuel used (Samuel 2006; Crowther 2012; Barton and Torrence 2015). Depending on the combination of these variables, the structure and morphology of starch grains may be affected to a greater or lesser extent. It has been demonstrated that, in general, starch grains heated in the absence of moisture do not undergo structural or morphological changes up to relatively high temperatures (greater than 200–220 °C) and that the amount of accessible water in the cooking method is one of the most important factors leading to gelatinisation, the irreversible structural and morphological change of the starch grains, evidenced by swelling, loss of crystallinity and irregularity of shape, which rarely occurs at water content levels under 30–35% (Crowther 2012 and references therein). Also, even when conditions are suitable for gelatinisation, this is not a homogeneous or immediate process, due to variations in the structure and composition of particular grains, even within the same plant species. For this reason gelatinisation of an entire sample may occur after an increase of 5/6 °C beyond that needed for alteration of the first starch grains (Reichert 1913; Crowther 2012 and references therein). As previously mentioned, subsequent post-depositional processes may also affect the grains after they have been archaeologically deposited. These processes may lead to disruption of the crystallinity, cavitations, centric implosion (degradation of the central part of the granule), granulation or lumps on the surface of the grain, long narrow cracks or fissures from the edge to the edge inwards and gelatinisation, among other visible features (Mercader et al. 2017, 2018). They may be caused by microorganisms, chemical reactions of the grains with other molecules, and by environmental factors such as soil temperatures of ~22 °C, which might lead to gelatinisation even in conditions of low moisture content (Mercader et al. 2017, 2018). From the section above it is clear that the histological features of starch grains, such as shape, size, hilum (attachment scar) and aggregation, together with other properties, depend on genetic composition and are often characteristic of a plant taxon (Reichert 1913; Loy 1994; Gott et al. 2006). However, identification should also consider that the structure and morphology of starch grains may be affected by the depositional and post-depositional factors mentioned above (Mercader et al. 2017, 2018; Copeland and Hardy 2018). It must also be considered that different taxa may have similar starch grains, and that starch grains can vary

within a single species (Reichert 1913; Hardy et al. 2018). Finally, any potential cross-contamination of the sample must be prevented (Samuel 2006; Mercader et al. 2017, 2018) (see materials and methods section).

When identifying starch grains, comparing the archaeological material with a modern starch reference collection is essential (Mercader et al. 2017; Kovárník and Benes 2018). However, no such collection existed for Argentine Patagonia. Therefore, the starch recovered from the Monte Loayza potsherd was first compared with the available published data on starch grains which could have been processed, stored or consumed in ancient Patagonian pots (see results section), however, no positive match was found. It was also found that except for the description of starch grains of Chilean specimens of *Alstroemeria aurea* by Reichert (1913), no published information exists comparing the starchy tubers of *Tropaeolum* (Tropaeolaceae), *Diposis* (Apiaceae) and *Alstroemeria* (Alstroemeriaceae) from Patagonia. These three genera, all from the Americas, along with *Arjona* and *Oxalis*, are the USOs most frequently mentioned in historical documents referring to Patagonia (Ochoa and Ladio 2011; Ciampagna and Capparelli 2012). Therefore, we have assembled a reference collection of *T. porifolium*, *A. aurea* and *D. patagonica* and described the morphology and anatomy of the tubers and morphology and size of the starch grains, to compare with the archaeological sample from Monte Loayza.

Underground storage organs and their archaeological relevance

It is well documented that USOs have played an important role in the diet of hunter-gatherer and foraging societies all over the world, from very early times and throughout hominid evolution (Laden and Wrangham 2005; Wollstonecroft et al. 2008; Singels 2013; Wadley et al. 2020; among others). Modern ethnographic works record the use of a wide range of wild USOs still used as food and medicine, for example, in Africa, Australia and North, Central and South America (Ugent 1994; Deur and Turner 2005; Laden and Wrangham 2005; Beck and Torrence 2006; Gott et al. 2006; Singels 2013; among others). Although USO taxa are managed either in rainforests or semi-arid regions, it is thought that there are larger numbers of them in the latter, where they have special importance for humans as fallback food, especially during summer (Laden and Wrangham 2005; Marlowe and Berbesque 2009; Molares and Ladio 2012). Modern ethnobotanical work carried out with Creole and Mapuche communities in Patagonia confirmed that *Tropaeolum*, *Diposis* and *Alstroemeria* are still consumed nowadays, although now less so compared to earlier times in the region (Ladio 2006; Ciampagna 2015). Despite their current marginal use, these taxa generally occupy an important place

in the social memory of local people. They are currently being redefined as plants, which represent a connection with ancestors and their practices (Ladio 2006; Rapoport et al. 1999, 2003; Ochoa and Ladio 2015). In contrast, archaeological evidence of remains showing the importance of USOs in Patagonia is limited to a few botanical finds, all of which come from the late Holocene and from the western steppe of Chubut and Neuquén provinces, as follows: one root tuber aff. *Oxalis articulata* Savigny, recovered in a charred state and associated with a hearth in the Sendero de Interpretación rock shelter site (Arrigoni 2002); one root tuber aff. *O. hypsophila* Phil. recovered from the Haichol cave (Ancibor 1988–1990) and two desiccated specimens, one of *Tropaeolum* sp. and another cf. *Arjona tuberosa* Cav. from Campo Moncada 2 rock shelter site (Fig. 1a; Pérez de Micou et al. 1992). The scarcity of tuber macroremains is probably due to several taphonomic, cultural and methodological factors that affect the visibility of parenchyma (structural plant tissue) in archaeology everywhere (Hather 1993; Berihuete Azorín et al. 2018). Among the most important are, first, the predominance of thin-walled parenchyma tissues resulting in low preservation potential of the tubers (Hather 1993), and second, past processing practices which usually involved grinding and/or cooking the tubers, leading to the loss of tissue integrity (Berihuete Azorín et al. 2018) and finally, the lack of sizeable parenchyma reference collections (Hather 1993, 2000).

Tropaeolum, *Diposis* and *Alstroemeria* in Patagonia

Ten species of *Tropaeolum* are native to Patagonia (Fig. 1a). *T. porifolium* (previously considered in the genus *Magallana* Cav., Watson and Flores 2010), grows from Mendoza to Santa Cruz provinces (Zuloaga and Morrone 1999) and to Aisén in Chile, and it is abundant in Patagonia (Muñoz-Schick and Moreira-Muñoz 2013), where it has been seen from the west (on the eastern slopes of the Andean mountains) to the eastern coast at Puerto Deseado, Puerto Madryn and Parque Jaramillo (Andersson and Andersson 2000; SIB 2002; Garralla and Bulacio 2011). Its common name is *curuhuilla* or *curuhuil*, and its tubers were reportedly consumed after either being roasted in charcoal or boiled in water (Martínez Crovetto 1982; Rapoport et al. 1999, 2003). Four species of *Alstroemeria* (Alstroemeriaceae) are native to Argentine Patagonia (Fig. 1a). *A. aurea* grows mainly on the eastern slopes of the Andes, from Neuquén to Río Negro and Chubut (Sanso 1996; Sanso et al. 2014). Its common name is *amancaes*. Together with *A. patagonica* (*liuto* or *chaquil*), its tubers were consumed raw or cooked in charcoal or boiled and mashed, or after being roasted and mashed to flour (Martínez Crovetto 1982; Rapoport et al. 1999, 2003). Finally, the only Patagonian native species of the genus *Diposis* is *D. patagonica*, which is distributed

throughout the steppe (Fig. 1a; Zuloaga and Morrone 1999). Its common name is *yocón*, and its tubers were reported to be consumed raw, soaked in milk, roasted, boiled whole or boiled and mashed (Rapoport et al. 2003; Ladio 2006; Ochoa and Ladio 2015).

In sum, this research is considered important for several reasons. Firstly, because of the scarcity of finds of pottery from the hunter-gatherer peoples of Patagonia, marking the turning point in cooking methods following the introduction of pottery during the late Holocene; secondly, because of the almost complete lack of microbotanical analysis of potsherd residues from the region so far; thirdly, because of the scarcity of knowledge about ancient starchy food and USOs of the area, despite being important in ethnohistorical and ethnobotanical evidence; and finally, because there is no comprehensive USO anatomical and starch grain reference collection for the region.

Materials and methods

Monte Loayza Site 3 and its environmental background

The archaeological area of Monte Loayza is located in the south of the Golfo San Jorge, Santa Cruz Province (Fig. 1a); it is an uninhabited area and not easily accessible, which lies nowadays within two zones of nature protection, Parque Provincial Monte Loayza and Reserva Asociada Cañadón del Duraznillo (Zubimendi and Ambrústolo 2017). The area has a cold temperate climate with a mean annual temperature of 10 °C (16 °C in summer, 4 °C in winter) and 200 mm precipitation per year (De Fina and Ravello 1973). The soils are of the aridisol type, shallow, stony, poor in organic and fine materials, pH neutral, and with moderate to high salinity (Oliva et al. 2001). The vegetation of the area is open shrub steppe of *Colliguaja integerrima* Gillies & Hook and *Mulinum spinosum* (Cav.) Pers., belonging to the Patagonian phytogeographical province (Cabrera 1971; Oliva et al. 2001). Pollen records demonstrate that during the late Holocene the vegetation of the area was similar to modern times (De Porras 2010). A potsherd was recovered from the ground surface at late Holocene Site 3 of Monte Loayza, located on a coastal terrace (Fig. 1b–c), and was sampled for this analysis. Site 3 is a shell midden still under study, where the soil pH is alkaline, making conditions favourable for preservation of organic residues (Babot 2007; Hammond 2015).

The potsherd and microbotanical recovery

The potsherd analysed here consists of a grey clay fragment 3.5 cm wide, 6.5 cm tall and 0.5 cm thick, which, together with three other fragments, formed part of the rim

of a bowl of approximately of 12–16 cm in diameter. It is a finely brushed, decorated ceramic piece of a type recovered from sites such as Monte Loayza, Bahía Lángara and Punta Medanosa, and rims are particularly frequent at Monte Loayza. The decoration consists of a continuous line under and parallel to the rim, and perpendicular to it, regular lines curved like claws (Fig. 2a; Roumec et al. 2020). The potsherd had a charred organic residue on its inner surface, which was considered as evidence that the pot had been used for cooking (Crowther 2005). This was the reason it was selected for analysis. Immediately after collection, it was placed in a separate plastic bag at the archaeological site to prevent cross-contamination. Once at the laboratory the surface of the potsherd was first cleaned with a synthetic cloth (Samuel 2006). Then the residue area was sampled by the technique suggested by Coil et al. (2003) in order to allow recovery of many microfossils with the least possible damage (Fig. 2b). Following Lema et al. (2012), two kinds of dry subsamples were obtained, the first by light brushing of the surface with a disposable brush and the second by scraping the ceramic cavities with a metal scalpel, which had been sterilized by heating to a high temperature and cooling. In the absence of sediment from the site, one sample was recovered in the same way as the previous one, but from the non-active external surface of the pot, and was used as a control. For each subsample, 20 µL of dry material was extracted and suspended in 50% glycerol (Ugent 1994) in a centrifuge tube, and then spread over four microscope slides with a micropipette with disposable tips and sealed with a cover slip. All the slides were scanned under a Leica DC/LM light microscope with polarized light at 200–500×, and photographed with a Toup 5MgPx camera. Sample recovery and slide mounting were carried out in a room free of any food substances (Loy 1994; Mercader et al. 2017) and inside an acrylic box specially arranged for the extraction of microremains. All manipulation of the fragments was performed with washed hands, since industrial maize starch is frequently found on Argentine gloves. Disposable laboratory supplies (brushes, bags, tubes, slides, cover glasses, tips) were first tested in order to prevent cross-contamination; they were washed with distilled water and the sample was scanned under a light microscope to ensure that it was starch free.

Tuber reference collection

Modern, naturally dried *Diposis patagonica* and *Tropaeolum porifolium* were collected, examined and identified by Ana Ladio from Cañadón Chileno (Pilcaniyeu Department, Río Negro) and the Mapuche Paineo community of La Amarga (Catan-lil Department, Neuquén), respectively. *Alstroemeria aurea* was also collected from Bariloche (Río Negro), and identified by A. Capparelli and L. Borrelli. A

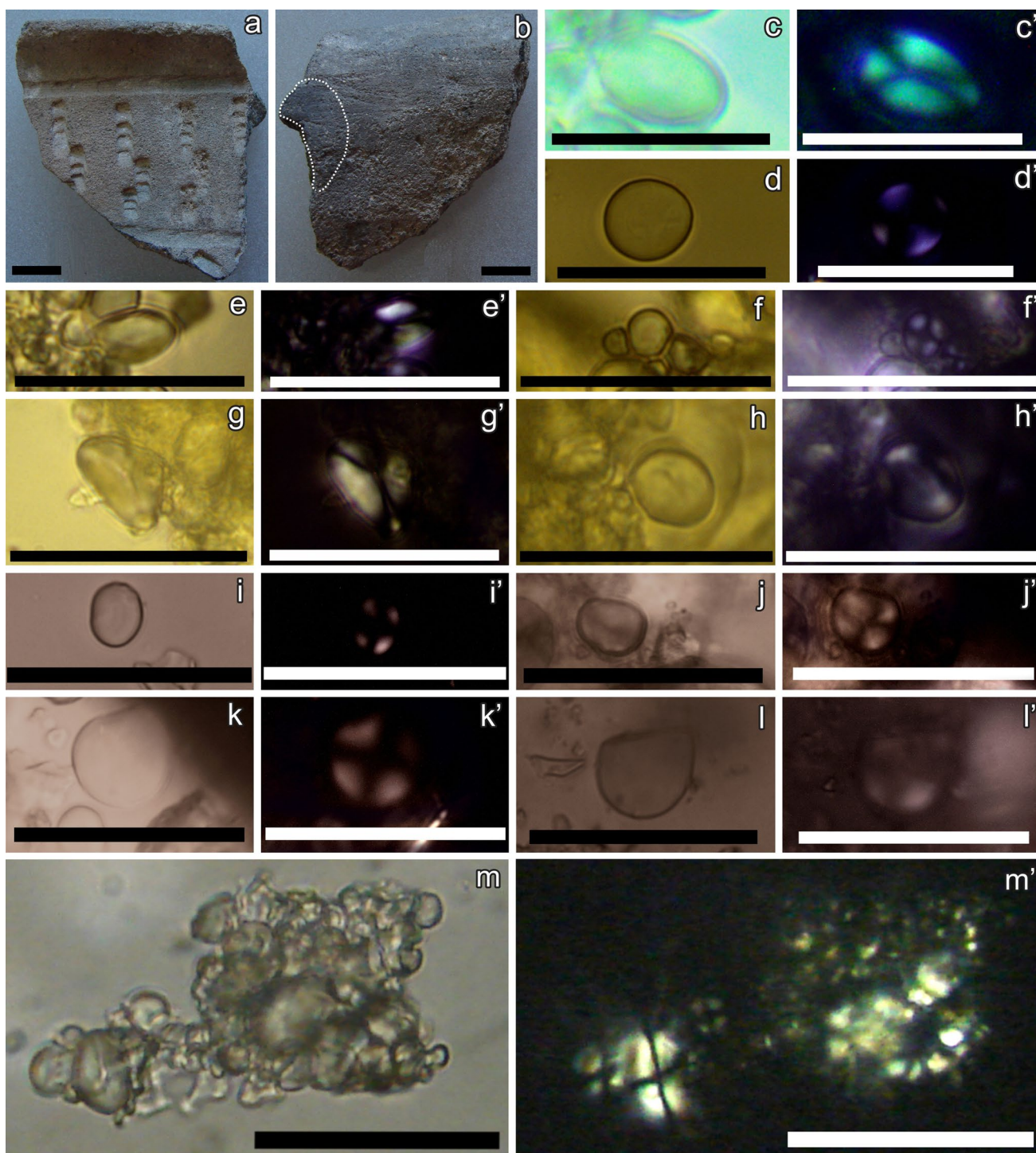


Fig. 2 Monte Loayza Site 3 potsherd, **a**, non-active external face; **b**, active internal face showing area sampled for charred residue (circled); **c–m**, starch grain residues under normal and polarized light; **c**, ellipsoid eccentric punctate hilum, **d**, spherical, diffused polarization

cross; **e**, ellipsoid eccentric elongate hilum; **f**, spherical and hemispherical grains; **g**, conical; **h–j**, ovoid, low birefringence; **k**, pyriform; **l**, hemispherical; **m**, conglomerate with high and low birefringent grains; scale bars, **a–b**, 10 mm; **c–m**, 50 μ m

voucher specimen of each one is deposited in Laboratory 129 of the Archaeological Division of La Plata Natural Science Museum, under the responsibility of A. Capparelli.

Tuber moisture content

The moisture content of *A. aurea* was determined as an average of three individual tubers collected in winter, on a

dry weight basis, methods according to AOAC (1990). The tubers were dried in a Dalvo oven at a constant temperature of 95 °C until a constant weight was reached, in six days. An Ohaus laboratory balance with 0.01 g readability was used to weigh the tubers every day. The percentage of water lost was obtained to evaluate the potential natural moisture content of Patagonian tubers when cooked without adding water (Crowther 2012).

Tuber morphology and anatomy

Morphological classification of the USOs followed Hather (2000). A quarter of each specimen was embedded in paraffin wax following standard protocols (D'Ambrogio de Argüeso 1986), after which transversal sections of 20 µm were obtained using a Spencer Lens Co. rotary microtome. These were coloured with safranin and IKI and mounted on slides in Canadian balsam, then studied and photographed. The anatomy was described, taking into account previous studies on related taxa as presented in Metcalfe and Chalk (1965), Weigandt et al. (2003), Bulacio and Ponessa (2012) and Bulacio et al. (2013, 2017).

Starch grain morphometric analysis

Starch grains were described across a transverse section of the reference tubers under a transmitted light microscope, in order to assess potential differences between tissues (Loy 1994). In this case, the sections were hand prepared with a razor blade, mounted in glycerol, observed under non-polarised and polarised light and photographed. In total, 100 starch grains per tissue were recorded, from the periderm/rhizoderm, cortical (cortex)/radial parenchyma and medullar (pit) parenchyma. The starch grains were turned in order to see their three dimensional shapes. The size range was considered as the length range that included more than 50% of all the grains. Since there is no internationally recognized, peer-reviewed nomenclature for morphometric characterization of starch (Mercader et al. 2017), the variables were

recorded following standard protocols and the nomenclature of Loy (1994), Torrence (2006), ICSN (2011) and Pagan Jiménez (2015). Starch grains recovered from the potsherd were characterized by morphology and size as they were isolated from the remaining material. The presence or absence of damage attributes, such as cavitation (hollows), fractures, radial fissures from the surface, loss of or faded birefringence and irregular or sac-like shape, were evaluated as a possible consequence of the actions of acid, enzymes, heat, freezing and/or grinding, according to the literature (Cortella and Pochettino 1994; Babot 2003; Messner and Schindler 2010; Crowther 2005, 2012; Hardy et al. 2018 and Mercader et al. 2017).

Results

Starch grains from the Monte Loayza potsherd

No other recognizable plant remains apart from starch grains were recovered from the potsherd. All the starch grains came from the sample obtained by scraping the charred residue matrix from the active (interior) face of the pot (Fig. 2c–m), but they were absent from the first brushed sample and the control sample. The recovered starch grains were mostly simple, although a few compounds of two together were observed. Ovoid (Fig. 2h–j, m) and spherical grains predominated (Fig. 2d, f), but ellipsoid (Fig. 2c, e), pyriform (pear-shaped) (Fig. 2k), hemispherical (Fig. 2l) and conical ones (Fig. 2g) were also present (Table 1). No pressure facets (flattened sides from being pressed together) were observed in the grains except for the hemispherical ones. The hilum (attachment point) was distinct or indistinct, spherical, with radial or stellate fissures in spherical and ovoid grains (Fig. 2m) or elongated fissures (Fig. 2h, i). The extinction cross seen with polarised light was centric (in the middle) mostly in spherical and ovoid grains (Fig. 2d, f, i–j) or eccentric in ellipsoid or conical ones (Fig. 2c, e, g), showing straight or curved lines sometimes widening near

Table 1 Relative frequencies of the shapes of starch grains from Monte Loayza Site 3 potsherd and from *Alstroemeria aurea*, *Tropaeolum porifolium* and *Diposis patagonica* tubers

	Monte Loayza Site 3 potsherd		<i>Alstroemeria aurea</i>		<i>Tropaeolum porifolium</i>		<i>Diposis patagonica</i>	
	count	%	count	%	count	%	count	%
Spherical	15	32.6	106	35.3	55	18.3	65	32.5
Hemispherical	1	2.2			5	1.6	15	7.5
Ovoid	24	52.2	194	64.7	218	72.6	96	48
Pyriform	3	6.6			1	0.3		
Ellipsoid	2	4.3			16	5.3	11	5.5
Conical	1	2.2			5	1.6	6	3
Polyhedral							2	1
Quadrangular							5	2.5

the edge (Fig. 2g). While some grains had high birefringence and sharply defined arms (Fig. 2c, m), others, especially in ovoid, truncate and pyriform grains, showed faded birefringence or none at all, but they all kept their shape (Fig. 2d, h–l). None of the grains were observed as fully gelatinised sac-like granules. The size distribution was in two parts, with more than 50% of the grains measuring between 2.5 and 6.49 μm and almost 30% between 12.5 and 16.49 μm (Fig. 3). Fewer grains lay between 16.49 and 33.75 μm . Spherical grains measured predominantly about 2.5 to 18.75 μm . The average diameter was 11.28 μm .

The assemblage was compared with all the available published data on starch grains which could potentially have been processed, stored, or consumed using ancient Patagonian pots. The list of plants to consider was drawn up by looking at the information published so far on plant macrofossils (Ciampagna and Capparelli 2012 and references therein; Capparelli and Prates 2015; Ciampagna 2015; Llano and Barberena 2019) and microbotanical remains (Pérez and Erra 2011; Lema et al. 2012), together with ethnographic reports of plant use written by travellers in the region from the 16th to 19th centuries (Pérez de Micou 1994; Ciampagna and Capparelli 2012; Ciampagna 2015; Prates et al. 2016). The archaeobotanical data from neighbouring areas such as La Pampa (Fig. 1a; Musaubach et al. 2013), were also considered. We were then able to compile information on the starch grain morphology of 11 starchy taxa, such as cereals, pseudocereals, fruits, nuts and a few USOs (*Oxalis* spp. and *Arjona tuberosa*) (starch grain morphology of each taxon is described in ESM Table 1). However, no positive match was found with the Monte Loayza starch grains. Then this archaeological assemblage was compared against the new reference collection created for this research project.

Reference collection

Tuber morphology and anatomy

Alstroemeria aurea (Fig. 4a–d) storage root tubers are cylindrical and elongated (Fig. 4b). The transverse section shows a rhizodermis (root epidermis) of 2/3 layers of quadrangular to oblong thin-walled cells with abundant equally distributed ovoid and spherical starch grains, about 10.5 to 42.5 μm in length/diameter (Fig. 4c). This is followed by several layers of cortical parenchyma (the outer part of the root) with thin-walled round cells, containing abundant predominantly ovoid starch grains of about 2.5 to 38.5 μm (Fig. 4c), which is limited internally by the endodermis (the inner part of the root) (Fig. 4d). The central cylinder of parenchyma has one layer of pericycle (Fig. 4d) and the vascular bundle is formed by a primary, polyarch stele (the central part of the root, bordered by the pericycle) with a medullar parenchyma (layer) of thin-walled cells smaller than those of the

cortical parenchyma, with predominantly ovoid starch grains about 2.5–30.5 μm in length. The average moisture content obtained for *A. aurea* tubers was 77.8% (ranging from 77.5 to 78%, STD=0.24, n=3).

Tropaeolum porifolium (Fig. 5a–d) stem storage tubers are rounded or conical (Fig. 5b). The transverse section shows a periderm (outer layer or skin) of 5/6 layers of radially compressed cells with small spherical starch grains of about 0.5 to 6.5 μm and ovoid grains of about 6.5 to 24.5 μm , followed by very thick-walled sclerenchyma cell tissue (Fig. 5c), then a cortical parenchyma of 4/5 layers of globular cells and the vascular system which is regularly distributed, with secondary xylem as solitary vessels alternating with starchy parenchyma rays of radially elongated cells (Fig. 5d), and finally a medullar parenchyma of globular cells (Fig. 5d). These structures contain abundant, predominantly but not only, ovoid starch grains (Fig. 5d, detail) (see below); starch grains from the medullar (pit) parenchyma have the largest size range, about 2.5 to 34.5 μm .

Diposis patagonica (Fig. 6a–c) has rounded stem storage tubers (Fig. 6a, b). The transverse section shows a periderm with 10/12 layers of radially compressed cells followed by a few (~8) layers of thin-walled oleoresin (essential oil) cells (Fig. 6b, c), and a central parenchyma in which vascular bundles are irregularly distributed. All the tissues contain many types and sizes of starch grains (see below); however, fewer grains were observed in the essential oil cell layers.

Comparing one against the other, the three USOs described here can be easily distinguished on the basis of their botanical structures, which are summarized in ESM Table 2.

Tuber starch grain morphology and size

Alstroemeria aurea (Fig. 4e–k). The starch grains are usually simple (Fig. 4e–h, k), although a few compounds exist (Fig. 4i, j). The surface may be wrinkled. Simple grains are spherical to ovoid in shape (Fig. 4e–f, h), rarely having one pressure facet (flattened side), giving the grain a domed or truncated shape (Fig. 4k; Table 1). Compound grains are ovoid and commonly made up of two or three parts. The lines where component grains join may be indistinct, composed of fused part-grains as in Reichert (1913) (Fig. 4i), or distinct as fissures or linear depressions which separate the granules from each other (Fig. 4j). Compound grains occasionally have small grains adhering to the surface (Fig. 4j). The hilum is distinct and punctate in simple spherical grains (Fig. 4e); however, it is frequently indistinct in other simple and compound grains, sometimes marked by a short longitudinal or transverse fissure (Fig. 4h), or two fissures crossing one another (Fig. 4k). It is centric or nearly centric (Fig. 4e, f) or eccentric (off-centre) by one-fifth of the longitudinal axis in ovoid grains (Fig. 4h). The lamellae (layers) are fairly

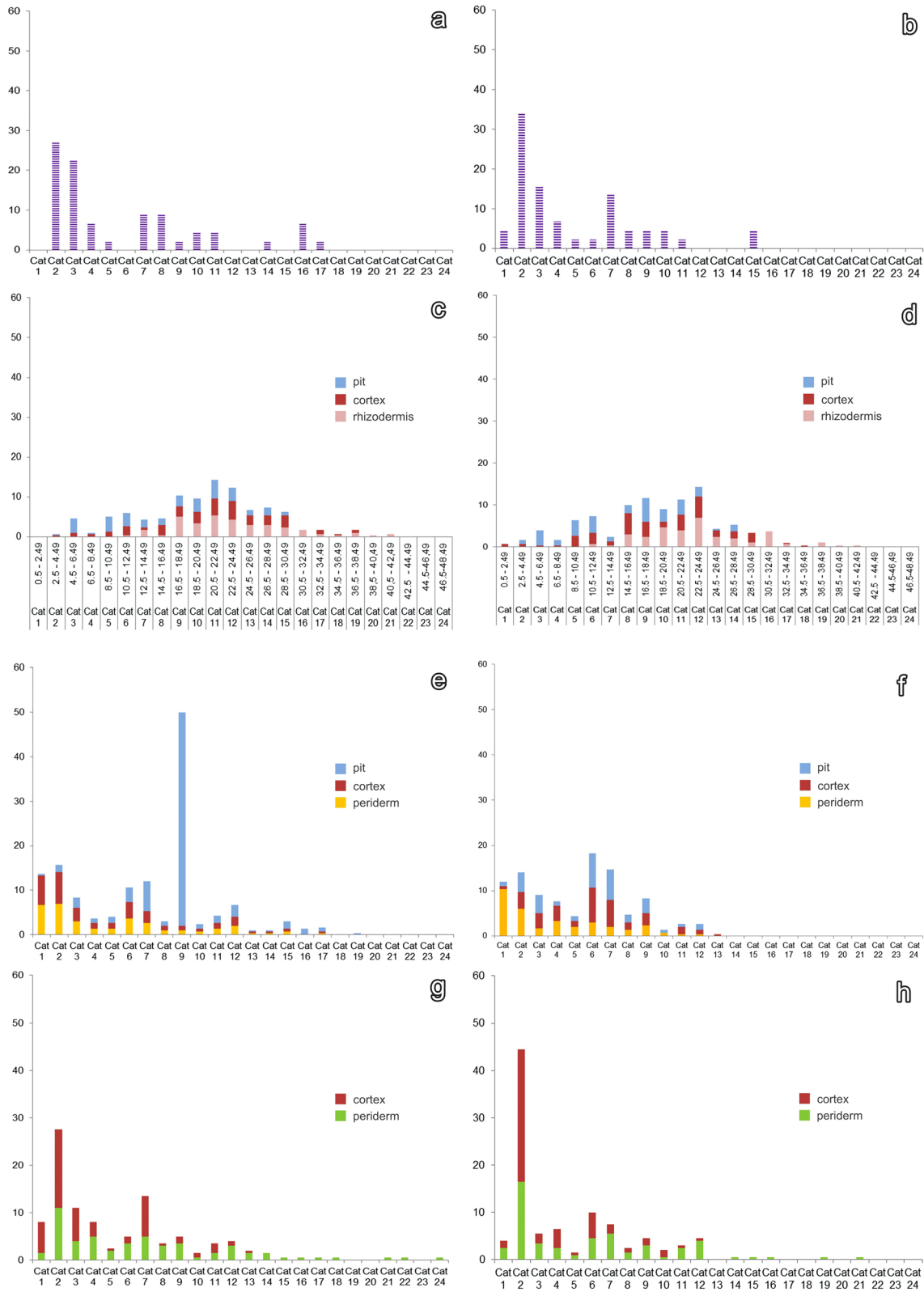


Fig. 3 Frequencies (%) of length and width of the recorded starch grains by diameter classes, **a–b**, Monte Loayza Site 3 potsherd; **c–d**, *Alstroemeria aurea*; **e–f**, *Tropaeolum portifolium*; **g–h**, *Diposis patagonica*

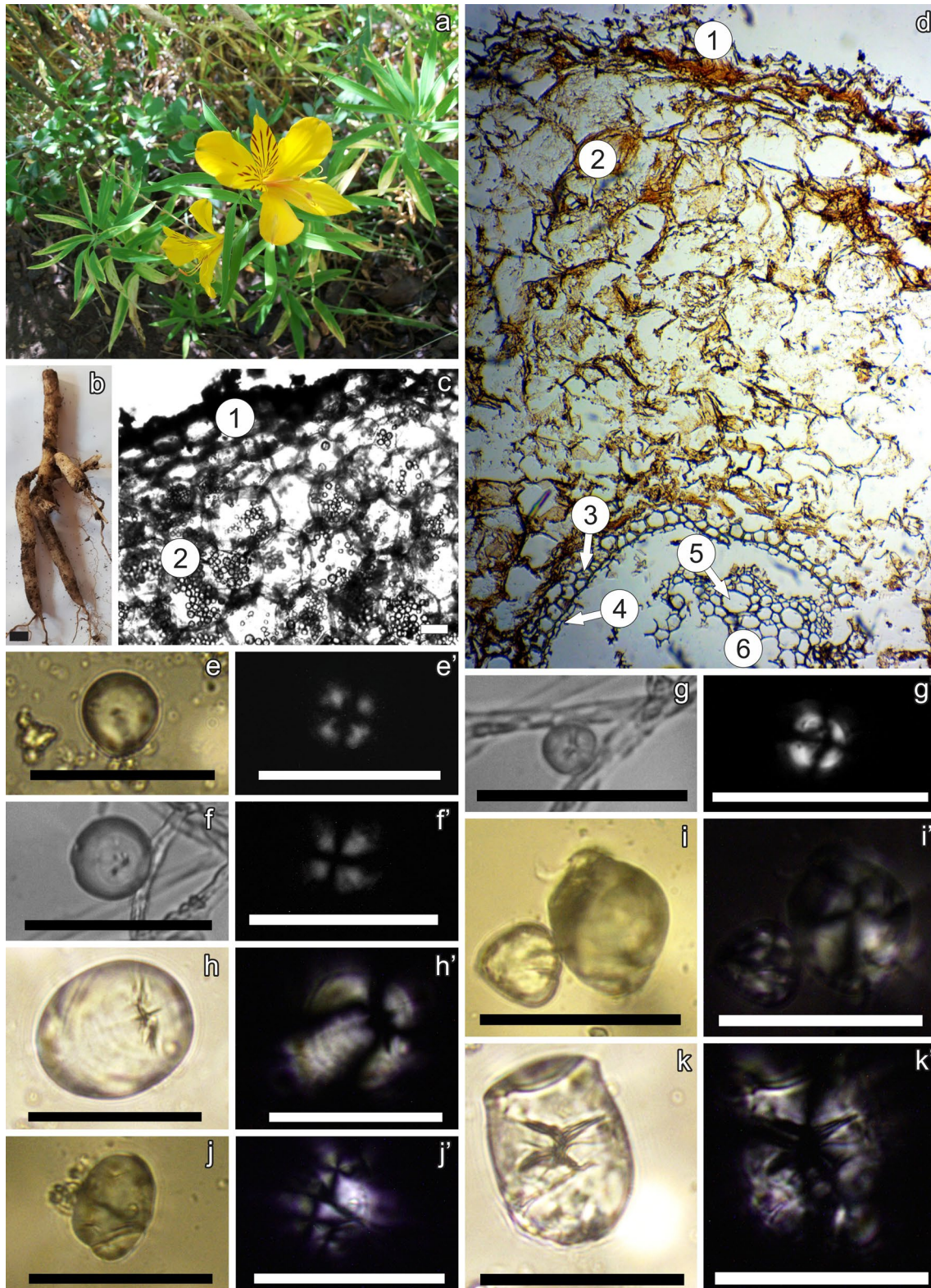


Fig. 4 *Alstroemeria aurea*; **a**, general view of the plant; **b**, tuber external view; **c–d**, tuber internal structure; **e–k**, starch grains under unpolarized and polarized light; **e–g**, spherical central hilum; **h**, ovoid eccentric hilum; **i**, ovoid fused compound grain; **j**, ovoid compound

of two grains; **k**, ovoid (truncated) simple grain; **1**, rhizodermis; **2**, cortex; **3**, endodermis; **4**, pericycle; **5**, polyarch central stele; **6**, pit; scale bars **b**, 10 mm; **c–k**, 50 μ m

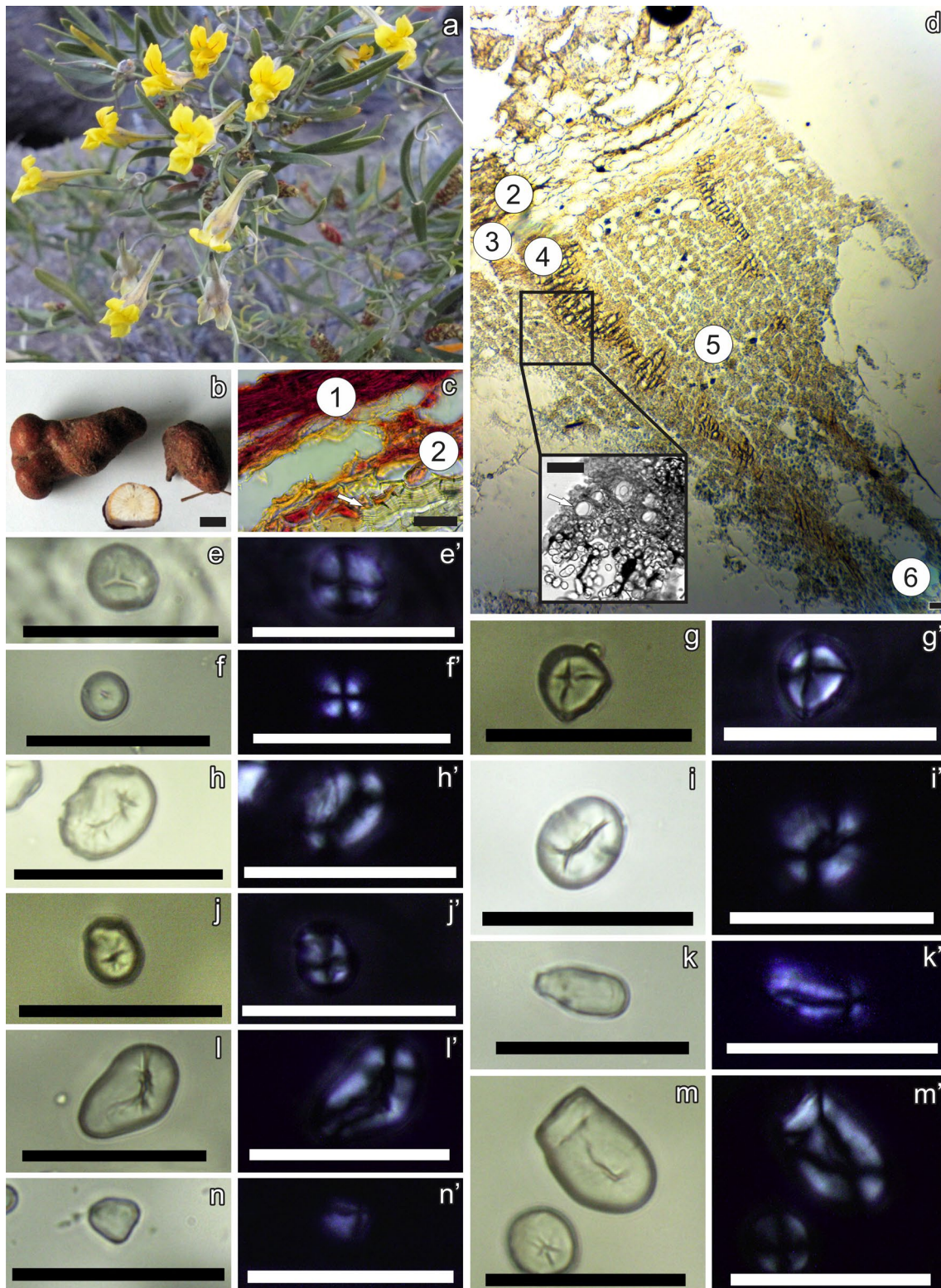


Fig. 5 *Tropaeolum porifolium*; **a**, general view of the plant; **b**, tuber external view; **c**, periderm and large sclereids (arrow); **d**, tuber internal structure with detail of solitary vessels of the secondary xylem tissue (arrow) and starchy parenchyma rays; **e–n**, starch grains under unpolarized and polarized light; **e–f**, spherical stellate and punctate central hilum; **g**, hemispherical stellate fissured hilum; **h**, ellipsoid

elongate hilum; **i**, ellipsoid elongate bifurcated hilum; **j**, pyriform stellate hilum; **k**, pyriform eccentric punctate hilum; **l**, pyriform elongate hilum; **m**, spherical stellate and ovoid (truncated) elongate hilum; **n**, pyriform punctate hilum; **n'**, pyriform punctate hilum; **1**, periderm; **2**, sclereid layer; **3**, cortex; **4**, secondary xylem tissue; **5**, parenchyma rays; **6**, pit; scale bars, **b**, 10 mm; **c–n**, 50 μ m

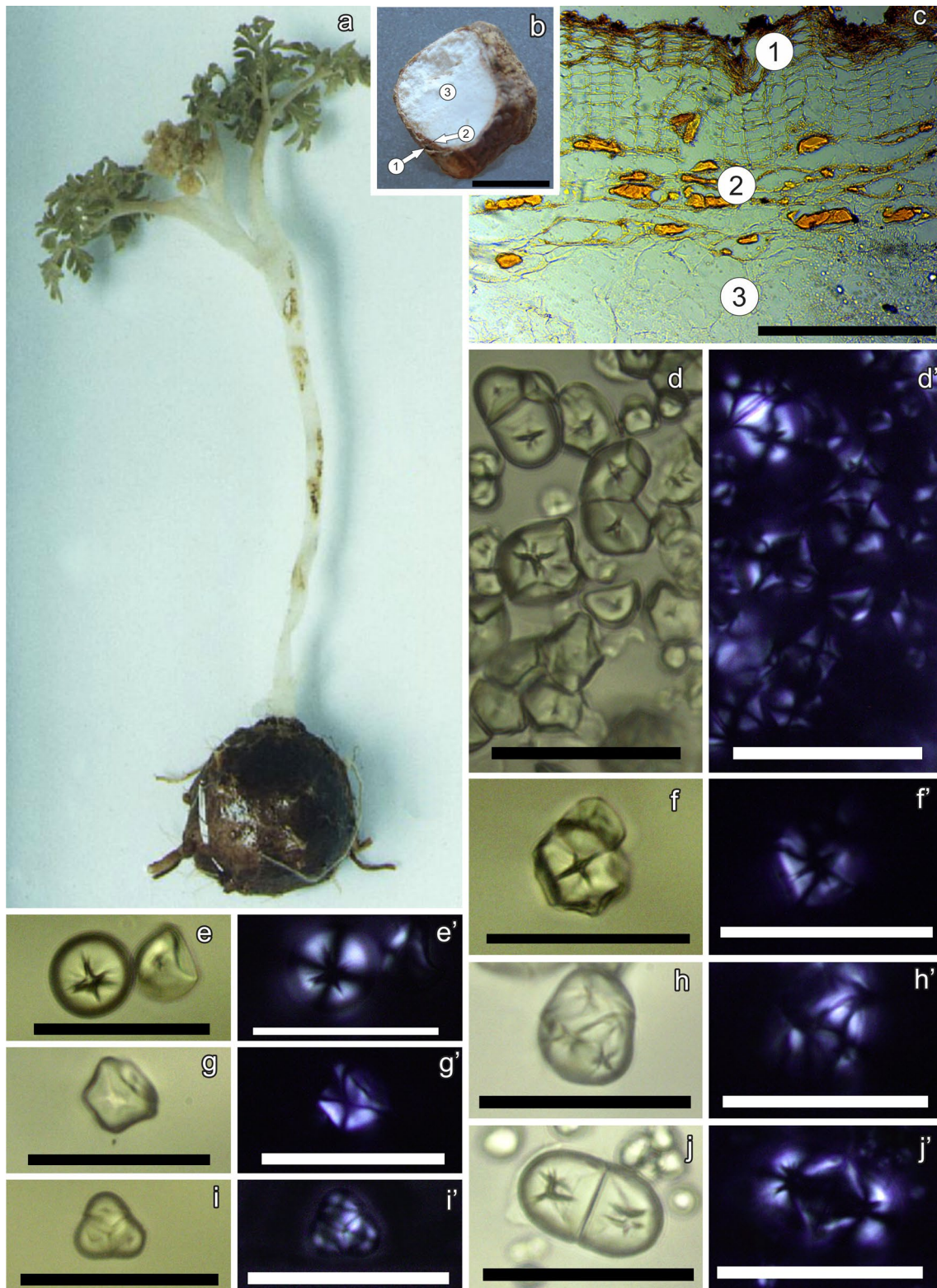


Fig. 6 *Diposis patagonica*; **a**, general view of the plant and tuber; **b**, tuber macroscopic transverse section; **c**, tuber internal structure; **d–j**, starch grains under unpolarized and polarized light; **d**, compound grains of 2–4 grains and part-grains; **e**, spherical simple stellate central hilum and hemispherical grains; **f**, polyhedral part-grain central

radial fissured hilum; **g**, quadrangular part-grain radial fissured hilum; **h**, ovoid fused compound radial fissured hilum; **i**, conical compound radial fissured hilum; **j**, ellipsoid (two, joined) stellate fissured hilum; **1**, periderm; **2**, oleoresin cell layer; **3**, cortex; scale, **b**, 10 mm; **e–j**, 50 μ m

distinct in a few grains; they appear as rather coarse, concentric rings (Fig. 4h). The grains vary in size; the spherical simple grains are about 3.65 to 20 μm , while the ovoid grains (simple and compound) are about 20 to 43.9 μm . The size distribution is in a single group, between 16.5 and 24.49 μm (Fig. 3). The extinction cross is central in the simple grains and centric or eccentric in the compound ones; in some grains it can be multiple (sensu Reichert 1913, p 662) and it is distinct and usually fairly clear-cut (Fig. 4f). The lines are straight or curved, and commonly broad, widening near the edge (Fig. 4f, h). The degree of polarization is high.

In *Tropaeolum porifolium* (Fig. 5e–m), the starch grains are usually simple, but very few are compound and the surface may be wrinkled (Fig. 5e). Simple grains are spherical (Fig. 5e–f) to ovoid (Fig. 5h, i), ellipsoid (Fig. 5m), conical (Fig. 5n) and pyriform (Fig. 5j–k; Table 1), the last two narrowing at the distal end. Some simple grains may have one pressure facet, giving them a domed, truncate (Fig. 5m) or hemispherical shape (Fig. 5g). Compound grains are usually pyriform (pear-shaped) and made up of two unequal part-grains, in which the lines where they join are usually indistinct (Fig. 5l). The hilum in spherical simple grains is usually distinct, centric and punctate (dotted) (Fig. 5e), but sometimes it may be obscured by radial fissures (Fig. 5m). In other grain shapes the hilum is usually eccentric by one or two fifths of the longitudinal axis, obscured by short longitudinal or transverse fissures (Fig. 5j–l). The hilum is elongated, with fissures at one or both ends in some ellipsoid grains (Fig. 5i). The lamellae (layers) are indistinct. The grains vary in size; the spherical simple grains are about 0.5 to 25 μm , while other shapes of simple and compound grains are about 12.5 to 38.75 μm . The size distribution is in three groups, the first between 16.5 and 18.49 μm , the second between 1.5 and 4.9 μm and the third between 10.5 and 14.49 μm (Fig. 3). The extinction cross is centric in simple spherical grains, ovoid (Fig. 5e–f) and hemispherical ones (Fig. 5g) and usually eccentric in pyriform or ellipsoid grains (Fig. 5k, m); in some grains it can be multiple. It is distinct, sharply or fairly well defined, with straight and/or irregular and jagged lines (Fig. 5l), commonly broad, less frequently fine, widening near the edge (Fig. 5f). The degree of polarization is high.

Diposis patagonica (Fig. 6d–j). The starch grains are either simple or compound. The surface may be wrinkled. Simple grains are spherical in shape (Fig. 6e). Compound grains are of several different types (Fig. 6d). Some are ovoid or ellipsoid in shape (Fig. 6h, j) and are made up of two, three to six or more equal (less frequently unequal) part-grains, which are separated by fissures (Fig. 6d, j). When separated, the part-grains may have one curved surface and one or more flattened pressure facets, making them hemispherical to conical (Fig. 6e, i), or several pressure facets, giving them polyhedral or quadrangular shapes (Fig. 6f, g;

Table 1). Other types of compound grain are ovoid and composed of fused part-grains (Fig. 6h). The hilum is usually centric, distinct and punctate in some simple spherical and in some part grains (Fig. 6i), but it is more frequently marked by a short longitudinal or transverse fissure (Fig. 6d, j), or two fissures crossing one another (Fig. 6e, f). In compounds of fused part-grains the hilum is indistinct or fairly distinct due to fissures (Fig. 6h). The lamellae are indistinct. The grains vary in size; the spherical simple grains are about 2.5 to 20 μm , while the compound ovoid or ellipsoid grains are about 20 to 50 μm and part-grains are about 5 to 14.49 μm . The size distribution is in two groups; the first is from 2.5 to 4.49 μm and the second one from 12.5 to 14.49 μm (Fig. 3). The extinction cross is usually centric, lines are fine and clear-cut, straight, less frequently curved, and commonly widen near the edge (Fig. 6e). The degree of polarization is high.

Comparative features of the starch grains from the Monte Loayza potsherd and from the three USO tubers analysed are summarised in ESM Table 3.

Discussion

The starch grain archaeological assemblage analysed here comes from a location with low moisture, neutral to alkaline pH, low ambient temperature and high salinity. This micro-environment is consistent with the conditions from which archaeological starch grain survival can be potentially expected (Copeland and Hardy 2018). Post-depositional processes affecting the starch grains either by enzymatic action or by the natural gelatinization of starch grains (Mercader et al. 2017, 2018) are improbable at Monte Loayza because of the unfavourable conditions there for microbial growth and the low air and soil temperatures, even in summer (De Fina and Ravello 1973). These factors, together with the fact that no plant microremains were recovered from the control or the brushed samples from the potsherd, and that heat damage was recognizable in some starch grains from the charred deposit on the inside face of the bowl, lead us to consider this assemblage as an archaeological residue of starchy food that had been cooked. This kind of residue is frequently recovered from pottery remains from archaeological sites (see also Crowther 2005, 2012; Zarrillo et al. 2008; Henry et al. 2009). Some of the heat damage features noted by Henry et al. (2009), Crowther (2012) and Messner and Schindler (2010) are compatible with those observed in starch grains from our site, where some of the grains kept their shape under normal light, but showed low or lost birefringence and very faded and wider centres of the extinction cross under polarized light. These damaged starch grains were found together with some unmodified intact grains. Various experimental analyses have demonstrated that intact

starch grains may remain after different types of cooking, and that when only partially damaged, the starch can still be assigned to morphological classes (Henry et al. 2009; Messner and Schindler 2010; Crowther 2012; Mercader et al. 2017). After comparing the Monte Loayza assemblage against our reference collection, we propose that the starch grains belong to *Tropaeolum* aff. *porifolium*, although *Alstroemeria* aff. *aurea* cannot be excluded. This assumption is based on several diagnostic characters as follows: 1. The presence of predominantly simple grains, together with a few compounds of two; 2. Simple grains predominantly spherical and ovoid, but ellipsoid, conical and hemispherical ones also present; 3. The presence of grains with elongate hilum with fissures; 4. Size range between 2.5 and 33.75 μm ; 5. Two size groups present, one of about 2.5 to 6.49 μm , corresponding to spherical grains and another larger one of about 12.5 to 16.49 μm , corresponding to a variety of shapes. Even when the morphology of spherical starch grains is ubiquitous in the plant kingdom and generally has no value in taxonomic identification (Reichert 1913), the distinctive grain size distribution in two groups was demonstrated to be a useful identification character of a plant source when both large and small grains occur together in the same archaeological sample (Hardy et al. 2018). *Alstroemeria aurea* cannot be excluded from the identification, since ovoid and spherical grains found in the Monte Loayza sample characterized not only *Tropaeolum*, but also *Alstroemeria*. Other wild species of these two genera should not be ruled out either, inasmuch as their natural distribution includes Patagonia, and they have similar morphologies of starch grains to the ones described here for *T. porifolium* and *A. aurea*. They include, for example, *A. ligtu* (L.) Curtis, *A. hookeri* Sweet ssp. *hookeri*, *A. brasiliensis* Spreng, *T. tuberosum* Ruiz et Pav. ssp. *tuberosum* Sparre and ssp. *sylvestre* Sparre (Reichert 1913; Melchiorre 1985; Cortella and Pochettino 1995; Bulacio and Ponessa 2012; Correa et al. 2013) (details in ESM Table 4). We discarded *Diposis patagonica* as a possibility because of the absence of part-grains with more than two pressure facets, with polyhedral or quadrangular shapes, or compound grains of several separated part-grains. However, it is worth mentioning as a particular feature observed in the reference collection made for this species that its polyhedral grains of 10–15 μm are similar in shape, hilum and fissures to the faceted grains of *Zea mays* (Cortella and Pochettino 1994), which might have archaeobotanical implications.

Returning to the cooking methods that may have been used for the Monte Loayza assemblage, the presence of both unmodified and heat damaged starch grains might be compatible with a short period of boiling. A low degree of starch gelatinization was observed, for example, by Henry et al. (2009) in legume seeds and cereal grains, depending on the taxon considered, after short periods of boiling, from 1 to 30 min. In the case of the USOs considered here, short

boiling times seem to be enough for the smooth texture of *Alstroemeria* parenchyma to cook, but not for those of *Tropaeolum* or *Diposis*, which are more fibrous or resinous. However, unless the tubers had been processed as an extract or infusion for herbal remedies, as is common in Patagonia, where the liquid is consumed after a short period of boiling, it is unlikely that these tubers would have been sufficiently cooked for consumption after a short boiling time (Molares and Ladio 2012). More experimental work is needed to test these possibilities in the future. On the other hand, the material from Site 3 at Monte Loayza may be more consistent with cooking methods involving low moisture content. Moisture content has a critical role in cooking, affecting the thermal conversion process of starch, as was demonstrated by Crowther (2012). Damage observed from ways of cooking with less than 30% moisture content led to the loss of, or a more diffuse polarization cross, with grains keeping their shape under transmitted light, and few gelatinized grains (Crowther 2012). This low-moisture cooking method may be more probable if flour instead of whole organs were used (Crowther 2012). However, none of the Monte Loayza starch grains appeared to show damage patterns compatible with having been ground, such as fractures or cavitation, among others observed from the experimental studies of Babot (2003); further experimental research needs to be carried out in the future to assess the specific response of these particular species to being ground. In contrast, low moisture content is unlikely when whole tubers are being cooked, even if they are cooked in their own water, since the moisture content of these organs is generally high (Messner and Schindler 2010; Crowther 2012). The moisture content of *A. aurea* measured here is compatible with this reasoning. Despite this, differences in the degree of gelatinization of starch may occur between different parts of the whole tuber tissues being cooked dry, by baking or grilling, such as a low degree of gelatinization on the surface, but high in the core (Crowther 2012). This might be true for the Monte Loayza sample, considering that the spherical grains, which are more likely to have come from the surface of the tubers, appear to have incurred less damage from heating, since they had kept their higher birefringence, compared with the ovoid, pyriform or hemispherical starch grains from the interior. Also, it is known that lipids or proteins may increase the gelatinization temperature of starch grains (Crowther 2012). The fatty acid values of potsherd residues from the lower course of the Río Colorado, Río Negro Province (Stoessel et al. 2015) and gas chromatography and isotopic analyses of other samples from the north coast of Chubut Province (Gómez Otero et al. 2014; Schuster 2014) reported plant residues together with signs of meat or fat processing from sea fish, marine mammals and *Lama guanicoe*. It can therefore be suggested that the absence of whole gelatinized starch grains in our material may have been produced by an

increase in the temperature needed for gelatinization of the USO starches, caused by the other substances being cooked together with the tubers so that gelatinization did not occur. Ethnohistorical written sources also mention the importance for Patagonian hunter-gatherer peoples of fat and salt, which also lead to an increase in the temperature needed for gelatinization (Prates et al. 2016). The sources also mention the practice of preparing tubers with lipid-containing substances, such as milk (see above), and finally, practices related to frying in fat, as known for *Prosopanche* fruits, for example (Ciampagna and Capparelli 2012).

In sum, as previously demonstrated, there are just a small number of universal changes to the starch associated with certain cooking methods (Henry et al. 2009), which show the need for a full range of cooking experiments for each plant starch that has to be studied. In the case of the USO species considered here, future experimentation with different processing methods and ways of cooking would contribute further to this preliminary discussion.

Conclusions

The archaeobotanical studies presented here enable us to explore the use and processing of wild plant resources by Patagonian hunter-gatherer communities, thus contributing to the discussion on the use of pottery by these societies. *Tropaeolum* aff. *porifolium*, has been identified, although *Alstroemeria* aff. *aurea* cannot be ruled out. Low moisture cooking techniques like roasting and frying, with the possible incorporation of other substances such as lipids used for frying to prepare these tubers, would allow us to reconstruct the ways of food preparation by the people on the coast of northern Santa Cruz and Patagonia, based on a larger number of case studies. In addition, the establishment of a USO reference collection and descriptions of the starch grains of *A. aurea*, *T. porifolium* and *Diposis patagonica* have enabled us to make a tentative identification of the archaeobotanical sample. It is therefore suggested that wild plant resources like USOs were used as food, and that they are probably under-represented in the archaeobotanical record due to their lack of visibility (Ciampagna and Capparelli 2012). It is important for this field of study that experiments are done to explore the association and cooking of plant, marine and terrestrial animal resources in different combinations and in pottery vessels, and to study their traceability through combined archaeobotanical and chemical analyses. This study constitutes a first approach to two types of archaeological record, which still raise many questions in this field of knowledge, where so few potsherd residues have been studied from this region in a context where the ceramic sample is fragmented and archaeobotanical analyses of microremains are scarce in Patagonia, Argentina.

Acknowledgements We thank CONICET (PIP 0319), ANPCyT (PICT 2015-2040) and UNLP (N866) for financial support to Aylen Capparelli. Also Mariana Weigandt, who carried out the initial anatomical descriptions of *Diposis patagonica*; Santiago Martínez, for his assistance to Aylen Capparelli in developing the technique of paraffin inclusion; Diego Gobbo for the assistance with the image quality; Diego Massone and Angela Martucci for their help with the moisture content measures. We thank also the anonymous referees for their comments towards improving the first version of this paper.

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