ORIGINAL RESEARCH
published: 16 May 2022
doi: 10.3389/feart.2022.815351

Description of Starch Granules From Edible Acorns (Oak), Palms, and Cycads in Southern China

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Specialty section:
This article was submitted to Quaternary Science, Geomorphology and Paleoenvironment, a section of the journal Frontiers in Earth Science

Received: 15 November 2021
Accepted: 26 April 2022
Published: 16 May 2022

Citation:
Li Z, Barton H, Wang W and Yang X (2022) Description of Starch Granules From Edible Acorns (Oak), Palms, and Cycads in Southern China. Front. Earth Sci. 10:815351. doi: 10.3389/feart.2022.815351

A review of ethnological and archaeobotanical evidence shows the potential for a range of starch-rich woody plants, beyond tuberous plants, to have been important foods in prehistoric south subtropical China. In this paper we review the size and shape characteristics of starch granules non-tuberous woody plants (Palms, Cycads and Acorns) that our research has identified as important sources of carbohydrates for prehistoric communities. The study sample consists of 34 modern starch reference samples across eight genera (Palms: *Arenga, Caryota,*; Cycads:*Cycas*; and, Acorns: *Castanopsis*, *Fagus, Lithocarpus, Quercus,* and *Quercus* section *Cyclobalanopsis*). Our descriptive criteria are developed granule descriptors standard in the literature and then assessed for their utility using multiple correspondence analysis. The results demonstrate that both morphometric characteristics and the maximum size of granules are valuable for distinguishing starch granules at various taxonomic levels. Of the five morphometric characteristics recorded in this study sample, granule shape is the most effective variable for granule identification.

Keywords: starch identification, non-parametric test, multiple correspondence analysis, woody plants, southern China

[1]Recent molecular studies of the oak grouping, *Cyclobalanopsis*, has revised this Southeast Asian group to the section level within the genus Quercus (see Denk et al., 2017; Hipp et al., 2020; Zhou et al., 2022). As we understand it, the use of the term *Cyclobalanopsis* refers in part, to the particular phenotypic expression of acorns in having acorns with distinctive cups bearing concrescent rings of scales; they commonly also have densely clustered acorns, though this does not apply to all of the species. Phenotypic differences will have mattered to hunter-gatherers collecting these plantswhile they may have processed and eaten them in similar ways to all other *Quercus*, the visual differences in the acorns, while not significant in terms of their molecular evolution, are likely to have featured in ethno-taxonomies. Differences in growth habitat will also have mattered to hunter-gatherers and be significant in our consideration of their use as a food in prehistory. To align our paper with previous studies and to reduce confusion in comparisons of starch granules, we continue to refer to *Cyclobalanopsis* sp. in this paper, while recognising it is technically inaccurate in current botanical scholarship.

1 INTRODUCTION

Starch grain analysis contributes to the understanding of several archaeological issues including prehistoric diet (e.g. Loy et al., 1992; Barton 2005; Lu et al., 2005; Liu et al., 2010; Yang and Jiang 2010; Yao et al., 2016), tool use (e.g. Piperno et al., 2004; Revedin et al., 2010; Buckley et al., 2014; Ma et al., 2014) and the emergence of agriculture and plant domestication (e.g. Denham et al., 2003; Pearsall et al., 2004; Perry et al., 2007; Yang et al., 2012). To contribute to these fields, it is normally necessary to attempt a taxonomic identification of recovered starch granules by direct comparison with available reference material and sometimes with published granule descriptions from other studies (e.g. Torrence and Barton 2006; Yang and Perry 2013; Yao et al., 2016; Guan et al., 2020; Lucarini and Radini 2020; Tsafou and García-Granero 2021). A morphometric analysis by granule description is the most common approach (e.g. Loy 1994; Piperno and Holst 1998; Torrence and Barton 2006; Liu et al., 2014; Mercader et al., 2018; Brown and Louderback 2020), but some limitations are recognized including the experience of the analyst, the breadth of the reference collection, the state of granule preservation (e.g. Lamb and Loy 2005; Barton 2009; Yang and Perry 2013; Ma et al., 2019), and complications arising from a similarity of granule size and shape between species (e.g. Liu et al., 2014; Guan et al., 2020). The development of expert systems for granule identification, or at least an increased reliance on quantitative approaches that are not so dependent on subjective decisions made by the analyst, have been recommended (Torrence et al., 2004; Wilson et al., 2010; Liu et al., 2014; Coster and Field 2015; Arráiz et al., 2016; Louderback et al., 2016). To date, no expert system has replaced the need for an experienced analyst to identify granules at a taxonomic level. With the growing importance of this technique in archaeology, and the potential it has to resolve important questions about tool use and diet in prehistoric China, here we apply some statistical procedures to assess the reliability of our recording variables so that we may improve the reliability of identification of ancient starch granules from our study region of south subtropical China.

Starch residue analyses of stone tools from a range of archaeological sites in south subtropical China, dating between 10,000 and 2,000 BP, has already provided direct evidence for the critical horticultural hypothesis, which emphasized the importance of tuberous plants, such as taros (e.g. *Colocasia* spp.), yams (e.g. *Dioscorea* spp.), and lotus root (*Nelumbo nucifera* Gaertn), in the indigenous diet and agricultural system (e.g. Li and Lu 1987; Yin 2000; Lu 2003; Zhao 2005; Yang et al., 2013; Li et al., 2016). Our review of the available historical texts and some archaeological finds from southern China, however, reveal that some lesser-known starch-rich woody plants, including acorns, palms and cycads, may have also contributed significant calories to the communities in this region (Cao et al., 2011; Ge 2015; Geng 2019; Liu 1983; Liu et al., 2012; Zhou et al., 2014; Fuller and Qin 2010; Yang et al., 2013).

Edible acorns (nuts of the Fagaceae family) were once an important food staple for hunter-gatherer and farming communities in the northern hemisphere. Archaeological records document the consumption of large quantities of acorns in the woodlands of Europe, North Africa, Southeast Asia and North America (e.g. Fuller and Qin 2010; Higham 2014; Messner 2011; Humphrey et al., 2014; Kawashima 2016; Lentfer et al., 2013; Noshiro 2016; Sasaki and Noshiro 2018; Stevens and Mcelreath 2015; Tushingham and Bettinger 2013). Preserved acorn exocarps (the tough outer shell) have been recovered from several waterlogged sites in southern China, including the Tianluoshan site (7,000–6,000 BP) in the Yangtze River Basin and the Guye site (5,900–5,000 BP) in the Pearl River Delta (Fuller et al., 2011; Institute of Cultural Relics and Archaeology of Zhejiang Institute of Cultural Relics and Archaeology 2004; Yang et al., 2018). At the Tianluoshan site, acorns (*Cyclobalanopsis/Lithocarpus/Quercus*) were stored in special purpose subterranean storage pits (Fuller and Qin 2010). Acorn starches have been identified from the surfaces of stone tools at the Xiaohuangshan site (9,000–7,700 BP), charred residues inside the ceramic pottery at the Kuahuqiao site (8,000–7,000 BP), and dental calculus at the Qihe Cave site (c. 9,500 BP), indicating long-term use of this plant resource in the local and regional diets of southern China (Guan et al., 2018; Liu et al., 2010; Yang and Jiang 2010; Yao et al., 2016). However, acorns, as food staples, were gradually replaced by other crops, including domesticated rice (Fuller and Qin 2010). In the historic period, acorns remained important in seasonal diets, but were relegated to a supplementary food or famine food, as noted, for example, in *Lu's Commentaries* of History (finished c. 239 BC), or the Compendium of Materia Medica (finished in 1578). Today, some ethnic minorities in southwest China, such as the Dong people and Wa people, still preserve the tradition of acorn consumption, albeit on a small scale (**Supplementary Table S1**) (Cao et al., 2011; Liu et al., 2012; Zhou et al., 2014).

Another important, though sidelined starchy plant foods in southern China is the palms which store starch in the trunk pith, recorded as *Guanglang* (桄榔), *Suomu* (莎木, the possible phonetic name of "sago"), and *Mianmu* (面木, meaning the trees with flour) (Ge 2015; Geng 2019; Lin 1997). The archaeological evidence for this foodstuff is confirmed only at the Xincun site in the Pearl River Estuary (5,300–4,400 BP) by recovered starch granules from at least two genera of palms (*Caryota* Lour. and *Coryphya* L.) (Yang et al., 2013). However, Chinese scholars have identified several genera of palms with edible pith, including guanglang (*Arenga* spp.), fishtail palms (*Caryota* spp.), as well as some species of cycads (*Cycas* spp.), from a series of Chinese historic texts (Ge 2015; Geng 2019; Lin 1997). For example, *Hua Yang Guo Zhi* (Eastern Jin Dynasty, 317–420 AD) recorded guanglang flour being consumed in the cities of the Yunnan-Guizhou Plateau where cereal crops were lacking. According to *Shu Du Fu*, this flour was already one of the critical commodities in the markets of the Sichuan basin, a region outside the natural range of these plants, at the time of the Wei-Jin dynasties (c. 220–315 AD), (Flora of China, online at: http://www.iplant.cn/info/%E6%A1%84%E6%A6%94?t=foc). This record shows the importance of this food, and that it was a traded commodity in some regions of southern China in the historic period. The Zhuang people in Longzhou, Guangxi still

TABLE 1 | Modern reference study sample.

Family	Genus	Species	Granules Size			
			Length Range/μm	Mean Length/μm	Median	Granule Count
Fagaceae	*Lithocarpus*	*Lithocarpus cleistocarpus*[a]	5.91–15.61	9.2 ± 1.9	8.7	135
Fagaceae	*Quercus*	*Quercus franchetii*[b]	5.13–24.93	10.0 ± 3.3	9.4	111
Fagaceae	*Quercus*	*Quercus oxyphylla*[b]	5.07–21.58	10.1 ± 3.0	9.5	107
Fagaceae	*Quercus*	*Quercus variabilis*[b]	5.07–21.17	10.2 ± 2.7	9.9	116
Fagaceae	*Lithocarpus*	*Lithocarpus litseifolius*[a]	5.54–17.69	10.5 ± 2.3	9.9	100
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis phanera*[a]	6.84–19.88	11.5 ± 2.5	11.0	110
Fagaceae	*Castanopsis*	*Castanopsis fargesii*[a]	6.6–19.5	12.0 ± 2.2	12.0	101
Fagaceae	*Castanopsis*	*Castanopsis platyacantha*[a]	8.3–19.2	12.5 ± 2.0	12.3	113
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis chapensis*[a]	6.29–20.83	12.9 ± 2.5	12.7	117
Fagaceae	*Quercus*	*Quercus cocciferoides*[b]	5.01–28.46	13.0 ± 3.8	12.7	121
Fagaceae	*Castanopsis*	*Castanopsis sclerophylla*[a]	6.5–22.1	13.2 ± 3.0	13.1	101
Cycadaceae	*Cycas*	*Cycas pectinata*	5.3–30.3	14.1 ± 4.8	13.6	112
Fagaceae	*Castanopsis*	*Castanopsis hystrix*[a]	6.1–28.7	14.3 ± 4.1	14.4	105
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis glauca*[a]	7.63–22.48	14.6 ± 2.6	14.6	122
Fagaceae	*Cyclobalanopsis*	*Cyclobalanopsis gambleana*[a]	7.69–24.88	14.7 ± 3.6	14.7	110
Palmae	*Caryota*	*Caryota obtusa*	8.0–47.3	18.1 ± 8.2	15.4	105
Cycadaceae	*Cycas*	*Cycas panzhihuaensis*	11.2–37.0	20.6 ± 4.9	20.2	113
Palmae	*Arenga*	*Arenga westerhoutii* (white)[d]	7.4–52.0	26.4 ± 11.3	24.8	100
Palmae	*Arenga*	*Arenga westerhoutii* (red)[d]	11.7–65.8	29.5 ± 11.3	27.3	116
Fagaceae	*Fagus*	*Fagus sylvatica*[c]	<5	-	-	>6
Fagaceae	*Lithocarpus*	*Lithocarpus chrysocomus*[a]	15.89	-	-	1
Fagaceae	*Castanopsis*	*Castanopsis fleuryi*[a]	-	-	-	0
Fagaceae	*Castanopsis*	*Castanopsis kweichowensis*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus crenata*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus engleriana*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus grandifolia*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus longipetiolata*[c]	-	-	-	0
Fagaceae	*Fagus*	*Fagus longipetiolata*[a]	-	-	-	0
Fagaceae	*Fagus*	*Fagus lucida*[c]	-	-	-	0
Fagaceae	*Fagus*	*Fagus orientalis*[c]	-	-	-	0
Fagaceae	*Lithocarpus*	*Lithocarpus balansae*[a]	-	-	-	0
Fagaceae	*Lithocarpus*	*Lithocarpus corneus*[a]	-	-	-	0
Fagaceae	*Lithocarpus*	*Lithocarpus fohaiensis*[a]	-	-	-	0
Fagaceae	*Quercus*	*Quercus* semecarpifolia[b]	-	-	-	0

[a]*Provided by Herbarium, Kunming Institute of Botany, CAS.*
[b]*Collected in the field with CAS, botanists.*
[c]*provided by Herbarium, Institute of Botany, CAS.*
[d]*Two landraces of guanglang palm flour were purchased from Longzhou, Guangxi. Red flour is extracted from guanglang palm trees over 10 m in height, which have low starch content, while white flour comes from high-yielding trees that grow to around five to 6 m tall.*

value guanglang flour as a traditional food and a medicinal supplement for treating diarrhoea (Ge 2015).

From the above review, we expand our range of important food plants in prehistoric southern China beyond roots and tubers to incorporate starchy palms, cycads and acorns, which may have been underestimated as important foods in previous studies. We therefore seek to expand our range of identifiable starches and look to the statistical analysis of modern reference materials to improve methods of identification as well as establish a reliable identification key for these species.

2 MATERIALS AND METHODS

2.1 Modern Starch Reference Collections

The starch reference collection for this study comes from a total of 31 reference samples of oak trees (Fagaceae, $n = 29$) and two new collections of guanglang landraces (*Arenga westerhoutii*). These specimens are supplemented for analysis by species of palm (*Caryota obtusa*) and cycads (*Cycas pectinata* and *Cycas panzhihuaensis*) already included in the Chinese Modern Starch Reference Database held at the Institute of Geographical Sciences and Natural Resources Research, Beijing (**Table 1**).

The oaks, Fagaceae, were primarily obtained from the Herbarium of the Kunming Institute of Botany while some *Fagus* samples were provided by the Herbarium of the Institute of Botany, Chinese Academy of Science. We also collected some *Quercus* nuts in the field with botanists, such as *Q. semecarpifolia* nuts from the woodland behind the Huanglong Temple in Yunnan Province and *Q. variabilis*/*Q. franchetii* from the Kunming Botanical Garden (**Table 1**). With the help of botanists, we selected as "intact and mature" samples as possible for this study. All of them were identified to species level by botanists from the Kunming Institute of Botany, Chinese Academy of Sciences. We

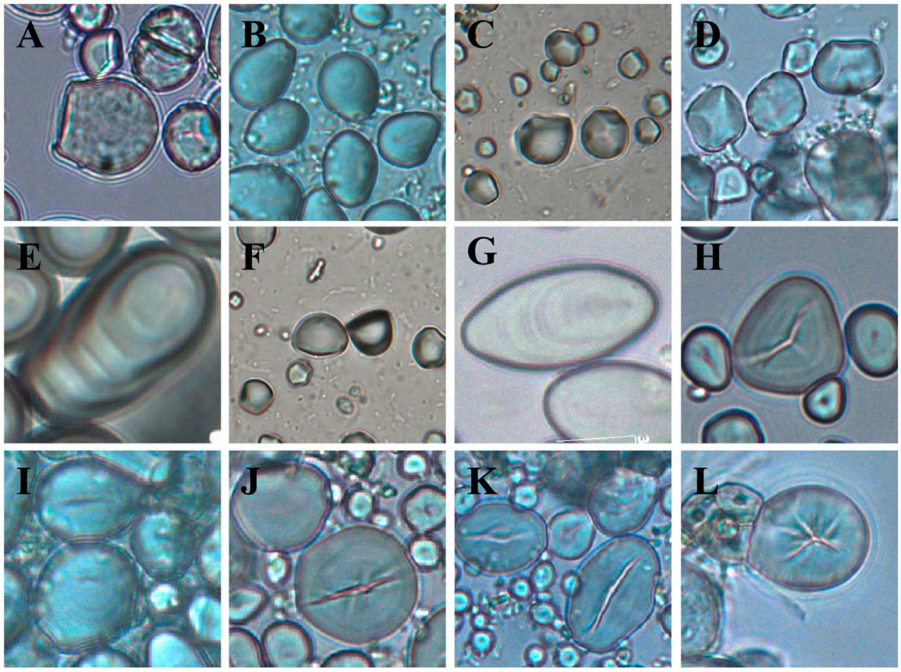

FIGURE 1 | Some of the morphological features considered in this paper: **(A)** bell-shaped granule with rough surface (*Cyclobalanopsis glauca*); **(B)** drop-shaped granule (*Quercus cocciferoides*); **(C)** faceted spherical granule (*Cycas pectinata*); **(D)** irregular polyhedron granule (*Castanopsis hystrix*); **(E)** pestle-shaped granule with visible lamellae (*Caryota obtusa*); **(F)** spherical cap granule (*Cycas pectinata*); **(G)** spindle-shaped granule with visible lamellae (*Arenga westerhoutii* (red)); **(H)**, triangle granule with v-shaped fissure (*Quercus variabilis*); **(I)** linear fissure; **(J)** and **(k)** longitudinal fissure; **(L)**, stellate fissure.

purchased two "landrace" samples of guanglang flour (*Arenga*) from local markets in Longzhou, Guangxi. The red sample is the reddish flour extracted from guanglang palm trees over 10 m in height, which have low starch content. It is said that this guanglang landrace is a rare wild landrace which is difficult to cultivate. The white flour is from high-yielding cultivated trees that grow to around five to 6 m tall. Other starch materials including *Caryota* and *Cycas* were analyzed from slides held in the China Modern Starch Grain Morphological Database.

2.2 Method of Extraction of Starches From Specimens

Following the protocols developed by Yang et al. (2009), we released the starch grains from the acorn kernels by the following method: 1) the nut, in a small plastic sealed bag, was broken with a hammer; 2) small pieces of broken nut were transported to new sterile test tubes with pure water for 24 h soaking; 3) after soaking, the samples were crushed with clean glass stirring rods to fully release the starches. The two purchased samples of guanglang palms were collected as ground flour. We dissolved 1 g flour into 20 ml of distilled water for the sample preparation.

All the starch solutions of acorns and guanglang flour were pipetted onto clean glass slides and mounted in a 50%/50% glycerine/water solution. The prepared slides were observed using a Zeiss AxioMAT at the Starch and Residue laboratory, School of Archaeology and Ancient History, University of Leicester. Images of the starch granules were taken with Zeiss AxioCam and Zeiss AxioVision software. For statistical purposes, a minimum of 100 granules of each sample were recorded. We measured the longest orientable measurement of each granule and recorded the following morphological features: granule shape; hilum position; presence/absence of fissures; the form of the fissure; presence or absence of lamellae; and surface texture (smooth/rough) (**Supplementary Table S2**).

2.3 Analyzing Method of Selected Variables

We observed all 34 samples including those newly collected for the database (**Table 1**), and expect at least 100 starch grains to be measured in each sample. If not enough granules were observed, we continued to sample until exhaustion of the sample itself. The collected data, including maximum granule length and the morphological variables (granule shapes, hilum position, lamellae visibility, fissure types, and surface texture) were recorded. The maximum granule sizes were measured using Zeiss Axiovision. We then tested the utility of granule size as a discriminating feature for starch identification statistically, despite the fact that much granule size overlap makes it difficult to use granule size as an independent discriminator. An assessment of the maximum granule sizes indicates that the values of most species tested do not have a normal distribution (**Supplementary Table S4**), violating the assumption required for parametric analysis (Wang 2013; Zhang 2014). Accordingly, we

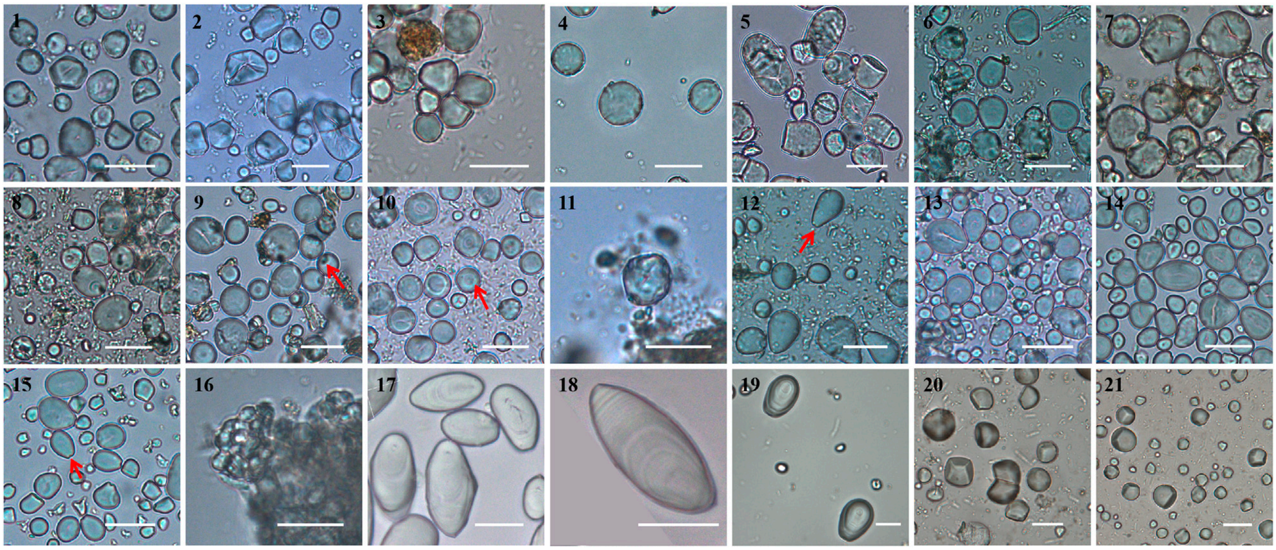

FIGURE 2 | Starch granules from woody starch plants. 1–16, acorn kernels:1, *Castanopsis platyacantha*; 2, *Castanopsis hystrix*; 3, *Castanopsis fargesii*; 4, *Castanopsis sclerophylla*; 5, *Cyclobalanopsis glauca*; 6, *Cyclobalanopsis chapensis*; 7, *Cyclobalanopsis gambleana*; 8, *Cyclobalanopsis phanera*; 9, *Lithocarpus cleistocarpus*; 10, *Lithocarpus litseifolius*; 11, *Lithocarpus chrysocomus*; 12, *Quercus cocciferoides*; 13, *Quercus oxyphylla*; 14, *Quercus variabilis*; 15, *Quercus franchetii*; 16, *Fagus sylvatica*; 17–18, guanglang palm: 17, *Arenga westerhouti* (red); 18, *Arenga westerhouti* (white); 19, fishtail palm: *Caryota obtusa*; 20–21, cycads: 20, *Cycas panzhihuaensis*; 21, *Cycas pectinata*. Scale, 20 μm. The red arrows shows the pits in the granules.

employ a non-parametric test to the dataset, the Kruskal–Wallis test for multiple independent samples. These statistical procedures are performed with SPSS v25.0.

We counted the proportion of each category of the recorded morphological variables to observe and summarize their distribution. In our descriptions of granule morphotypes, we follow the approach of previous studies (e.g. Torrence et al., 2004; Torrence and Barton 2006; Yang and Perry 2013; Liu et al., 2014; Mercader et al., 2018), including overall shape, fissure types, lamellae visibility, hilum position and surface texture (**Figure 1** and **Supplementary Table S2**). We further use a geometric typology that separates overall granule shapes into four categories: irregular-shape type; ovate types; polygonal types; and faceted types. The ovate types include drop-shaped (an ovate with a wide shoulder and one pointed end; **Figure 1B**), pestle-shaped (an ovate with a shoulder at the hilum, tapering toward the distal end; **Figure 1E**), spindle-shaped (an ovate with two symmetrically pointed ends; **Figure 1G**), sphere and oval-shaped. And the faceted types are granules with a spherical or ellipsoid surface but abutting each other in the plant cell causing facets to form (**Figure 1**), including bell-shaped (**Figure 1A**), faceted spheres (**Figure 1C**), and spherical caps (**Figure 1F**). We then calculate the percentage of all the features observed in the starch samples at a species level, in order to identify diagnostic features.

Besides the granule description, we applied statistical procedures to reduce dependence on subjective decisions. As multivariate analysis has been effectively applied in previous starch reference analyses (Devaux et al., 1992; Liu et al., 2014; Torrence et al., 2004), we also select a multivariate analysis approach, multiple correspondence analysis (MCA), to analyse our morphometric data through SPSS v25.0. The MCA results are scatter plots in two dimensions (e.g. **Figure 5**), presenting a data set with categorized variables in a two-dimensional graph (Beh and Lombardo 2014; Devaux et al., 1992; Greenacre 2007; Le Roux and Rouanet 2005; Macheridis 2017). The variables (shown in the columns) are plant taxa, granule shape, fissure pattern, lamellae visibility, hilum position and surface texture, and each object (shown in the rows) represents an individual granule from our dataset. Inertia measures an approximate level of homogeneity within the dataset, the higher the value the greater the correspondence among the data (Greenacre 2007). The centroid of the result plot represents the average distribution in each row, which means that the closer the point comes to the centroid, the less different it is from the rest of the observations (Zhang and Dong 2013). The closer the distribution of the category points in the plot, the more relevant they are in the dataset (Franco 2016). The input dataset for the analysis of all productive species is a 2115∗6 matrix. As the main body of our samples is from Fagaceae samples, we therefore analyzed the Fagaceae samples separately. To avoid outliers, we exclude 107 rows where the shape features have high frequency in palm and cycad starches, as distributional patterns of these plots would give emphasis to these uncommon features (Greenacre 2007). Hence, a 1462∗6 matrix was analyzed using SPSS 25.0.

3 RESULTS AND DISCUSSION

3.1 Broad Trends in the Observation Data

In our study sample, a total of 19 species, from seven genera, produced slides with over 100 granules (**Table 1**). We note that

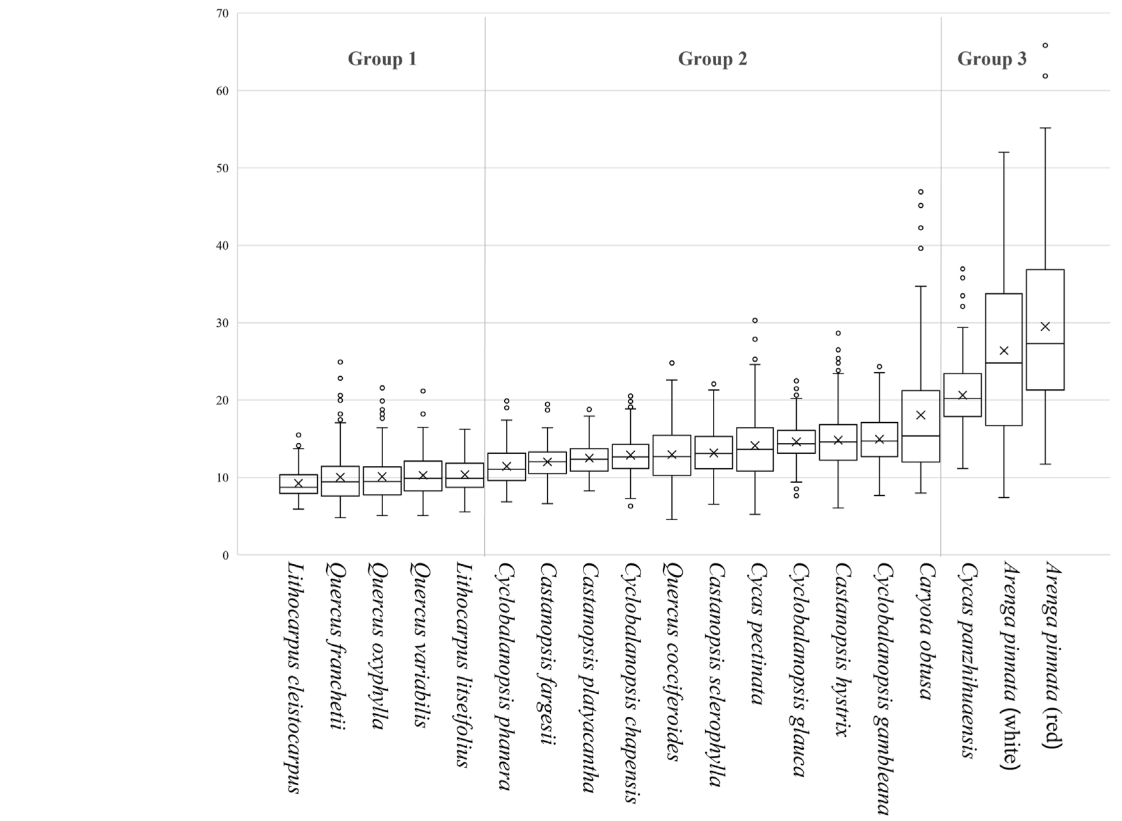

FIGURE 3 | Box plot of the granule sizes of the starches examined.

FIGURE 4 | Percentage distribution of morphological features among plant species (Data shown in **Supplementary Table S2**).

for 15 species of Fagaceae nuts, no, or only few, starch granules were recorded. The *Lithocarpus chrysocomus* sample released only one 15.89 μm granule (**Figure 2**: 11) and *Fagus sylvatica* produced only a few tiny agglomerated granules (**Figure 2**: 16). The lower starch content of some species, such as *Fagus* species, is in accordance with published data (**Supplementary Table S3**).

TABLE 2 | Adjusted significance values of pairwise comparison results of the Kruskal–Wallis test.

Table 2 Adjusted significance values of pairwise comparison results of the Kruskal-Wallis test

	Lithocarpus cleistocarpus	*Quercus franchetii*	*Quercus oxyphylla*	*Quercus variabilis*	*Lithocarpus litseifolius*	*Cyclobalanopsis phanera*	*Castanopsis fargesii*	*Castanopsis platyacantha*	*Cyclobalanopsis chapensis*	*Quercus cocciferoides*	*Castanopsis sclerophylla*	*Cycas pectinata*	*Castanopsis hystrix*	*Cyclobalanopsis glauca*	*Cyclobalanopsis gambleana*	*Caryota obtusa*	*Cycas panzhihuaensis*	*Arenga westerhoutii*(white)	*Arenga westerhoutii*(red)
Lithocarpus cleistocarpus		1.000	1.000	0.339	0.156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quercus franchetii	1.000		1.000	1.000	1.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quercus oxyphylla	1.000	1.000		1.000	1.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quercus variabilis	0.339	1.000	1.000		1.000	0.213	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lithocarpus litseifolius	0.156	1.000	1.000	1.000		0.454	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cyclobalanopsis phanera	0.000	0.009	0.021	0.213	0.454		1.000	0.147	0.004	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Castanopsis fargesii	0.000	0.000	0.000	0.000	0.000	1.000		1.000	1.000	1.000	0.644	0.307	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Castanopsis platyacantha	0.000	0.000	0.000	0.000	0.000	0.147	1.000		1.000	1.000	1.000	1.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Cyclobalanopsis chapensis	0.000	0.000	0.000	0.000	0.000	0.004	1.000	1.000		1.000	1.000	1.000	0.038	0.003	0.001	0.000	0.000	0.000	0.000
Quercus cocciferoides	0.000	0.000	0.000	0.000	0.000	0.011	1.000	1.000	1.000		1.000	1.000	0.015	0.001	0.000	0.000	0.000	0.000	0.000
Castanopsis sclerophylla	0.000	0.000	0.000	0.000	0.000	0.000	0.644	1.000	1.000	1.000		1.000	0.413	0.049	0.017	0.001	0.000	0.000	0.000
Cycas pectinata	0.000	0.000	0.000	0.000	0.000	0.000	0.307	1.000	1.000	1.000	1.000		1.000	1.000	1.000	0.127	0.000	0.000	0.000
Castanopsis hystrix	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.038	0.015	0.413	1.000		1.000	1.000	1.000	0.000	0.000	0.000
Cyclobalanopsis glauca	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.049	1.000	1.000		1.000	1.000	0.000	0.000	0.000
Cyclobalanopsis gambleana	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.017	1.000	1.000	1.000		1.000	0.000	0.000	0.000
Caryota obtusa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.127	1.000	1.000	1.000		0.004	0.039	0.000
Cycas panzhihuaensis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004		1.000	1.000
Arenga westerhoutii (white)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039	1.000		1.000
Arenga westerhoutii (red)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	

Asymptotic significance (2-sided tests) is displayed. The significance level is 0.05. Significance values are adjusted by the Bonferroni correction for multiple tests. p < 0.05 suggest that the two species have statistically significant differences in size distribution.

Ida et al. (2017) tested the nutritional content of some edible beechnuts (*Fagus* spp.) which were once consumed in central Honshu, Japan, indicating that these nuts are high in fats, but low in carbohydrate. But there are some inconsistencies (**Supplementary Table S3**). For example, *Lithocarpus corneus*, which has been found to have a starch content of 47.78% of the kernel (Yang 2014), did not release any granules in our study (**Table 1**).

From the productive starch samples, we first review the broad trends in the data, specifically the morphological data and granule size for each category of plant in our sample. In terms of maximum length, *Lithocarpus cleistocarpus* has the smallest starch range in our sample, both in terms of mean size (9.2 μm) and median size (8.7 μm). The starch granules of the *Arenga landraces* are much larger than those of other plants (**Table 1**). However, there is, generally, a lot of overlap in the sizes of our samples, especially of acorns, which may make it difficult to identify them by granule size alone (**Figure 3** and **Table 1**).

The first feature we note is surface texture, which differs between acorn and palm/cycad starch granules (**Figure 4**). It is clear that all palm/cycad starch grains have smooth surfaces, whereas in the acorn samples, there are some granules with a rough surface (**Figure 4**). Among the starches examined, the palm granules are most likely to have visible lamellae. They also have a higher percentage of granules with an eccentric hilum. Conversely, the fissure patterns make it difficult to classify the granules recorded, as the majority do not bear any fissures (**Figure 4**).

We chart the four categories of granule shapes with different colors (**Figure 4**). The grey type includes irregular-shape granules. The faceted types are in blue, while the ovate-types in orange, the polygonal types (e.g. **Figure 1D**) in red. Of the facet types, bell-shaped granules are concentrated in the *Cyclobalanopsis*and *Casntanospsis* sample, especially *Cyclobalanopsis glauca* (**Figure 2**: 5) which accounts for 56% of its total sample (**Figure 4** and **Supplementary Table S2**). The faceted spherical granules are mainly found in the cycad samples, which separately account for 46 and 56% of the study sample (**Figure 4** and **Supplementary Table S2**). Spherical cap granules occur in small proportions in both *Cycas* and *Lithocarpus*. For ovate types, oval and spherical granules are widely distributed in every sample. Drop-shaped granules are primarily observed in *Quercus* while pestle-shaped and spindle-shaped granules are most frequent in palms. Most species produce some form of polygonal granules (**Figure 4**). However, we note an irregular type, with wavy edges or concave/convex surfaces (**Figure 1D**), mostly observed in *Castanopsis*. The two species of this genus, *C. hystrix* (**Figure 2**: 2) and *C. fargesii* (**Figure 2**: 3) also have a high percentage of normal polygonal granules.

3.2 Non-Parametric Test of Maximum Granule Size

Although the overlap in size of our samples cannot be ignored (**Figure 3**), the Kruskal–Wallis test indicates significant differences among the granule size data (H = 1422.199; df = 18; $p < 0.05$). Further pairwise comparison results (**Table 2**) reveal the differences in the size distributions. Combining the median granules size (**Table 1**) and adjusted significance values (**Table 2**), we first note the large-sized granule group containing *Cycas panzhihuaensi* and two landraces of *Arenga* (**Figure 3**, Group 3), for which the median size is larger than 20 microns (**Table 1**). However, there is no significant difference between the three samples. The group of two *Lithocarpus* samples and three *Quercus* samples (**Figure 3**, Group 1) have the same size distributions with a median size smaller than 10 microns (**Table 1**).

The remaining species produce medium-sized granules (**Figure 3**, Group 2), which can be roughly classified into two groups. The first contains five species, *Castanopsis fargesii, Castanopsis platyacantha, Cyclobalanopsis chapensis, Quercus cocciferoides*, and *Castanopsis sclerophylla*, which have similar size distributions, but can be distinguished from other species (**Table 2**). The second contains four species, *Castanopsis hystrix, Cyclobalanopsis glauca, Cyclobalanopsis gambleana*, and *Caryota obtusa* (**Table 2**). The median size of the former group (12.0–13.1 microns) is smaller than the latter (14.4–15.4 microns) (**Table 1**).

The size of *Cyclobalanopsis phanera* has a similar distribution to *Quercus variabilis, Lithocarpus litseifolius, Castanopsis fargesii*, and *Castanopsis platyacantha,* the median size of which is 11.0 microns, while *Cycas pectinata* (median size = 13.6 microns) has no statistically significant difference in size from the most abundant species (n = 9) (**Table 2**).

3.3 Multiple Correspondence Analysis of Morphological Data

3.3.1 Discrimination of all Starches at Species Level

The first-time analysis indicates that the variable fissure pattern has the least discrimination value within the dataset (**Supplementary Table S5**), which corresponds to our overview of the data (**Figure 4**). Hence, we exclude this column from the second analysis. The second-time analysis at species level shows the differences in our reference starches, with the first dimension explaining 52.3% of the inertia and the second dimension explaining 38.8% (**Supplementary Table S5**). The result plot (**Figure 5**) shows that we can distinguish species within the genus Fagaceae.

The Cycadaceae cluster is located in the first quadrant, i.e. the positive direction of both dimensions. This cluster contains two granule shapes, faceted spherical type and spherical cap type, indicating the close correlation between these and the two cycad samples. The other morphological features, according to the discrimination measures (**Supplementary Table S5**) and the point locations (**Figure 5**), which characterise the granules in this cluster are no lamellae, centric hilum, and smooth surfaces. These characteristics are fully consistent with our observations of the overall trends (**Figure 4**).

Along the first dimension, we note another cluster of three Palmae samples, with the points of pestle-shaped, spindle-shaped, and visible lamellae indicating the major morphological features of the Palmae granules (**Figure 5**). As this cluster is also located in the second quadrant, the Palmae granules are distinguishable by other variables, including those that strongly affect the negative direction of the first dimension, eccentric hilum, and the positive direction the second dimension, smooth surfaces. But the results of the two guanglang landraces show a high degree of consistency, so we cannot distinguish them further by their morphological features.

The points representing Fagaceae species are far away from the palm and cycad samples, revealing evident differences in morphological features (**Figure 5**). The most consistent difference is that the granules from edible acorns tend to have rough surfaces, which can be seen in the percentage distribution of the morphological features (**Figure 4**). We also note that the four *Cyclobalanopsis* species and *Castanopsis platyacantha*, in the dashed circle of **Figure 5**, may be distinguishable from other species, as they are relatively far away from the centroid. The granules may have a bell shape or irregular polygonal shape, with rough surfaces. However, the Fagaceae species are relatively close to the centroid of the plot, suggesting that they may represent the average features of our dataset. This may be because the sample of Fagaceae is larger than the other two genera. Hence, we decide to analyse the Fagaceae independently, to find their morphological differences.

3.3.2 Discrimination of Fagaceae Granules at Both Species Level and Genus Level

The analysis of the Fagaceae starches, at both the species level and the genus level, shows that the variables fissure type and lamellae visibility have weak discriminant value (**Supplementary Table S5**), consistent with **Figure 4**, that is, the Fagaceae starches commonly have no fissures or lamellae. Therefore, we focus on the variables of shape, hilum position and granule surface texture within the dataset which could be used to discriminate acorn starches.

The results at the species level show the differences in our reference starches, with the first dimension explaining 51.7% of the inertia and the second dimension explaining 41.0% (**Supplementary Table S5**). Of the two binary variables, based on the discrimination measures (**Supplementary Table S5**), dimension 1 is related to hilum position, i.e. species along the positive direction of dimension 1 tend to have centric hilum, while dimension 2 is surface texture. In this case, we note five rough clusters of Fagaceae starches in the plot (**Figure 6A**).

Cluster 1 contains the points for two *Lithocarpus* species with spherical granules. This indicates a correspondence between spherical granules and these two species. These granules also have a centric hilum and smooth surface. Cluster 2 shows the associations between bell-shaped granules and the species *Castanopsis platyacantha, Cyclobalanopsis glauca* and *Cyclobalanopsis gambleana*. These granules tend to have a centric hilum but rough surface. Along dimension 1, there are two clusters in the negative direction, representing granules with eccentric hilum. Cluster 3 indicates that the granules of *Cyclobalanopsis chapensis* and *Cyclobalanopsis phanera* are similar in morphology, characterized as oval with rough surfaces. Cluster 4 includes *Quercus cocciferoides, Q. variabilis* and *Q. franchetii*. These granules mainly have a drop shape but can be classified further. Compared to the other two species, the starches of *Q. cocciferoides* are more likely to have rough surfaces, consistent with the percentage calculation (**Figure 4**).

Cluster 5 is located in the positive direction of the second dimension, representing an overall trend for granules with smooth surfaces. This cluster contains two species of *Castanopsis*, *C. hystrix* and *C. fargesii*, and has polygonal features, showing their correspondence. According to the point locations, *C. hystrix* tends to produce normal polygonal granules, but *C. fargesii* may produce irregular types. This result is consistent with the trends represented by the granule shape percentages. We note that the *C. hystrix* granules may have a higher occurrence of an eccentric hilum. Based on the percentage calculation (**Supplementary Table S2**), the difference in this variable between these two species is minor.

The result at the genus level (**Figure 6B**) show that discrimination is possible at this taxonomic level, with the first dimension explaining 48.8% of the inertia and the second dimension explaining 38.6% (**Supplementary Table S5**). In the results plot, the feature hilum position, which has a large measure of discrimination in the first dimension, distinguishes *Quercus* from others, specifically, *Quercus* granules have a higher probability of having an eccentric hilum than the other three genera. Similarly, *Cyclobalanopsis* starches are more likely to have rough surfaces.

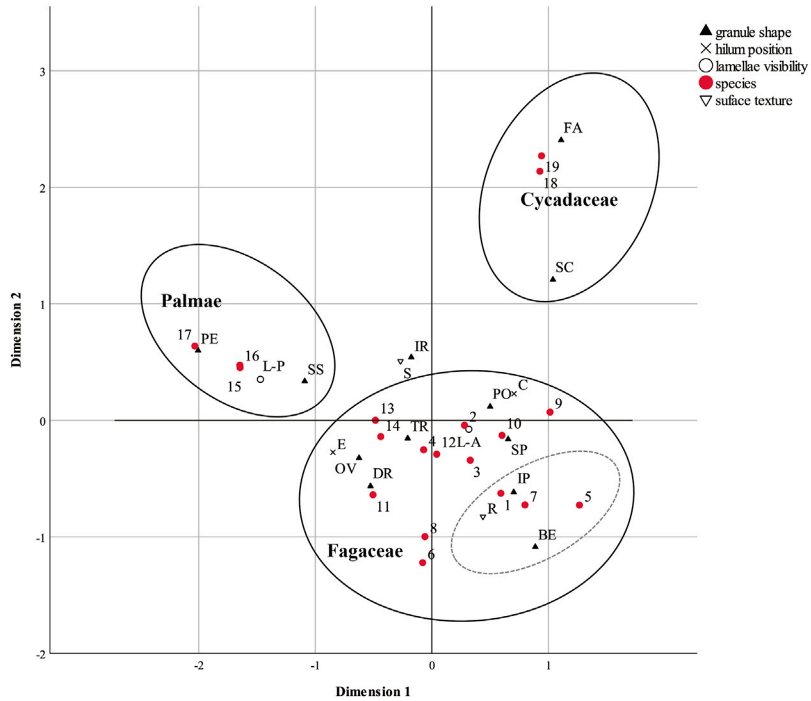

FIGURE 5 | MCA result of morphometric data of starches at the species level. Labels: Species: 1, *Castanopsis platyacantha*; 2, *Castanopsis hystrix*; 3, *Castanopsis fargesii*; 4, *Castanopsis sclerophylla*; 5, *Cyclobalanopsis glauca*; 6, *Cyclobalanopsis chapensis*; 7, *Cyclobalanopsis gambleana*; 8, *Cyclobalanopsis phanera*; 9, *Lithocarpus cleistocarpus*; 10, *Lithocarpus litseifolius*; 11, *Quercus cocciferoides*; 12, *Quercus oxyphylla*; 13, *Quercus variabilis*; 14, *Quercus franchetii*; 15, *Arenga westerhoutii* (white); 16, *Arenga westerhoutii* (red); 18, *Caryota obtusa*; 18, *Cycas pectinata*; 19, *Cycas panzhihuaensis*. Shapes: BE: bell shape, DR: drop shape, FA: faceted sphere, IP: irregular polyhedron, IR: irregular shape, OV: oval shape, PE: pestle shape, PO: polygonal shape, SP: sphere, SC: spherical cap, SS: spindle shape, TR: triangle. Lamellae visibility: L-A: lamellae absence, L-P: lamellae presence. Surface texture: R: rough surface, S: smooth surface. Ellipses are not statistical, but hand-drawn to isolate key woody plant forms (Acorns, Cycads, Palms).

This result also reveals the most identifiable shape features of the four Fagaceae genera. The identification keys include bell-shaped granules for *Cyclobalanopsis* and drop-shaped granules for *Quercus*. The *Castanopsis* and *Lithocarpus* granules seem to be similar in shape, irregular polyhedrons, polygons and spheres. However, according to the percentage distribution of the shape features (**Figure 4**), we suggest that spherical granules are common in *Lithocarpus* starch sample, while polygonal granules, especially irregular types, are *Castanopsis*. This result matches our previous analysis of morphological characteristics at the species level.

4 Identification Scheme of Woody Plant Starches

The identification of productive woody starch granules in this study combines two methods of mutual verification: a traditional morphmetric analysis made by an expert and automated computer-based statistical analyses. The statistical analyses confirms that both the numerical variables of maximum granule size and nominal morphological variables are useful to discriminate starches of various plant taxa, supporting previous attempts to identify plants using a morphometric method (e.g. Torrence et al., 2004; Yang and Perry 2013; Liu et al., 2014; Coster and Field 2015). Non-parametric tests statistically evaluate the distribution differences of granule sizes between the samples examined. Multiple correspondence analysis can be used to visualize the correlation between morphometric data and plant taxa in a two-dimensional plot and calculate the contribution of each categorized variable, which can help an analyst select those that are most critical for discriminating starches.

In our cases, the fissure types of the starches from woody plants make the least contribution in starch identification. However, we can reasonably identify granules from tested edible acorn kernels, cycad pith and palm pith in southern China on the basis of size and other morphological features. The identification scheme is summarized as follows.

(1) The spindle-shaped or pestle-shaped granules, with eccentric hilum, clear visible lamellae, smooth surface and non-fissure are the typical starches from starchy palm pith including *Caryota obtuse* (**Figure 2**:19) and the two landrace samples of *Arenga westerhoutii* (**Figure 2**:17–18). The size data can be used to make an inter-genus distinction, as the maximum granule sizes of the two guanglang landraces (mean size = 26.4/29.5 microns) are statistically larger than those of fishtail palm (mean size = 18.1 microns) (**Table 1**).
(2) The diagnostic morphological features of the two *Cycas* species are fully identical. They have faceted sphere or spherical cap shapes with a smooth surface and centric hilum, but without any fissures or visible lamellae (**Figure 2**: 20–21). However, the two *Cycas* are statistically

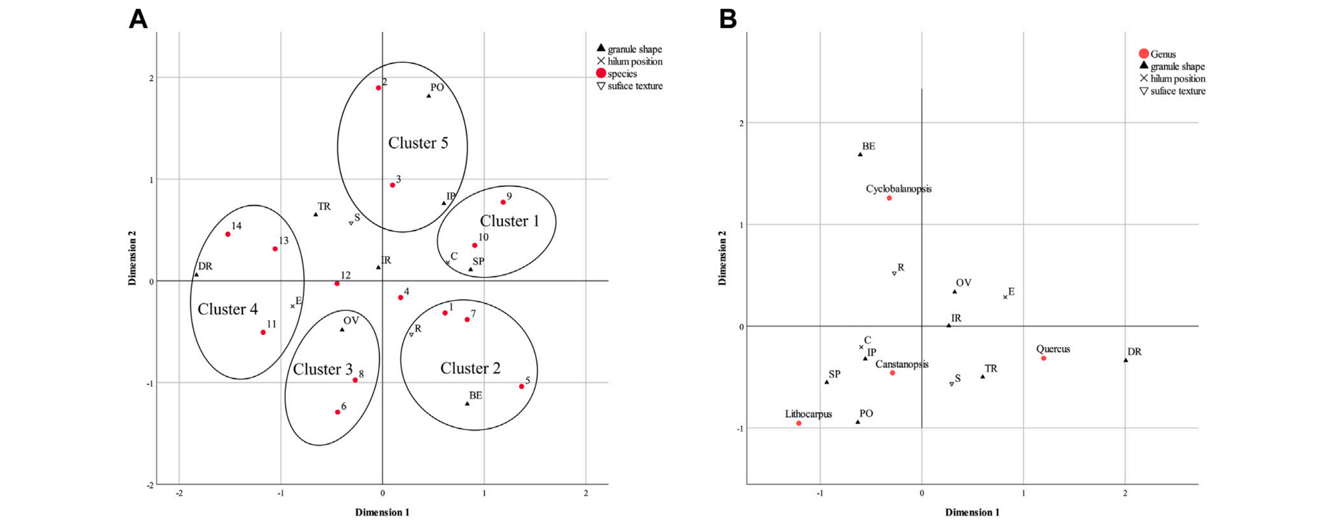

FIGURE 6 | MCA result of morphometric data of Fagaceae starches: **(A)** at the species level; **(B)** at the genus level. Labels: Species: 1, *Castanopsis platyacantha*; 2, *Castanopsis hystrix*; 3, *Castanopsis fargesii*; 4, *Castanopsis sclerophylla*; 5, *Cyclobalanopsis glauca*; 6, *Cyclobalanopsis chapensis*; 7, *Cyclobalanopsis gambleana*; 8, *Cyclobalanopsis phanera*; 9, *Lithocarpus cleistocarpus*; 10, *Lithocarpus litseifolius*; 11, *Quercus cocciferoides*; 12, *Quercus oxyphylla*; 13, *Quercus variabilis*; 14, *Quercus franchetii*. Shapes: BE: bell-shape, DR: drop-shape, FA: faceted sphere, IP: irregular polyhedron, IR: irregular shape, OV: oval shape, PE: pestle shape, PO: polygonal shape, SP: sphere, SC: spherical cap, SS: spindle-shape, TR: triangle. Lamellae visibility: L–A: lamellae absence, L–P: lamellae presence. Surfaces texture: R: rough surface, S: smooth surface.

different in size, with granules from *C. panzhihuaensis* (mean size = 20.6 microns) larger than those from *C. pectinata* (mean size = 14.1 microns) (**Table 1**), which can be used to make an inter-species identification.

(3) Starch granules from acorn kernels are more likely to have rough surfaces and various morphological features by comparison. Based on our analyses, though, we can discriminate further at genus level.

(a) *Quercus* granules (**Figure 2**:12–15) are most distinguishable within the acorn starches, as they have a typical granule form, which has drop shape, eccentric hilum, but no fissures or visible lamellae. In our observations, some of them have a small pit at their pointed ends (**Figure 2**:12), making them more identifiable. Among the species collected, *Q. cocciferoides* are distinguishable, because the maximum granule size of this species (mean size = 13.1 microns) is statistically larger than the other three species (mean size = 10.0/10.1/10.2 microns) which are in the smallest granule sample of our dataset.

(b) A rough bell-shape granule with centric hilum is the most identifiable type of *Cyclobalanopsis* at the genus level (**Figure 2**: 5–8). Of the four species studied, the starch samples of *C. glauca* (**Figure 2**: 5) and *C. gambleana* (**Figure 2**: 7) are dominated by these typical granules. The maximum size data do not show a statistically significant difference between them (**Table 2**). The other two species, *C. chapensis* (**Figure 2**: 6) and *C. phanera* (**Figure 2**: 8), have identifiable granules, but are more likely to produce oval granules, according to both traditional analyses (**Figure 4**) and the MCA results (**Figure 6A**). Their granule sizes are also smaller than the former two species, of which *C. phanera* is the smallest (mean size = 11.5 microns).

(c) The granules of *Lithocarpus* are statistically smaller (with size medians smaller than 10 microns) (**Figure 3**), while the two species *L. litseifolius* and *L. cleistocarpus* cannot be distinguished by size. They are also similar in morphology, having spherical shapes, centric hilum and smooth surfaces, without any fissures or visible lamellae (**Figure 2**: 9–10). Such granule form seems to be widespread in many other plants, for example, lotus seeds (*Nymphaea tetragone*) (Wan et al., 2011). Therefore, we return to the granule images, and note that there is a small pit at the centre of the granule (**Figure 2**: 10) which may help to distinguish this genus.

(d) The *Castanopsis* granules (**Figure 2**: 1–4) are characterized by irregular polygonal shapes, with centric hilum and smooth surfaces, although the percentage distribution of shape features suggests that this is not the major form in some species (**Figure 4**). On the basis of size, *C. hystrix* granules (mean size = 14.3 microns) are larger than those of the other three *Castanopsis* species.

The study shows that it is possible to identify starches among plant taxa using both numeric size data and nominal morphological features. Traditional identification is effective for setting up an identification scheme, and multiple correspondence analysis applied to morphological data is also a useful approach in the early stage of discriminating identifiable features of modern reference collections.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: HB and XY. Performed the experiments: ZL and WW. Analyzed the data: ZL, HB and XY. Contributed reagents/materials/analysis tools: XY and HB. Wrote the paper: ZL, HB and XY. Collected the samples in fieldwork: ZL, WW and XY.

FUNDING

Funding for this research was provided by National Natural Science Foundation of China (Grant No. 41771231 and 4137121) and China Scholarship Council (201604910602).

ACKNOWLEDGMENTS

We thank Prof. Changjiang Liu from Institute of Botany, Chinese Academy of Sciences for providing *Fagus* nuts; Lu Li from Southwest Forestry University for other modern sample collection in Yunan and identification some modern samples to species. We also thank the University of Leicester School of Archaeology and Ancient History Starch and Residue Laboratory.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2022.815351/full#supplementary-material

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