Palmitone as a potential species-specific biomarker for the crop plant taro (Colocasia esculenta Schott) on remote Pacific islands

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A B S T R A C T

The Pacific Island ecosystems of Remote Oceania were dramatically transformed following the arrival of humans within the last ~3000 years, as the new settlers required technological innovations and environmental modifications to maintain their populations. These modifications included the introduction of numerous exotic species, including the important crop Colocasia esculenta Schott (taro) and the development of infrastructure suitable for its cultivation. Archaeological reconstruction of C. esculenta use in the Pacific has been challenging because of the low-specificity of fossil starch granules and its limited pollen production during periods of intense cultivation. Here, we assess a lipid biomarker approach to trace C. esculenta cultivation in the past. We characterized the neutral lipid compositions of leaf samples from common cultivars and widespread indigenous species from the archipelago of Vanuatu by gas chromatography–mass spectrometry (GC–MS). The compound palmitone (hentriacontan-16-one) was a major leaf wax constituent in C. esculenta cultivar samples (mean concentration of 402 ± 63 μg g⁻¹ dry wt) and was only detected in one other species, the ornamental tree Cananga odorata (175 μg g⁻¹ dry wt). The structure of palmitone is favorable for its long-term stability and we demonstrate its preservation potential in a 55 cm sedimentary record from Lake Vesalea on Espiritu Santo, Vanuatu, where C. esculenta is grown today. Palmitone concentrations in this core fluctuated up to 4.1 μg g⁻¹ dry wt. Our results indicate that in appropriate environmental contexts, sedimentary palmitone concentrations could be used to reconstruct C. esculenta cultivation and to provide insights about past horticultural innovations in Remote Oceania.

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1. Introduction

The islands of Remote Oceania were settled relatively recently, within the last three thousand years, and provide important microcosms in which to study human–ecosystem interactions (Kirch, 2017). The first known human settlers on the westernmost islands, including the archipelago of Vanuatu, were the Lapita people, who had an unprecedented impact on the pristine natural environments (Stevenson, 2004; Fall, 2005; Anderson, 2009; Prebble and Wilmshurst, 2009; Summerhayes et al., 2008; Horrocks et al., 2017). Many questions remain about the subsistence activities of these first settlers, the initial role and subsequent development of horticultural systems, and in particular the production of the starchy corm crop Colocasia esculenta (taro). The initial assumption was that the Lapita people relied on wild food procurement, particularly of marine resources and that crop production became more important as these resources declined (Groube, 1971). Later studies have shown that the Lapita people brought domesticated plants and animals to Remote Oceania, which allowed them to rapidly adapt to the local ecology and to settle previously uninhabited islands (Spriggs, 1997; Kirch, 2017). The successful establishment of crop production might have been delayed as the first settlers had to adapt to sparse conditions as they moved eastwards (Sheppard, 2011; Kirch, 2017). The wild terrestrial foods available during colonization consisted mainly of leafy greens, fruits and some nuts that were supplements to meals, whereas starchy vegetables such as yam and taro comprised the major carbohydrate sources, and were introduced throughout the Pacific by early human colonizers (Pollock, 1992;
Horrocks and Bedford, 2005; McClatchey, 2012). However, the natural distribution and human translocation of many domesticated plant species remains uncertain.

Different approaches have been used to find evidence of the arrival and subsistence activities of the first settlers and their descendants. The excavation of archaeological sites has provided valuable insights through the analysis of ceramics, stable isotopes and ancient DNA from human skeletal remains, and plant microfossils (Bedford et al., 2006, 2007; Horrocks et al., 2009; Skoglund et al., 2016; Valentin et al., 2016; LeClerc et al., 2018). Information about past diet has been obtained by examining plant microfossils (such as starch residue, pollen, and phytoliths) from archaeological and palaeoecological sites including lakes, swamps and former gardens, located across the Pacific Islands. In many cases, starch granules represent the first direct evidence of horticulture and the introduction of agricultural crops such as taro at many Lapita sites (Horrocks and Bedford, 2005; Horrocks and Nunn, 2007; Clark et al., 2015). The comparative collections used to identify fossil starch granules, however, have been drawn almost exclusively from cultivated plants, and given the potential variety of indigenous plant starches with overlapping granule morphology, starch identification has been problematic (Horrocks and Weisler, 2006). Fossil starch granules also provide little ecological information of the growing conditions of this crop. In contrast to starch, taro pollen has a highly specific surface ultrastructure and is readily identified using compound microscopy (Grayum, 1992).

Fossil pollen has been retrieved from stratified sediments from the Hawaiian Islands (Athens and Ward, 1997), Tonga (Fall, 2005), French Polynesia (Prebble and Wilmshurst, 2009; Prebble et al., 2013, 2016, in press), Rapa Nui (Horrocks et al., 2017) and New Zealand (Prebble et al., in press). However, limited taro pollen enters the sedimentary record during periods of high intensity production, most likely because the perennial combs of the plant are rarely allowed to flower by cultivators due to frequent harvesting (Prebble et al., in press). Additional methods are needed to help identify when taro was introduced to the islands, as well as past periods of high intensity production.

An alternative approach that could provide information about past taro cultivation is the use of sedimentary lipid biomarkers, here defined as compounds specific to certain biotic sources which retain their essential chemical structure and stable isotopic composition through preservation in sediments. Lipid biomarkers are an important tool in paleoenvironmental research, limnology and oceanography (e.g., Prahl et al., 1992; Jacob et al., 2008; Dubois and Jacob, 2016). Lipids are often used as biomarkers due to their stability through diagenesis compared to other biochemical components of organic matter, making them more long-lived in sedimentary records (Meyers, 1997). Molecular distributions and the stable isotopic composition of lipids biomarkers have a long history as indicators of past climatic and ecological changes (reviewed by Meyers, 1997; Castañeda and Schouten, 2011). More recently, biomarkers have been employed to reconstruct past human agricultural activities (Jacob et al., 2008; Dubois and Jacob, 2016; Motuzaite-Matuzevičiute et al., 2016). For example, the pentacyclic triterpenoid miliacin is used as a species-specific biomarker for millet and has been used to reconstruct the history of cultivation in the French Alps and in the floodplain of the River Donets, eastern Ukraine (Jacob et al., 2008; Motuzaite-Matuzevičiute et al., 2016). However, biomarkers like miliacin that are specific to only one plant in a specific environmental context have only been rarely established, and no source-specific biomarkers suitable for tracing horticultural developments in Remote Oceania have been developed. The aim of the present study was to find a species-specific biomarker for C. esculenta that is well preserved in sediments. In order to do this, we extracted the lipids from 161 plants from Vanuatu, including the most common cultivars and indigenous species, and analyzed their lipid compositions, as well as the uniqueness of individual lipids and their down core presence in a sedimentary record from a modern garden site.

2. Materials and methods

2.1. Sample collection

A total of 161 samples from cultivars and non-cultivated indigenous plants, representing 87 different genera, were collected in 2012 and 2017 from the islands of Espiritu Santo (15°9′S, 166°49′E), Efate (17°40′S, 168°25′E), Aniwa (19°15′S, 169°34′E) and Thion (15°1′S, 167°4′E) in the archipelago of Vanuatu (Fig. 1). The most economically important cultivars from Vanuatu are included in the sample set. The 45 cultivar samples were collected at the Vanuatu Agricultural Research and Technical Center (VARTC, Espiritu Santo, 15°26′53″S, 167°12′47″E) and included samples from C. esculenta (taro), as well as Artocarpus altilis (breadfruit), Bambusa sp. (bamboo), Dioscorea bulbifera (air yam), Ipomoea batatas (sweet potato), Manihot esculenta (cassava), Musa AAA (banana), Piper methysticum (kava) and Terminalia catappa (sea almond). The leaves were collected in Ziploc bags and placed in an insulated box with ice in the field and were subsequently stored at −20 °C until they were freeze-dried, directly prior to analysis.

In order to assess the potential preservation of plant-based biomarkers in sedimentary settings from the tropical Pacific Islands, we retrieved a 55 cm long sediment core with a percussion corer (UWITEC, Mondsee, Austria) equipped with a PVC tube (diameter 63 mm) from the center of a small pond near the village of Vesalea (15°9′32″S, 166°39′18″E) on the west coast of Espiritu Santo (Fig. 1). This core was sectioned at 1 cm intervals, which were stored in Whirl Paks at −20 °C until the samples were freeze-dried.

Fig. 1. Map of the archipelago of Vanuatu indicating the plant sample locations on the islands of Espiritu Santo, Efate, Aniwa and Thion (triangles) and the Lake Vesalea coring site (circle). The plant cultivar samples were collected at the Vanuatu Agricultural Research and Technical Center (VARTC, Espiritu Santo).
As the pond does not have an official name it is referred to as Lake Vesalea in this study. At the time of coring, the pond was covered by mats of aquatic plants, primarily *Persicaria cf. attenuata, Salviniola molestia, Hibiscus tiliaeus* and *Calystegia soldanella*. Secondary vegetation surrounding the pond consisted of *Aristochitum aureum, Veitchia sp., Macaranga spp., Dracontomelon vitensis, Diospyros sp., Machaerina sp., Acacia spirorhiz*, and *Abrus precatorius*. Gardens situated within ~100 m of the pond contained *Musara* sp., *Cocos nucifera, Citrus × paradisi, Citrus × sinensis*, and *C. esculenta*. *C. esculenta* is the staple crop of this region, and the west coast of Espiritu Santo is unique in having a continuing tradition of irrigated gardens that are now restricted to only a few locations in the Western Pacific (Spriggs, 2002).

The earliest regional evidence for taro production in Vanuatu comes from Efate and Malekula islands in the form of starch grains from archaeological sites (e.g., Horrocks and Bedford, 2005). In Vanuatu, radiocarbon dating of agricultural features including stone-wall irrigated garden features constructed for taro production is limited to Aneityum in the south (Spriggs, 1981, 2002). Farther afield, similar evidence dating from the past 1000 years is found in the Western Solomon Islands, to the north of Espiritu Santo (Bayliss-Smith et al., in press). Similar (but undated) archaeological remains exist in the western part of Espiritu Santo, and irrigated taro garden features are currently used on the western Santo (Bayliss-Smith et al., in press). Similar (but undated) archaeological remains exist in the western part of Espiritu Santo, and irrigated taro garden features are currently used on the western Santo (Bayliss-Smith et al., in press).

2.2. Lipid extraction and purification

A recovery standard including 5a-androstan-1, 1-nondecanol, heneicosanolic acid and 3-ecosane was quantitatively added to ~2 g of freeze-dried, homogenized leaf material from each plant sample. The lipids were extracted with a mixture of dichloromethane/methanol (DCM/MeOH) (4:1, v/v) in an ultrasonic bath during three cycles of 20 min each. The same recovery standard was added to 3 g of dried sediment from every second cm of the sediment core. Sediment samples were extracted in a mixture of DCM/MeOH (9:1, v/v) with a High Performance Microwave Digestion Unit (MLS 1200 MEGA, Milestone, Sorisole, Italy), which was heated to 70 °C over 2 min and kept at 70 °C for 5 min (Kornilova and Rosell-Melé, 2003). The results from the two different methods of lipid extraction are comparable (Kornilova and Rosell-Melé, 2003; Ma et al., 2014). However, due to the large number of leaf samples and the limited availability of Teflon tubes for the microwave system, the ultrasonic bath was more efficient for processing our leaf samples. The total lipid extracts (TLEs) from both sediment and plant samples were dried under a gentle stream of nitrogen. All samples were then saponified with 3 mL of 1 N KOH in MeOH and 2 mL of solvent-extracted Milli-Q water at 70 °C for 3–16 h. The neutral fraction was extracted from the aqueous phase with three subsequent hexane rinses (~2 mL each).

While the neutral fractions from the leaf samples were not separated into different compound classes, the neutral fractions from the sediment samples were purified using silica gel column chromatography. The neutral fraction was dissolved in hexane and loaded onto a 500 mg/6 mL pre-packed Si column (Biotage, Uppsala, Sweden). The total volume of the glass cartridge was 6 mL, with a small dead volume of Si gel itself. The hydrocarbons and n-alkanes were eluted with 4 mL of hexane, the ketones and aldehydes with 4 mL of hexane/DCM (2:1, v/v), the sterols and other alcohols with 4 mL of DCM/MeOH (19:1, v/v), and the more polar compounds with 4 mL of MeOH. The entire neutral fraction of the plant samples as well as the second and third Si gel fractions of the sediment samples were converted into trimethylsilyl derivatives prior to analysis by adding 25 μL each of N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine to each sample and placing them in the oven at 60 °C for one hour.

2.3. Compound identification and quantification

The neutral fractions of the plant samples as well as the n-alkane, ketone and alcohol fractions from the sediment samples were analyzed and quantified by gas chromatography–mass spectrometry (GC–MS). The GC–MS analyses were performed on an Agilent 7890B gas chromatograph with an Agilent HP-5MS column (30 m × 0.25 mm × 0.25 μm film thickness) coupled with an Agilent 5977B mass spectrometer (MS) and a flame ionization detector (FID) (Agilent Technologies, Santa Clara, USA). The oven was heated from 70 °C to 210 °C at 15 °C/min then to 320 °C at 3 °C/min and held for 10 min before cooling to 100 °C at a rate of 100 °C/min. The injection volume was 1 μL and the column effluent was split between the FID and the MS (1:1).

Compounds were identified by comparison of their mass spectra with reference compounds using the NIST 14 Mass Spectral Library from the National Institute of Standards and Technology (NIST), and quantified relative to one of the four recovery standards. The mass spectra of the identified compounds were added to a library created for the project using the Agilent ChemStation Enhanced Data Analysis software. Only compounds that had a score higher than 90% in comparison with the NIST 14 library reference spectrum were added to the project library. The library entries included the mass spectra, the retention times and the molecular masses. All samples were subsequently compared with those compounds in the project library using the Agilent Mass Hunter Workstation Qualitative Analysis (B.07.00) software and the resulting compound identification and peak areas were used for further analysis. The compound palmitone was initially identified by comparison with the NIST 14 library by the m/z 71 + 239 + 255 extracted ion chromatogram (Fig. 2) and confirmed by comparison to an authentic standard (abcr GmbH, 16-Hentriacontanone; lot 1398514).

2.4. Radiocarbon dating

Fifteen macrofossils from 13 different depths of the Lake Vesalea sediment core were handpicked and radiocarbon dated (Supplementary Table S2). Standard procedures previously described were followed (Lavrieux et al., 2017). The measurements were performed using accelerator mass spectrometry (AMS) at ETH Zurich. 137Cs radionuclide activity was measured using a high-purity Germanium Well Detector (HPGe, Gamma spectrometer) (Supplementary Fig. S13). An age model was constructed from these values using the rbaco R package version 2.3.6 (Supplementary Fig. S14) (Blauuw and Christen, 2019).

3. Results

3.1. Neutral lipid distributions in leaf samples

A total of 177 different compounds were identified in the plant samples, 54 of which were present in the cultivar samples (Fig. 3; Supplementary Table S3). Phytol was present in all sampled plants (n = 161), and was typically one of the most abundant compounds, with a mean concentration of 2191 μg·g⁻¹ dry wt. The other most common compounds in the cultivars and other plants were squa-lene (n = 109), n-alkanes (n = 118), n-alkanols (n = 135) and the phy-tosterols campesterol (24α-methylcholest-5-en-3β-ol) (n = 140), stigmasterol (24α-ethylcholesta-5,22E-dien-3β-ol) (n = 144), and sitosterol (24α-ethylcholesterol-5-en-3β-ol) (n = 143). These
compounds occurred in varying amounts with mean concentrations ranging from 165 μg g⁻¹ to 785 μg g⁻¹ (Fig. 3; Supplementary Table S3). Many of the other compounds were produced by a more limited number of plants in the sample set. However, those compounds were not unique to a single genus. For example, many of the plants sampled did not produce any pentacyclic triterpenoids (produced by n = 89), but the ones that did only produced the same small group of compounds such as α-amyrin (3β-urs-12-en-3-ol) (n = 55), β-amyrin (3β-olean-12-en-3-ol) (n = 47) and lupeol (lup-20(29)-en-3β-ol) (n = 42). The same is true for ketones, where the most commonly found ketone was pentadecan-2-one (n = 46) and for amides, where oleamide (octadec-9Z-enamide) (n = 4) was the most common compound. In addition to the most common sterols listed above, several plants produced 9,19-cyclolanost-24-en-3β-ol (n = 35), cholesterol (cholest-5-en-3β-ol) (n = 38) and lanosterol (4,4,14τ-trimethyl-5x-cholesta-8,24-dien-3β-ol) (n = 18). The most commonly identified sesquiterpenes and diterpenes were caryophyllene (n = 13), farnesol (n = 10) and kaur-16-ene (n = 6).

Of the 177 identified compounds, there was one compound that was abundant in C. esculenta and only present in one other species (Cananga odorata), namely palmitone (hentriacontan-16-one) (Figs. 2 and 3). Palmitone is a ketone with the molecular formula C₃₁H₆₂O and has a molar weight of 450.836 g mol⁻¹. It was present in all of the six C. esculenta cultivar specimens in this sample set. Palmitone in the C. esculenta cultivar samples had a mean concentration of 402 ± 63 μg g⁻¹ dry wt (Fig. 3), making it one of the most abundant compounds in C. esculenta, along with some of the most common plant compounds such as phytol, triacontan-1-ol and sitosterol (Figs. 3 and 4). Palmitone was also identified in the ornamental garden plant C. odorata, where it had a concentration of 175 μg g⁻¹ dry wt.

Additionally, there were many compounds that could not be identified by comparison with the mass spectra in the NIST 14 library. One of those unidentified compounds in C. esculenta had a retention time of ~3.6 min relative to the external cholesterol standard (Fig. 4). This compound could not be found in any other plant in the sample set (Supplementary Table S2). Eleven other unidentified compounds were present in all replicate samples from one species of cultivar (Supplementary Table S1). However, these compounds were neither source specific within our sample set, nor were identified in the sediment.

3.3. Age model

137Cs was present in the upper 15 cm of the sediment core but was not detected below this depth (Supplementary Fig. S13). The presence of 137Cs in this region is associated with nuclear weapon testing in the Pacific, beginning in 1956 C.E. (Leslie and Hancock, 2008). Depths below 15 cm were therefore considered pre-1956 C.E. There were several age-depth reversals among the 15 radiocarbon dates obtained from macro-fossils. These may be due to inclusion of relatively young root material among the plant fibres that were dated. Based on the 137Cs results, negative 14C dates below 15 cm were considered likely root intrusions and not included in the age model. The age-depth model for the Lake Vesalea sediment core (Supplementary Fig. S14) was derived with the rbacon package version 2.3.6 (Blaauw and Christen, 2019) from the remaining 8 radiocarbon dates, the onset of bomb testing, and assumption that the surface sediment was contemporaneous to the core collection year of 2017 (Supplementary Table S2). Other age reversals were included in the model as there was no clear rationale to exclude them. The bottom of the sediment core was radiocarbon dated to 940 ± 78 cal years BP, and the mean accumulation rate was 20 y/cm (Supplementary Fig. S14).

4. Discussion

The distributions of neutral lipids among the plants in our sample set are very heterogeneous and differ significantly between species (Fig. 3). There are only a few compounds that are unique to one species or genus. This is consistent with the fact that biomarkers restricted to an individual species or genus have rarely
been discovered on a global scale. Therefore, the identification of high amounts of palmitone in *C. esculenta* samples, but not in other widespread species from Vanuatu (Fig. 3), is a valuable discovery.

In addition to being unique, a biomarker must be well preserved in sediment in order to be useful for reconstructing crop production changes over time. Fluctuating concentrations of palmitone...
in the upper portion of the sediment core from Lake Vesalea (Fig. 5) are indicative of its preservation potential.

4.1. Overall potential for plant species-specific lipid biomarkers

The analysis of the lipid composition of a broad range of plant species from Vanuatu demonstrates that the majority of species possess only a few lipids in high concentrations (Fig. 3). There are only a few compounds that are relatively rare in the sample set and only present in a small number of species. An example of a rare compound in the sample set is diploptene (hop-22(29)-ene), which was only found in two fern samples (*Cyclosorus interruptus* and *Cyathea lanulata*) and is already used as a biomarker for ferns (Ageta and Arai, 1983), although it can also indicate bacterial activity (Prahl et al., 1992; Elvert et al., 2001). Therefore, this compound does not represent a new, species-specific biomarker. Another unique compound in this sample set was 4-methoxy-benzaldehyde (*p*-anisaldehyde), which was only produced by *Piper methystichum* (kava). This compound is a flavoring ingredient and can naturally be found in a variety of different plants including American cranberry, anise oil, fennel and vanilla (Bilia et al., 2000; Rodrigues et al., 2003; De Jager et al., 2008). As no known producers of 4-methoxy-benzaldehyde besides *P. methystichum* are cultivated or naturally abundant in Vanuatu, it could serve as a biomarker for kava cultivation. However, 4-methoxy-benzaldehyde is a highly functionalized, volatile compound, meaning that it is unlikely to be preserved on any significant timescales, and is therefore not promising as a sedimentary biomarker.
Palmitone was only detected in two species in the sample set, but as discussed below (Section 4.2), even this compound is not unique to these two species, and it has been identified in other plants from other countries. However, it can still be used as a species-specific biomarker in areas with well-defined botanical settings where none of the other plants have been recorded. This approach was also used by Jacob et al. (2008) who used miliacin as a biomarker for millet cultivation around Lake Le Bourget. While miliacin has also been detected in a few other plants around the world, archaeobotanical studies supported broomcorn millet as the sole possible source in the catchment in Bronze Age Europe.

In general, all the compounds that are listed in the NIST 14 library have been previously identified in natural or synthetic products and are therefore unlikely to be unique to an individual species. As such, during our compound screening we also focused on compounds that were present in the lipid extracts from cultivars, but that did not have a match in the NIST 14 library (Supplementary Table S1). Eight compounds were present in a single species of cultivar and were absent from all other plants in the combined cultivated and wild sample set. However, none of these compounds were detected in the sediments from the modern garden site at Lake Vesalea, suggesting that they may have limited use as sedimentary proxies of past horticultural activities. Until the preservation potential of these compounds can be established, determining their structures and identities is not warranted for the purpose of developing lipid biomarkers that are relevant for studying the history of horticulture in the tropical Pacific.

4.2. Uniqueness of palmitone to C. esculenta

While palmitone was unique to C. esculenta in this sample set from Vanuatu (Fig. 3), it has previously been identified in the leaves of other plants (e.g., Chibnall et al., 1937; Mackie and Misra, 1956; González-Trujano et al., 2006, 2009), although it is generally considered to be a rare component of plant leaf waxes (Barthlott et al., 2017). Palmitone has not been identified in C. esculenta, nor in the Areaceae family, prior to our study. Palmitone was first reported to be present in Santalum album (sandalwood) (Chibnall et al., 1937) and was later also detected in Allium porrum (Rhee et al., 1998), Laurus nobilis, Aristolochea gigantea, Paeonia officinalis, Paeonia mlokosewitschii (Meusel et al., 1999), Rollinia mucosa (Estrada-Reyes et al., 2002), and Aristolochia esperanzae (Oliveira et al., 2003). More recent studies have focused on the identification of palmitone in different species from the Annonaceae family. Palmitone extracted from Annona diversifolia, A. cherimolia, A. diversifolia, and A. squamosa has been identified to possess anticonvulsant properties (González-Trujano et al., 2001, 2009; Cano-Europa et al., 2010), anxiolytic-like effects (González-Trujano et al., 2006; López-Rubalcava et al., 2006), antiinflammatory activity (Carballo et al., 2010) and antibacterial activity (Shanker et al., 2007).

Although palmitone is not produced exclusively by C. esculenta, none of the other known producers is expected to have contributed significant amounts of organic matter to sediments in Vanuatu. Sandalwood comprises 16 species distributed across India, Indonesia, Papua New Guinea, Oceania and Australia (Harbaugh and Baldwin, 2007). While one of those 16 species (Santalum austrocaledonicum) is native to Vanuatu and New Caledonia, small-scale plantations of S. album were only established in the last 20 years (Page et al., 2012). Allium porrum (leek) is native to the Near-East and other four species of Allium found in Vanuatu today are exotic species and were introduced post-European contact (Walter and Lebot, 2007). Of the five Allium species introduced to Vanuatu, A. cepa (onion) is the one commonly used in local cuisine. However, most onions are still imported and are relatively rarely used in rural areas of Vanuatu (Walter and Lebot, 2007). Laurus nobilis (bay laurel), Aristolochea gigantea and A. esperanzae, Paeonia officinalis and P. mlokosewitschii, and Rollinia mucosa are not listed in the National Herbarium of Vanuatu (PVNH), which includes over 20,000 specimens from plants in Vanuatu. Regarding the Annonaceae, the Vanuatu National Herbarium lists only Annona muricata (soursop) as present on the archipelago. This plant was introduced to Vanuatu at the end of the 18th century and is now cultivated on a small scale (Walter and Lebot, 2007).

Several studies focused on the hydrophobic properties of plant waxes have found that palmitone helps form surface structures known as transversely ridged rodelts and is a characteristic wax component in some plants from the Magnoliaceae (Gülfz et al., 1992; Barthlott et al., 1998; Barthlott et al., 2017), Annonaceae (MacKie and Misra, 1956; Hennig et al., 1994), Lauraceae (Hennig et al., 1994), and Aristolochiaceae families (Hennig et al., 1994). Within these plant families, 22 species are listed in the Vanuatu National Herbarium. Two of those species were also included in this sample set (Cananga odorata and Cordyline fruticosa). While Cananga odorata possessed small amounts of palmitone, Cordyline fruticosa did not. This indicates that although some species in these families produce palmitone, it is not universally present among them. None of the species listed from these four families of palmitone producers are cultivated on large scales, and therefore are not expected to make high amounts of palmitone that could accumulate in sedimentary archives.

C. odorata is a plant that is native to Indo-Malaysia and has most likely been introduced to Vanuatu (Manner and Eivijtch, 2006). It is mostly grown as an ornamental and symbolic tree adjacent to villages, but was not observed in the vicinity of Lake Vesalea. The absence of houses or remains of houses around the lake suggests that C. odorata was unlikely to have been introduced to the site in the past. Therefore, we can assume that C. esculenta cultivated around Lake Vesalea was the main source of palmitone detected in the lake’s sediment (Fig. 5).

In sedimentary systems it is also possible that compounds are derived from the degradation of other compounds, rather than being an indication of direct environmental inputs. Mid-chain ketones such as palmitone can be produced from the oxidation of algal-derived long-chain diols (LCDs) (Zhang et al., 2016; Zhang and Volkman, 2017). In particular, the oxidation of algal derived C23 1,17-diol can also be a source of palmitone (Zhang et al., 2016; Zhang and Volkman, 2017). If C23 1,17-diol were a significant source of palmitone in Lake Vesalea sediments, we would expect the unoxidized diol to be present in at least trace amounts in the upper portion of the core. The C23 1,17-diol and other LCDs were not detected in any of the alcohol fractions from the Lake Vesalea core, suggesting that an LCD source for palmitone is unlikely here. However, LCDs should be screened for in other settings where palmitone might be used as an indicator of C. esculenta cultivation.

4.3. Preservation potential of palmitone in sediment

Fossil biomarkers are only useful for reconstructing past environments if they are well preserved in sedimentary archives. If they are degraded rapidly in the sediment, it is not possible to use them to trace the input of the source organism over time, as degradation could alter or attenuate the molecular stratigraphic record (Dubois and Jacob, 2016). The preservation potential of palmitone in sediment has not been investigated previously. However, palmitone has been identified in a Late Saxon/Early Medieval vessel from West Cotton site in Northamptonshire and in an early bronze age cooking vessel from an excavation at St Veit-Klinglberg in Austria (Evershed et al., 1992, 1995). Although long-chain ketones in archaeological pottery can be derived from the heating of free fatty acids or tracyglycerols during cooking (Evershed et al., 1995), these findings suggest that palmitone has the potential to be well preserved on millennial timescales. Additionally, the
likelihood that palmitone is preserved in sediment can be assessed by comparing it to other compounds with similar molecular structures whose degradation paths are better understood. The most frequently used ketone biomarkers are long-chain alkenones, which are produced by haptophyte algae and form the basis of the well-established sea-surface temperature proxy, the $\delta^{13}C_P$ index (Marlowe et al., 1984; Prahl and Wakeham, 1987). Like alkenones, palmitone only has one carbonyl group. In contrast to alkenones, the hydrocarbon chains of palmitone are fully saturated, likely making it less prone to degradation.

Evidence for the persistence of palmitone in sediment comes from the profile of its concentration in the sediment core from Lake Vesalea, where *C. esculenta* is grown today (Fig. 5). The concentration of palmitone increased with depth in the upper ~20 cm of this core (Fig. 5). If degradation was the only control on palmitone concentrations with depth, its concentration would be expected to decline exponentially with depth (Meyers and Ishiwatari, 1993). However, this was not the case with palmitone in the Lake Vesalea sediment core, and the fluctuations in its concentration throughout the upper 35 cm of the core suggest that it is recording variable inputs to the sediment, rather than a simple degradation curve. Given the sediment accumulation patterns in this particular site, it is not possible to speculate on the causes of the fluctuations in the palmitone concentrations with depth, nor to attribute them to specific historical intervals. Rather, these data are presented solely as a demonstration that palmitone can be preserved in sediments, and that its concentrations have the potential to be used as a biomarker for past cultivation.

Nevertheless, the palmitone concentrations in the Lake Vesalea sediments are consistent with what is known on the cultivation of taro on the west coast of Espiritu Santo, where the lake is located. Although taro in the area has been cultivated throughout the past two centuries (Macdonald, 1891; Weightman, 1989), archaeological evidence based on remains of past irrigation systems throughout the region (e.g., Spriggs, 2002; Bayliss-Smith et al., in press) suggests that taro was more intensively cultivated in the area. The degradation of such systems, most likely linked with human population decline following the introduction of European disease epidemics, is related to a decrease in taro cultivation in the region (Speiser, 1923), and elsewhere. These findings are consistent with the decrease of palmitone in the upper part of the sediment core.

Additional support for using palmitone to trace *C. esculenta* inputs to sediment comes from comparing its concentrations to those of other plant derived compounds in the sediment core. Palmitone concentrations do not covary with those other plant-derived compounds (Fig. 5), suggesting that fluctuations in the down core palmitone concentrations are not an artifact of organic matter preservation. Sedimentary palmitone concentrations are 1–2 orders of magnitude smaller than the concentrations of the other main compounds produced by *C. esculenta*, specifically phytol, stigmatasterol, sitosterol, and triacontan-1-ol (Fig. 5). These compounds are among the most common lipids produced by plants in our vegetation survey (Fig. 3), and it is unsurprising that they are so much more abundant than palmitone in the sediment, because they are sourced from most or all of the plants in the catchment, and not limited to a single source.

### 4.4. Implications for paleoenvironmental reconstructions in Remote Oceania

Overall, palmitone has only been identified in a few plants in the global flora, and none of those species are widespread in Vanuatu or elsewhere in Remote Oceania, in contrast to *C. esculenta*, which is an important staple crop of the region (Champagne et al., 2011). Furthermore, while some other plant families make palmitone, our results indicate that the compound is not universally produced by all species within those families. In order to be preserved in the sediment in quantities sufficient for eventual detection by organic geochemists, it is necessary that the plant produces palmitone in high amounts and that the plant is abundant in the surrounding ecosystem. Although *C. esculenta* is being replaced by more productive crops, such as cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*), it is still considered an essential part of every meal on Pacific islands and in southeast Asia and cultivated on large scales (Caillon et al., 2006). Therefore, the presence of palmitone in sedimentary archives is likely from *C. esculenta* as it seems to be the only plant widespread in Remote Oceania that produces this compound in such high amounts.

The validation of palmitone as a species-specific biomarker for *C. esculenta* is not only useful for the reconstruction of the introduction and production of this plant in Vanuatu and other Pacific islands, it can also contribute to clarify the complex interplay between humans and remote tropical environments throughout Oceania. The use of palmitone as a biomarker for *C. esculenta* could be combined with other fossil proxies to give new insights about the role of agricultural production in this region over time. Such a multiproxy approach could employ pollen, compound specific stable isotope composition, leaf wax distributions and coprostanol concentrations. Together, these proxies can give an overall picture of the past, relating climatic and ecological shifts with human activities. A species-specific biomarker for *C. esculenta*, such as palmitone, could be used in this context to identify introduction of horticulture relative to arrival of humans on the islands, and to determine the significance and intensity of crop production over time, ultimately providing insights about the complex relationships between people and their island environments.

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### Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orggeochem.2019.03.006.
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