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Starch granules from human teeth: New clues on the Epi-Jomon diet

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This study examined starch granules from the dental calculus of specimens from the Epi-Jomon (Zoku-Jomon in Japanese, ca. 350 BCE–350 CE) period in Japan for taxonomic identification of plant food items and the reconstruction of human socioeconomic practices. Dental calculus was extracted from 21 individuals across six Epi-Jomon sites in Hokkaido. Moreover, 12 starch granules and starch clusters were recovered from nine individuals. The morphologies of the extracted starch granules were then classified into five types: elliptical, angular circular, polygonal, pentagonal, and damaged. Morphometric analysis indicated that a small portion of these starch granules may have derived from acorns, nuts, and bulb or tuber plants, with one starch granule supposedly from rice. Although extracted starch granules are poor predictors of food diversity at the individual level, the results can identify potential food sources of the surveyed population. This is the first study to determine how well plant microremains in dental calculus reflect a plant diet in the Epi-Jomon population. The starch granules discovered at the surveyed sites provide essential information about the utilization of plant species and cultural contacts in Hokkaido during this period. This is of great significance in reconstructing the Epi-Jomon subsistence patterns in Hokkaido and exploring cultural interactions between hunting-gathering-fishing and agrarian societies.

KEYWORDS
dental calculus, diet, Epi-Jomon period, plant food, starch granules

Introduction

The Japanese Archipelago extends over 3,000 km from north to south and comprises four main islands: Hokkaido, Honshu, Shikoku, and Kyushu (**Figure 1A**). The islands exhibit differences in geography, climate, and environment, as well as regional culture. Recent radiocarbon dating results (e.g., Fujio, 2005; Sakamoto, 2007) revealed that irrigated rice farming took place in northern Kyushu, Japan, in the late Tenth century BCE (Leipe et al., 2020). After the 850s BCE, when rice farming culture spread from mainland China to northern Kyushu via the Korean Peninsula, cereal cultivation spread across most of Honshu, Shikoku, and Kyushu.

Conversely, in Hokkaido, the Epi-Jomon culture (Zoku-Jomon in Japanese, ca. 350 BCE–350 CE) flourished alongside the Jomon culture (Gjesfjeld, 2014; Takase, 2014, 2022; Crawford, 2018). The Epi-Jomon culture was characterized by a hunter-fisher-gatherer economy which included domesticated crop utilization introduced from outside Hokkaido (Crawford and Takamiya, 1990; Leipe et al., 2017, 2018; Aono, 2021). The Epi-Jomon culture included participation in extensive trade to procure prestigious goods (e.g., glass and stone beads and shell bracelets) and iron tools (Shitara, 2003; Hudson, 2004; Takase, 2014; Junno et al., 2020).

Takase's studies(2014, 2022) reveal that the early Epi-Jomon people exploited resources in southern, central, and northern Hokkaido in unique ways and placed more importance on fisheries than did the Jomon people. In central Hokkaido, more foreign commodities may have been available from the first half of the Epi-Jomon period, and an economy that focused on a network of fisheries throughout the different areas of Hokkaido and on salmon fisheries may have led this area to flourish in the latter half of the period (Takase, 2014). According to the conceptual framework of Smith (2001), this hybrid subsistence economy in the Epi-Jomon period may have belonged to the "middle ground" subsistence economy (Leipe et al., 2018). In addition, Epi-Jomon sites comprise a mixture of burials and evidence of short-term occupations. This culture consisted of seasonally mobile forager communities with gradually declining demographics and moderate social differentiation (Junno et al., 2020). These short-term occupations are evidenced by few artifact deposits and burned structural remains, together with concentrations of faunal and plant remains (Crawford and Takamiya, 1990; Takase, 2014; Abe et al., 2016).

In Hokkaido—more than in other regions—extensive information on archeological plant remains has been accumulated through the active practice of flotation and wet sieving (Crawford, 2011). Many case studies and reports have been published since the key primary studies (e.g., D'Andrea, 1995b; Leipe et al., 2017, 2018) first appeared in the literature. Plant utilization in the Epi-Jomon period mainly included nuts and acorns (*Juglans ailanthifolia*, *Castanea crenata*, Fagaceae, *Quercus* sp., *Corylus* sp., and *Aesculus turbinata*), shrub and vine fruits (*Phellodendron amurense*, Lamiaceae, and *Cannabis sativa*), and domesticated crops (*Oryza sativa*, *Hordeum vulgare*, *Echinochloa esculenta*, *Panicum miliaceum*, and *Setaria italica*) (e.g., Crawford and Yoshizaki, 1987; Yoshizaki, 1988; D'Andrea, 1995a; Sakakida, 2018). The relatively few finds of domesticated crops from Epi-Jomon cultural layers are likely the result of exchange, and show evidence of imported grains of low-level use with either small-scale or no cultivation (Leipe et al., 2018). Among the aforementioned cultivated crops, at the K135–4 Chome site within the city of Sapporo, a carbonized hulled barley (*Hordeum vulgare* var. *vulgare*) grain was collected from the Epi-Jomon level (typologically dated to ca. 200–400 CE) (Crawford and Takamiya, 1990), but it has not been directly dated (Leipe et al., 2017). At the Hamanaka 2 site on northern Rebun Island, a single charred grain of naked barley (*Hordeum vulgare* var. *nudum*) revealed an age range of 375–203 BCE (95% confidence interval) by AMS ^{14}C dating (Leipe et al., 2017). This seed represents compact naked barley, and it probably originated from the continental Russian Far East, rather than from the Japanese Islands region south of Hokkaido (Leipe et al., 2017, 2018). In addition, rice (*Oryza sativa*) and millets, which were reported at the Mochiyazawa site (D'Andrea, 1995a), have not been precisely dated. As impressions of domesticated cereal seeds on Epi-Jomon pottery have not yet been reported (e.g., Takase, 2011b; Sakakida, 2018), limited evidence exists for the use of domesticated crops by Epi-Jomon populations (Takase, 2014; Leipe et al., 2018).

This study aimed to reconstruct food consumption among the Epi-Jomon population by applying existing reference data to the samples obtained from Epi-Jomon individuals. By applying several previous analysis guidelines, this study examined starch granules from the dental calculus of individuals from the Epi-Jomon period in Japan for taxonomic identification of plant food items and the reconstruction of human socioeconomic practices. This study also clarified contact between hunter-gatherers and agricultural populations in the northern part of the Japanese Archipelago. Samples showing contact between the two cultures were selected chronologically and regionally.

Archeological starch studies in East Asia

Over the last two decades, analysis of starch residues has become progressively widespread across East Asia (Shibutani, 2017). Similarly, in Japanese archeology, extensive starch data have been accumulated from stone artifacts, pottery sherds, wooden materials, sediments, and dental calculus (Shibutani, 2022). Most reports examine starch data obtained from stone tools used for grinding, possibly because starch granules in surface roughness and cracks appear to be less affected by microbial activity than those in the soil (Haslam, 2004; Barton and Matthews, 2006). Since the 2000s, research has focused on the utilization of starch granules to determine tool functions (e.g., Yang et al., 2009; Liu et al., 2010; Shibutani, 2014), identification of plant domestication (e.g., Yang et al., 2012, 2015; Liu et al., 2015), and human exploitation and production of plant food (e.g., Wong et al., 2011; Shibutani, 2017; Liu et al., 2018). Although the utilization of starch granules as an indicator of plant food cooked in pottery has been less reported than that of stone tool residues, these reports have focused on cooking with pottery in the early years (e.g., Shoda et al., 2011; Shibutani, 2014; Yang et al., 2014) and beer production (Wang et al., 2016; Liu et al., 2020). The functional analysis of wooden tools has been performed less frequently within and around East Asia; however, in recent years, starch residue analysis has been attempted using ethnographic tools (e.g., Kamijo, 2014). Additionally, an experimental analysis of starch granules from archeological sediments was attempted to identify field crops cultivated by the Ainu people (Aono et al., 2021).

Starch granules from dental calculi are useful for identifying plant-derived components, particularly starchy plant food sources (Zhang et al., 2021). Dental calculus is a calcified dental plaque (Suga, 1981; White, 1997) that is mainly composed of calcium phosphate in oral indigenous bacteria and its metabolites in saliva, calcium carbonate, and magnesium phosphate (Little et al., 1963; Little and Hazen, 1964; White, 1997). The calculus becomes mineralized during an individual's lifetime, and after death, it can be preserved if the environment is relatively stable. This means that a protected environment contributes to the long-term preservation of starch granules (Henry, 2020). Due to their chemical structures, starch granules can be preserved in conditions ranging from extremely dry to wet, and in soils and sediments with moderate to extreme pH levels (Beck and Torrence, 2006; Gott et al., 2006). Thus, studying dental calculus helps researchers understand the dietary habits and health conditions of people and resident groups, dietary changes, history of diseases, and changes in the social environment and sanitary conditions (White, 1997). Increasingly, case studies focus on the detection of phytoliths, pollen, diatoms, mineral particles, bacterial mycelium, and viruses extracted from human and animal dental calculus (e.g., Warinner et al., 2015; Hardy et al., 2017; Hutschenreuther et al., 2017). Dental calculus is considered worldwide to be a new resource for research (Yamazaki and Takahashi, 2015).

Starch granules found in the residues of stone tools and pottery sherds must be verified in conjunction with other analytical studies, and cannot always identify the species levels of processed plants or the details of their utilization (Shibutani, 2017). However, starch granules extracted from dental calculus are derived from plants that were present in the oral cavity of humans and animals and can provide evidence of ingested plants (e.g., Henry et al., 2011; Power et al., 2015; Zhang et al., 2021) and non-dietary debris, thus revealing environmental and cultural contexts (Radini et al., 2017). Although conventional methods of analysis involving starch granules are enlightening and have been applied to many archeological studies, research on starch granules from human dental calculus in East Asia remains limited, as this analysis can only be conducted by specialized researchers. In Japanese archeology, research on starch from human and animal bone remains scarce (e.g., Shimono and Takenaka, 2014; Shibutani, 2020; Yamazaki et al., 2021). This is largely because detailed analysis of dental calculus from human and animal bones is a relatively new field that emerged in the 2000s, and challenges remain in detecting starch granules from human and animal teeth. For instance, starch granules derived from dental calculus may be poorly preserved, and taxonomic identification of plant food at the species level is often difficult. Furthermore, as the Japanese Archipelago is covered extensively with acidic soil made of volcanic ash (e.g., Shibutani, 2014; Mizuno et al., 2021), the acidic conditions make bone material analysis challenging (Nafte, 2000). Thus, the reliability of starch granule analysis of dental calculus has not been adequately validated. Researchers and analysts have been seeking innovative methodologies to advance studies of starch granules in ancient remains.

Furthermore, the completeness of starch reference databases varies from region to region, with data that can be used in one region but not another. For instance, digital reference collections have been published primarily in Europe and the United States (e.g., Warinner et al., 2011; Cagnato et al., 2021). Regarding reference data in East Asia, various studies have presented candidate species of starchy plants for taxon identification of archeological starch granules (e.g., Li et al., 2020, 2022; Shibutani, 2020; Yasui, 2021); however, the criteria used for assessing starchy species of plants are sometimes subjective. With more openly accessible resource data, researchers now require more fundamental data to taxonomically identify starch and capture data provenance and reproducibility. Therefore, to further develop starch granule analysis in East Asia, researchers must share resource data and apply a comprehensive methodology.

Materials and methods

Study sites and sample collection

This study compiled 21 dental calculus samples from the following six archeological sites in Hokkaido, northern Japan: Usu-moshiri, Usu-oyakotsu, Minami-Usu 6, Rebunge, Onkoromanai, Oshonnai 2 (**Figure 1B**). These sites were primarily dated between the Final Jomon (ca. 1050–350 BCE) and the Epi-Jomon (ca. 350 BCE–350 CE) periods.

The Usu-Moshiri site (42°31′4″N, 140°46′34″E) is located in the Usu district of Date City on the eastern shore of Funkawan Bay (Uchiura Bay) on a small island of approximately 10,000 m^2 at the mouth of Usu Bay (**Figure 1C**). An active volcano, Mount Usu is located northeast of this site, and Mount Komagatake in Mori Town is situated to the south. During excavations by the Second Department of Anatomy of Sapporo Medical University in 1985–1989, gravesites and burial goods dating from the Final Jomon to the Epi-Jomon periods were unearthed. Among the burial goods, bracelets made of cone shells (Conidae) from the South Seas and realistic carved wooden bear spoons revealed an exchange between the Jomon and Yayoi cultures (Oshima, 2003; Aono and Nagaya, 2021).

The six analyzed human bones in this study belong to the Final Jomon and the first half of the Epi-Jomon periods.

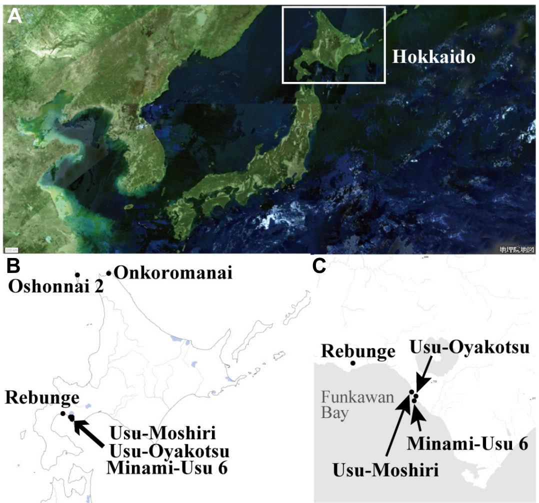

FIGURE 1
Geographic location of the surveyed sites in Hokkaido, Japan. **(A)** Map of northeast Asia showing Japanese Archipelago; **(B)** surveyed sites; **(C)** location of the Rebunge, Usu-Moshiri, Usu-Oyakotsu, and Minami-Usu 6 sites. The base map is from the Geospatial Information Authority of Japan.

Individual nos. 1–6 were uncovered in grave nos. 4, 7 (1), 13, 14, 16, and 17 (**Figures 2A–G** and **Table 1**). The cultural periods of grave nos. 4, 7 (1), 13, and 17 represent the early Epi-Jomon period, and the bones of individual nos. 1, 2, 3, and 6 belonged to Epi-Jomon residents. Grave nos. 14 and 16 belong to the Final Jomon period, and individual nos. 4 and 5 represent the final Jomon people.

The Usu-Oyakotsu site (42°30′26″N, 140°46′58″E) is situated on a sand dune facing Funkawan Bay in the Usu district of Date City and is located approximately 1 km south of the Usu-Moshiri site (**Figure 1C**). The site was excavated by the Date City Board of Education in 1981, and shell mounds and gravesites dating from the Epi-Jomon, Satsumon, and 17th century periods were discovered. Initially called the Minami-Usu 7 site, the name was later changed to the Usu-Oyakotsu site, because it was identified as an integral part of the adjacent Oyakotsu site (Date City Board of Education, 1984). The three analyzed human remains (individual nos. 7, 8, and 9)—reported to be bones excavated from the Minami-Usu 7 site—are attributed to the first half of the Epi-Jomon period. The conditions of their dental calculus are illustrated in **Figures 2H–J**.

This site's excavation spot GP016 contained human bone specimen no. GP016 (individual no. 9), well-preserved human bone remains excavated in the prone flex position from an oval pit. Although burial goods and other artifacts were not unearthed, the previous report concluded the site to be of the Epi-Jomon period, based on the location of the excavation surface (Mineyama et al., 1984). Another excavation spot, GP021 (containing human bone individual nos. 7 and 8), had an irregular circular-planar shape with a diameter of approximately 1.4 m. Possibly a joint grave holding multiple bodies, it was dated to the first half of the Epi-Jomon period based on excavated artifacts. The partial human bones uncovered in this grave were labeled from A to H.

A unique feature of the Usu-Oyakotsu site is that the artifact inclusive layer yielded Inakadate-style pottery from the Yayoi culture located in northern Honshu. The pottery is a neckless jar, which may have been used to store seed rice by this agrarian society. This pottery serves as evidence of contact between the Epi-Jomon and Yayoi cultures in Honshu, and is significant in pursuing the possibility that non-agricultural Epi-Jomon people consumed rice.

The Minami-Usu 6 site (42°30′4.67″N, 140°46′55.78″E) is located in the Usu district of Date City, at the base of Cape Altori, the western tip of the flow topography formed by the Zenkoji debris avalanche of the Usu volcano (Dodo, 1983; Mitsuhashi, 1983). Cape Altori is a land-locked island with an elevation of approximately 27 m, and may have been recognized as a landmark from offshore. The site is situated on a low terrace face (elevation of approximately 6 m) at the northern base of the cape with the Usu-Oyakotsu site located approximately 700 m above the dune line to the north (**Figure 1C**).

The Minami-Usu 6 site was excavated by Sapporo Medical University in 1981 after it was discovered during the renovation of a private museum (Dodo, 1983; Mitsuhashi, 1983). During the excavation, gravesites with a shell midden and human remains, and a pit dwelling were found in a survey area of more than 68 m^2. One pottery sherd, two pieces of gravel, and a stone axe were found buried at the base of a gravesite (Mitsuhashi, 1983), revealing specific features of the first half of the Epi-Jomon period. An analyzed human bone (individual no. 10) excavated from a grave pit was attributed to the first half of the Epi-Jomon period (**Figure 2L**). A previous study (Dodo, 1983) identified the bone as belonging to an adolescent female.

The Rebunge site (42°34′38″N, 140°35′22″E), occupied in the first half of the Epi-Jomon period, is a shell mound site located on the northern shore of Funkawan Bay (**Figure 1C**), near the mouth of the right shore of the Rebunge River. The area comprises a river terrace (1 km wide and 8 km deep), which developed at the mouth of the Rebunge River and the surrounding hills. Situated on a micro-elevation composed of floodplain sediments, the site was excavated by Sapporo Medical University from 1963 to 1965. Gravesites, shell mounds, human bone remains, and artifacts dating from the first half of the Epi-Jomon period were unearthed during this excavation (Mineyama et al., 1972; Matsuda and Aono, 2003).

The three analyzed human bone remains (individual nos. 11, 12, and 13) were excavated from gravesites attributed to the first half of the Epi-Jomon period (**Figures 2N–P**). In 1991, the university conducted another survey (Matsuda and Aono, 2003), but did not analyze any human bone remains.

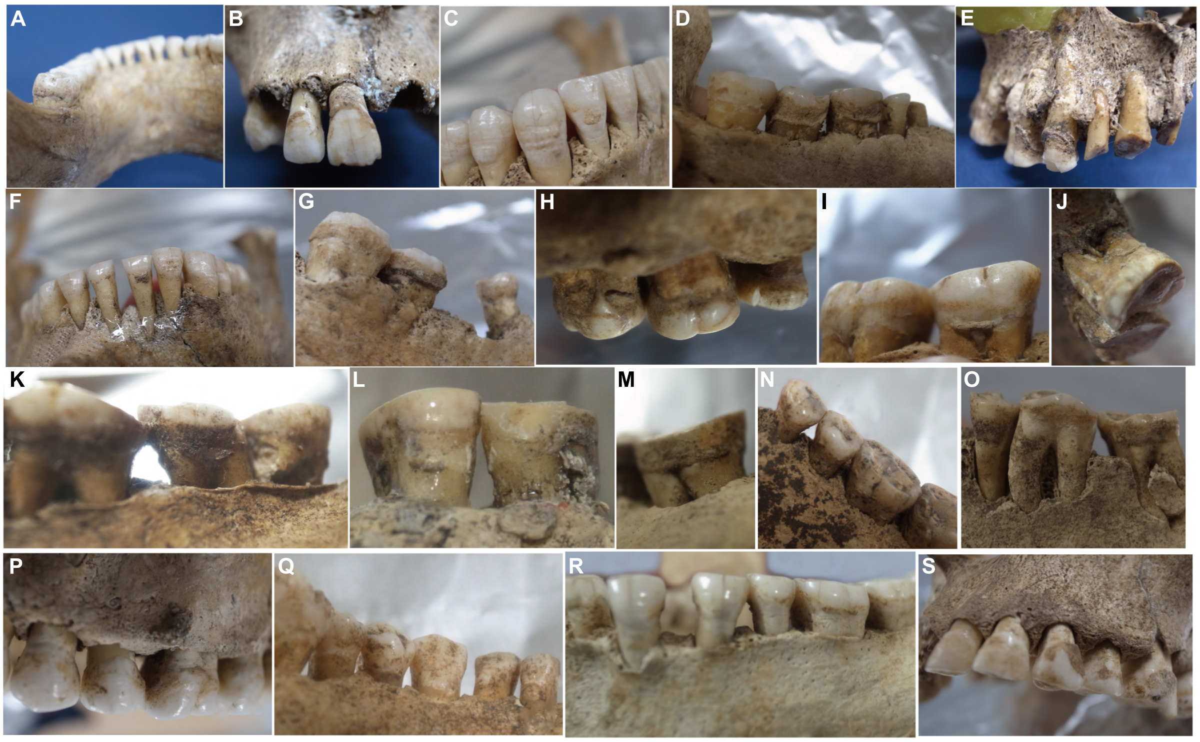

FIGURE 2
Conditions of dental calculus on the sampled human bone remains. **(A)**, ID 1; **(B)**, ID 1; **(C)**, ID2; **(D)**, ID 3; **(E)**, ID 4; **(F)**, ID 5; **(G)**, ID 6; **(H)**, ID 7; **(I)**, ID 8; **(J)**, ID 9; **(K)**, ID 9; **(L)**, ID 10; **(M)**, ID 10; **(N)**, ID11; **(O)**, ID 12; **(P)**, ID 13; **(Q)**, ID 14: **(R)**, ID 15; **(S)**, ID 16.

The Onkoromanai site (45°30′0″N, 141°53′24″E) is situated in coastal sandy soil on the left bank of the mouth of the Onkoromanai River (**Figure 1B**). This site was excavated by the Department of Cultural Anthropology of the University of Tokyo in October 1959.

Excavated from a soil layer attributed to the Epi-Jomon period, five human bone remains were associated with this period (Yamaguchi, 1963) and later stored at Sapporo Medical University. Two analyzed human bone remains (individual nos. 14 and 15) were uncovered in the soil layer attributed to the latter half of the Epi-Jomon period (**Figures 2Q,R**).

The Oshonnai 2 site (45°26′23″ N, 141°2′23″ E), mainly occupied in the early half of the Epi-Jomon period, is situated in the northern part of Rebun-cho, on a coastal dune at an elevation of 3.5 m (**Figure 1**). The famous Oshonnai and Funadomari sites of the Late Jomon period lie to the west of this site, which was excavated by the Rebun-cho Board of Education in 2000 to construct the new Funadomari post office. Three graves containing human bone remains from the first half of the Epi-Jomon period were discovered (Fujisawa, 2001). Identified as those of an adolescent male (**Figure 2S**), analyzed human bone remains (individual no. 16) excavated from grave no. 2 were associated with the first half of the Epi-Jomon period.

The surveyed human bone remains excavated from the Usu-Moshiri site are owned, managed, and stored by the Date City Institute of Funkawan Culture, to which one of the authors (YN) belongs. Bone remains from the other sites are owned by the municipalities where the sites are located and stored by Sapporo Medical University, enabling physical anthropological analysis. Permission was obtained from these institutions for the analysis of dental calculus. Human bone remains with the following two conditions were selected: (1) good state of preservation with dental pieces from which a sample could be obtained, and (2) availability of other samples for reproducibility in future analyses. A total of 21 dental calculus samples were selected from 16 individuals (**Table 1**).

Starch extraction and identification

For the extraction of dental calculus, the authors followed a methodology proposed by established laboratory operations (e.g., Madella et al., 2014; Power et al., 2014; Leonard et al., 2015), with minor modifications:

1. The sample was weighed, 40 μl of 10% hydrogen chloride (HCl) was added, and the mixture was left in a clean booth at room temperature for 24 h.
2. After 24 h, the sample was centrifuged (13,500 rpm for 10 min), and the supernatant liquid was

TABLE 1 Dental calculus sample information (Sapmed no.: Sapporo Medical University material no.).

Individual no.	Site	Lab no.	Figure 2	Cultural period	Excavation spot	Sapmed no.	Specimen no.	Sex	Starch
1	Usu-Moshiri	USM-SRP1-DC1	(A)	Epi-Jomon	Grave No. 4		No. 4	Male	0
1	Usu-Moshiri	USM-SRP1-DC2	(B)	Epi-Jomon	Grave No. 4		No. 4	Male	1
2	Usu-Moshiri	USM-SRP2-DC1	(C)	Epi-Jomon	Grave No. 7(1)		No. 7(1)	Male	0
3	Usu-Moshiri	USM-SRP3-DC1	(D)	Epi-Jomon	Grave No. 13		No. 13	Male	2
3	Usu-Moshiri	USM-SRP3-DC2	(D)	Epi-Jomon	Grave No. 13		No. 13	Male	0
4	Usu-Moshiri	USM-SRP4-DC1	(E)	Final Jomon	Grave No. 14		No. 14	Female	0
5	Usu-Moshiri	USM-SRP5-DC1	(F)	Final Jomon	Grave No. 16		No. 16A	Female	0
5	Usu-Moshiri	USM-SRP5-DC2	(F)	Final Jomon	Grave No. 16		No. 16A	Female	0
6	Usu-Moshiri	USM-SRP6-DC1	(G)	Epi-Jomon	Grave No. 17		No. 17	Male	1
7	Usu-Oyakotsu	OYK-SRP1-DC1	(H)	Epi-Jomon	GP021		GP021D human bone	Unknown	2
8	Usu-Oyakotsu	OYK-SRP2-DC1	(I)	Epi-Jomon	GP021		GP021H human bone	Unknown	0
9	Usu-Oyakotsu	OYK-SRP3-DC1	(J)	Epi-Jomon	GP016		GP016 human bone	Male	1
9	Usu-Oyakotsu	OYK-SRP3-DC2	(K)	Epi-Jomon	GP016		GP016 human bone	Male	1
10	Minami-Usu 6	MNU-SRP1-DC1	(L)	Epi-Jomon	Grave pit		Minami-Usu 6 human bone	Female	0
10	Minami-Usu 6	MNU-SRP1-DC2	(M)	Epi-Jomon	Grave pit		Minami-Usu 6 human bone	Female	0
11	Rebunge	RBG-SRP1-DC1	(N)	Epi-Jomon	Unknown	EPJ-73	A	Unknown	2
12	Rebunge	RBG-SRP2-DC1	(O)	Epi-Jomon	Unknown	EPJ-73	B	Unknown	0
13	Rebunge	RBG-SRP3-DC1	(P)	Epi-Jomon	Grave No. 1	EPJ-71	No.1	Male	2
14	Onkoromanai	OKN-SRP1-DC1	(Q)	Epi-Jomon	Layer in the latter half of the Epi-Jomon	EPJ-63	No. 1	Male	1
15	Onkoromanai	OKN-SRP2-DC1	(R)	Epi-Jomon	Layer in the latter half of the Epi-Jomon	EPJ-64	No. V	Female	0
16	Oshonnai 2	OSN-SRP1-DC1	(S)	Epi-Jomon	Grave No. 2	EPJ-33	No. 2	Male	0

removed. Subsequently, 40 μl of distilled water was added and stirred.

3. The stirred sample was centrifuged again (13,500 rpm for 10 min). The supernatant liquid was removed, and 40 μl of purified water was added.
4. Soon after the addition of purified water, a third centrifugation was conducted (13,500 rpm for 10 min). The supernatant liquid was removed and the sample was dried naturally at room temperature in a clean booth.
5. Finally, 40 μl of purified water was added to the dried sample.

This paper reported the results using the aforementioned method. Although this method has been applied to multiple other cases and the results showed well-preserved starch granules (e.g., Yamazaki et al., 2021; Shibutani et al., 2022), comparative experiments should be conducted using other extraction methods, such as ethylenediaminetetraacetic acid (EDTA) decalcification (Tromp et al., 2017) and weaker chemical extraction, to confirm greater quantitative and qualitative reproducibility. These methods are demonstrated to be less destructive; thus, our future research will apply them to samples from the same archeological sites used in the current study. If more well-preserved starch granules can be extracted from dental calculus samples by these other methods, it may be possible to draw more conclusions regarding the Epi-Jomon diet.

All samples were prepared utilizing the same method applied to create the modern starch reference collection (Shibutani, 2006, 2010b) for preparing permanent slides. After centrifugation (1,300 rpm for 1 min), 8 μl of the sample were sealed with 8 μl of glycerol gelatin (refractive index 1.46–1.48) as a mounting medium, and at least two slides per sample were prepared. In addition, slides without any samples were prepared to check for contamination of the glass slides, coverslips, and mounting medium. An Olympus BX53-33Z light microscope (OLYMPUS, Japan) with a polarizer and a WRAYCAM-NF500 microscope camera (Wraymer, Japan) were used to perform two-dimensional (2D) measurements and other examinations. All slides were scanned at 100–1000 × magnification, and the starch slides were photographed at 400 × magnification.

To identify archeological starch granules, previous studies have employed an image comparison method, such as taxonomic identification, followed by shape, size, location of the hilum, surface conditions, and extinction cross shapes. As part of this identification method, modern starch image data have been published to confirm the significant comparative reference points of archeological data (e.g., Lentfer, 2009; Warinner et al., 2011; Cagnato et al., 2021). However, these publications cannot adequately explain starchy plant utilization at Japanese sites or the morphological variations in archeological starch granules. The available starch data cannot be applied to the archeological context in Japan, and an original reference collection is required. Furthermore, taphonomic problems associated with starch granules in Japan must be resolved. In this study, species identification and classification of archeologically-derived starch granules were based on comparisons with a modern starch reference collection established by one of this study's authors (e.g., Shibutani, 2006, 2010a) and with previously published starch data in East Asia (e.g., Yang et al., 2014; Liu et al., 2019; Li et al., 2022).

Results

As shown in **Tables 1**, **2**, 13 starch granules were extracted from nine out of 16 individuals. Among these starch granules, four were recovered from three individuals (nos. 1, 3, and 6) of the Usu-Moshiri site, four from two individuals (nos. 7 and 9) of the Usu-Oyakotsu site, four from two individuals (nos. 11 and 13) of the Rebunge site, and one from an individual (no. 14) of the Onkoromanai site. Seven granules were identifiable by comparison with reference data. The other six granules were degraded, and their original morphologies remain unknown. In addition to starch granules, unidentifiable plant fibers and cells were observed in a much smaller proportion.

Starch granules

The morphologies of the extracted starch granules were classified into five types: elliptical, angular circular, polygonal, pentagonal, and damaged (**Figure 3** and **Table 2**). Extinction crosses were mostly vertical. Gammadion, reverse gammadion, and vanished crosses were also observed.

According to the reference collection and published starch data, many 2D shapes of nut (e.g., *Castanea crenata*, *Aesculus turbinata*, and *Corylus heterophylla* var. *thunbergii*) and acorn (*Quercus* sp. and other Fagaceae) starch granules are primarily round, elliptical, or semi-elliptical with vertical and diagonal extinction crosses. The center of the extinction crosses in the *Castanea crenata* and *Quercus* species appears as a dark linear area (Shibutani, 2010a). These types of starch granules were found in the Usu-Moshiri (**Figure 3B2**) and Rebunge samples (**Figures 3I,J**), of which the Usu-Moshiri granule may represent the *Quercus* species, and the Rebunge granules may be derived from the *Castanea crenata* or *Quercus* species.

Other elliptical and angular circular starch granules may be derived from bulbs or tubers. These extinction crosses are bent like gammadions, and their lamellae and eccentric hila exhibit a dark linear area. These starch granules were extracted from Usu-Moshiri (**Figure 3C**) and Usu-Oyakotsu samples (**Figure 3F**). Many tubers and roots contain this starch granule morphotype, and further precise identification is not currently possible.

The polyhedral body of starch granules is principally produced by Japanese barnyard millet (*Echinochloa esculenta*), foxtail millet (*Setaria italica)*, broomcorn millet (*Panicum miliaceum*), or rice (*Oryza sativa*). Other Poaceae starch granules, e.g., starch granules of barley (*Hordeum vulgare*), of which carbonized remains were reported in the Epi-Jomon sites (e.g., D'Andrea, 1995a; Sakakida, 2018), consist mainly of a mixture of large lenticular granules (10–30 μm) and smaller irregularly shaped (less than 6 μm) granules (Vasanthan and Hoover, 2009; Zhu, 2017). Starch granules with smaller diameters (5.0–8.3 μM approximately), sharper edges, and hexagonal shapes have been observed in rice (Shibutani, 2010a). The reference collection and published starch data indicate that the sizes of polygonal starch granules from *Echinochloa esculenta*, *Setaria italica*, and *Panicum miliaceaum* are larger than those of rice starch. Based on these, although the number

TABLE 2 Extracted starch granules from dental calculus samples.

Individual no.	Site	Lab no.	Figure 3	Shape	Vertical diameter	Horizontal diameter	Extinction cross	Candidate species
1	Usu-Moshiri	USM-SRP1-DC1	(A)	Damaged	Unknown	Unknown	Vanished	Unknown
3	Usu-Moshiri	USM-SRP3-DC1	(B)1	Elliptical	16.88 μm	13.78 μm	Unknown	Unknown
3	Usu-Moshiri	USM-SRP3-DC1	(B)2	Elliptical	18.38 μm	14.62 μm	Vertical	*Quercus* sp.?
6	Usu-Moshiri	USM-SRP6-DC1	(C)	Elliptical	23.63 μm	27.68 μm	Gammadion	Bulb/tuber
7	Usu-Oyakotsu	OYK-SRP1-DC1	(D)	Polygonal	6.3 μm	8.16 μm	Vertical?	*Oryza sativa*?
7	Usu-Oyakotsu	OYK-SRP1-DC1	(E)	Damaged	Unknown	Unknown	Vanished	Unknown
9	Usu-Oyakotsu	OYK-SRP3-DC1	(F)	angular circular	16.97 μm	17.69 μm	Gammadion	Bulb/tuber
9	Usu-Oyakotsu	OYK-SRP3-DC2	(G)	Pentagonal	20.81 μm	22.23 μm	Vertical	*Juglans ailanthifolia*?
11	Rebunge	RBG-SRP1-DC1	(H)	Damaged	Unknown	Unknown	Vanished	Unknown
11	Rebunge	RBG-SRP1-DC1	(I)	Elliptical	19.74 μm	14.26 μm	Vertical	*Castanea crenata* or *Quercus* sp.?
13	Rebunge	RBG-SRP3-DC1	(J)	Semi-elliptical	11.22 μm	10.71 μm	Vertical	*Castanea crenata* or *Quercus* sp.?
13	Rebunge	RBG-SRP3-DC1	(K)	Damaged	Unknown	Unknown	Vanished	Unknown
14	Onkoromanai	OKN-SRP1-DC1	(L)	Damaged	Unknown	Unknown	Reverse gammadion?	Unknown (Poaceae? Possibly not *Oryza sativa*)

of recovered starch granules was limited, a polygonal (possibly hexagonal) starch granule from Usu-Oyakotsu (**Figure 3D**) may be identified from an *Oryza sativa*.

Pentagonal shapes (near-crushed rectangular shapes) with closed and centrally located hila are also present in Japanese walnut species (*Juglans ailanthifolia*), based on the previously reported starch data (e.g., Shibutani, 2010a; Yasui, 2021). This particular shape of starch granules recovered from Usu-Oyakotsu (**Figure 3G**) was possibly derived from *Juglans ailanthifolia*.

Damaged starch granules were found at the Usu-Moshiri (**Figure 3A**), Usu-Oyakotsu (**Figure 3E**), Rebunge (**Figures 3H,K**), and Onkoromanai sites (**Figure 3L**). Broken or gelatinized, their lamellae, hilum, and extinction crosses were fuzzy and invisible. A cracked starch granule from Onkoromanai (**Figure 3L**) likely derived from Poaceae with polygonal-shaped starch granules (possibly not *Oryza sativa*); however, its whole shape is unknown and it could not be identified at the species level. A broken and expanded starch granule found at the Rebunge site (**Figure 3K**) was affected by heat treatment, such as cooking.

Other plant organ fragments

Fibers, cells of unknown biological origin, and other fragments (or non-plants) were identified in all the surveyed samples. Lacking biological attributes and not identifiable to plant species, these fragments are not discussed in this study.

Discussion

The results of this study are limited, but provide preliminary evidence for the Epi-Jomon diet and contact with agrarian cultures. A total of 13 starch granules were extracted from nine individuals, and a possible *Oryza sativa* starch granule indicates the possibility that rice may have been imported from outside Hokkaido. However, given the billions of starch granules that enter the mouth in each bite, it is difficult to confirm that a small portion of identified starch granules represented part of a regular diet. The possibility that the decalcification process in this study has affected the recovered starch granules and other plant organ fragments must be re-examined. If future studies apply more

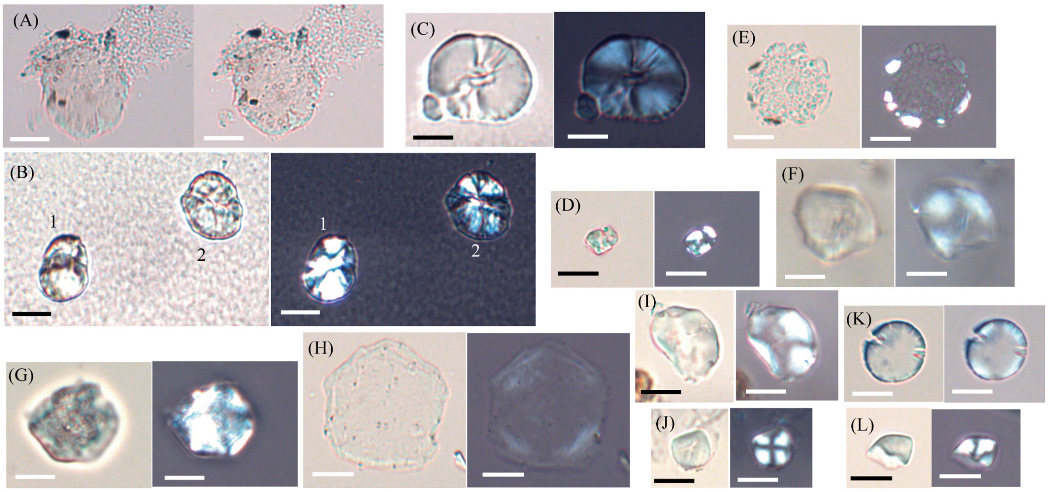

FIGURE 3
Starch grains from the surveyed dental residue samples. Scale bar: 10 μm; photographed at 400× magnification; left: brightfield, right: brightfield with cross-polarized light. **(A)** Damaged starch grain of unknown species from ID 1; **(B1)** starch grains of unknown species from ID 3; **(B2)** possibly Quercus starch grain from ID 3; **(C)** bulb or tuber starch grain from ID 6; **(D)** possibly Oryza starch grain from ID 7; **(E)** damaged starch grain of unknown species from ID 7; **(F)** bulb or tuber starch grain from ID 9; **(G)** possibly Juglans starch grain from ID 9; **(H)** damaged starch grain from unknown species from ID 11; **(I)** possibly Castanea or Quercus starch grain from ID 11; **(J)** possibly Castanea or Quercus starch grain from ID 13; **(K)** damaged starch grain from unknown species from ID 13; **(L)** damaged starch grain of unknown species (Poaceae type?) from ID 14.

current techniques in starch extraction to the samples at the same sites, better preserved starch granules might be recovered and other new evidence might indicate plant food sources in the Epi-Jomon period.

Plant food resources by human dental calculus analysis

Wild plant resources, such as root plants, acorns, and nuts, may have been crucial to the Epi-Jomon people as well as the Jomon people. This is especially true of roots and tubers which are easy to collect and process, and contain rich starches. Since prehistory, these starchy plants have been one of the most common food sources. However, they decompose easily due to the soil conditions of the Japanese Archipelago. Hence, their evident macrobotanical remains are difficult to locate at the sites, except for carbonized crusts that adhere to pottery (Sasaki et al., 2007; Kudo and Sasaki, 2010; Shibutani, 2014).

Starch granules and a few other plant microfossils extracted from human dental remains in this study provide preliminary information concerning human diets and plant food resources during the Epi-Jomon period. Acorns (*Quercus* sp.), nuts (*Castanea crenata*, *Juglans ailanthifolia*), bulbs or tubers, and cereals (*Oryza sativa*, other species in Poaceae with polygonal starch granules) may have been used by Epi-Jomon populations at the surveyed sites. In contrast to some reports outside Japan, such as in China (e.g., Shibutani, 2020; Chen et al., 2021; Wu et al., 2021), the number of starch granules detected in this study was small, and the total proportion of extracted starch within the entire assemblage from the Usu-Moshiri, Usu-Oyakotsu, and Rebunge sites was insufficient to directly compare variations in food resources. However, acorns (*Quercus* sp.), nuts (*Castanea crenata*, *Juglans ailanthifolia*), bulbs or tubers, and cereals (*Oryza sativa*, other species in Poaceae with polygonal starch granules) may have been used by Epi-Jomon populations at these sites.

Previous studies (e.g., D'Andrea, 1995a; Crawford, 1997, 2011) indicated that plant remains from Epi-Jomon sites primarily represent nuts, shrub and vine fruits, and cultivated cereals introduced from outside Hokkaido. Carbonized remains of such plants from the relevant period were found at the sites in Hokkaido (e.g., Yoshizaki, 1988; Crawford, 2011; Sakakida, 2018). However, many details of these plant food sources among Epi-Jomon populations are not yet understood. Additional dental calculus samples must be examined in a future study to further examine this issue.

Contact between hunter-gatherer and agricultural cultures

A related project containing this study result is currently reconstructing social transformation through contact between hunter-gatherer and agrarian cultures. Similar to comparisons between different pottery cultures in the Jomon period (e.g., Okamura, 1997; Kobayashi and Sakamoto, 2015), Japanese archeological research has focused on elucidating the actual state of interaction between different regional cultures in any period. The presence of many similar underlying cultural elements with small differences between regional cultures, determined through artifact analysis, indicates cultural influence; however, it is difficult to determine the specific content and process of exchange of these influences. To solve this issue, studies must select an appropriate period and region with clear cultural differences and show specific examples of social transformation caused by cultural contact.

Various discussions on the Yayoi and Epi-Jomon economies (e.g., Shitara, 2003; Makibayashi, 2010; Takase, 2014) have explained differences in their subsistence (i.e., comparison of agrarian and hunting-gathering-fishing societies) as being related to variations in latitude, temperature, and regional distance from the cultural cores. According to a previous study (Aono, 2011), after the final Jomon period, the number of shell mounds decreased throughout the Japanese Archipelago due to temporary rapid warming and subsequent climate change.

Under these environmental conditions, rice farming began in the Tsugaru plain of Aomori Prefecture, in the northern part of Honshu, from the middle of the 4th century BCE to the 3rd century BCE (Fujio, 2004; Shitara, 2014; Aono, 2021). For instance, the Sunazawa site in Hirosaki City, Aomori Prefecture, is the northernmost and oldest site in eastern Japan and is representative of the Yayoi rice paddy site (Takase, 2011a; Shitara, 2014). After rice farming began in Honshu, various social changes occurred, including the location and structure of settlements, interaction with hunter-fishers, and rituals (Shitara, 2014; Mizoguchi, 2020). In Hokkaido, people continued the tradition of hunting and gathering, and fishermen introduced various elements of Yayoi culture (metal tools, ornaments, and burial systems), diverging from the lifeways of the Final Jomon period (Crawford, 2011, 2018; Junno et al., 2020; Aono, 2021). Thereafter, differences and some exchanges in the Yayoi and Epi-Jomon cultures may have developed, even in the utilization of plant resources.

According to previous studies (Crawford, 1997, 2018; Takase, 2014), plant utilization actively continued between the Jomon and Epi-Jomon cultures, and the use of starchy plants such as acorns and nuts may have been prevalent even in the Epi-Jomon period. The starch residues in the present study suggested that starchy plant resources were consumed by their residents; however, such residues were not evident for domesticated plants introduced from agricultural areas. When more starch data from samples in other Epi-Jomon sites are analyzed, this discussion will be re-examined.

Conclusion

This was the first study to determine how plant microremains in dental calculus reflected plant diet at the Epi-Jomon sites in Hokkaido. This study identified several possible plant species from starch granules in dental calculus samples from nine human bone remains, providing evidence of plant foods consumed by the Epi-Jomon inhabitants in Hokkaido. Morphometric analysis indicated that a small portion of these starch granules may have been derived from acorns, nuts, and bulb or tuber plants with one starch granule supposedly from rice.

The Epi-Jomon people of Hokkaido likely employed subsistence strategies until the Final Jomon period, while being concurrently affected by agrarian societies, such as in the Tohoku area. Based on previous archaeobotanical research conducted in Hokkaido, this study indicated the possibility that the subsistence economy in the Epi-Jomon period continued to be a hunting-gathering-fishing economy that was influenced by contact with agrarian societies. The starch granules recovered at the surveyed sites provided valuable insight regarding the utilization of starchy food sources and cultural contacts in northern Honshu in this period. This is of great significance in reconstructing the Epi-Jomon subsistence patterns in Hokkaido and exploring cultural interactions between hunting-gathering-fishing and agrarian societies.

This study detected few starch granules, for which the preservation was mostly poor. One of the main reasons may have been that the starch granules were damaged by heat-treatment. In addition, a decalcification chemical utilized in this study may have affected the surveyed samples. As mentioned in the Introduction section, in Japan, research on starch from human and animal bone remains is scarce, and acidic soil conditions created by volcanic ash can make bone material analyses challenging in many cases. Thus, comparative experiments of decalcification methods will be conducted to ensure less destructive analysis of archeological dental calculus samples in future studies.

This study indicated that microfossil analyses of dental calculus have significant potential for future studies of human dietary composition in the Hokkaido region. Future studies on contact between other hunter-gatherer and agrarian societies may be able to build upon these techniques to demonstrate cultural interactions.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AS conducted the research and investigation process, performed the analyses and data and evidence collection, and wrote the initial draft. TA and YN investigated the provision of study materials, conducted a critical review, and provided commentary and revision. All authors contributed to the overall manuscript and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.907666/full#supplementary-material

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