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ZooMS as a tool for understanding prehistoric pelagic fishing: Insights from archaeological shark and scombrid remains on Fais Island, Micronesia, over the last two millennia

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ABSTRACT

The capture of fast-moving marine predators, such as sharks and scombrids, played a crucial role in human subsistence and cultural evolution, with advanced fishing techniques emerging alongside the maritime expansions of Neolithic populations across the Pacific. However, challenges in identifying their remains in the archaeological record have constrained our understanding of their significance. Fais, a raised coral island in Micronesia, has been inhabited for 1800 years, with archaeological evidence revealing a reliance on fishing strategies targeting inshore taxa but also pelagic taxa including some species of sharks and scombrids. Using ZooMS, this study analysed archaeological bones, mostly vertebrae, from the Powa (FSPO) archaeological site, Fais, with 100 % and 93 % success rates of retrieving collagen fingerprints in scombrids ($n = 77$) and sharks ($n = 54$) respectively. The scombrids were overwhelmingly dominated (97 %; $n = 75$) by skipjack tuna (*Katsuwonus pelamis*), with the remaining specimens deriving from two distinct species, yellowfin tuna (*Thunnus albacares*) and wahoo (*Acanthocybium solandri*). In contrast, the shark remains were more taxonomically diverse and evenly balanced, with at least five distinct taxa across much fewer samples. Although lacking a complete enough reference database to make confident assignments to species, we could infer that at least more than one half of the identifications were to groups that closely match the silky shark (*Carcharhinus falciformis; n = 20*), and the Galapagos shark (*C. galapagensis; n = 11*); these two sharks have well-known associations with tuna and their identifications are consistent with some of the dominant species inferred through morphology. A third relatively abundant type ($n = 17$) yielded spectra that could not be matched to our reference material, though plausibly of the only other relatively abundant tuna-associated taxon, the silvertip shark (C. *albimarginatus*). A further two species were represented by one sample each, one of which was a good match for the whitetip reef shark (*Triaenodon obesus*), but the other also not close to any of the reference material included in this study. Nonetheless, the categorization of the shark remains in this study using ZooMS disagrees with the categorization by morphology reported elsewhere, where multiple 'types' are found in previously identified morphological types and *vice versa*. From a methodological viewpoint, this study clearly demonstrates the substantial difference in confidence that can be assigned to a taxonomic identification that well-curated ZooMS databases can offer, particularly when supported by genomic sequence information. By improving the taxonomic resolution of archaeological fish identifications these findings enhance our understanding of ancient fishing practices while suggesting a need for expanded research to address gaps in species-level identification and ecological data.

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1. Introduction

1.1. Scope

The capture of fast-moving marine predators, such as sharks (Elasmobranchii) and representatives of the Scombridae family (including tunas, bonitos and mackerels), particularly within pelagic and outer-reef environments, is believed to have been pivotal in human subsistence and cultural evolution (e.g., O'Connor et al., 2011). The Pacific region in particular provides a rich archaeological record of prehistoric fishing innovations (Boulanger, 2021, 2023). Pelagic fishing activities might have been happening as early as 42,000 BP (Before Present) at Asitau Kuru (Jerimalai), Timor-Leste, which is supported by the presence of scombrid remains in these sites (O'Connor et al., 2011). While some suggest that these fishing activities were instead coastal, they still required a high degree of technological sophistication (Anderson, 2013; Boulanger et al., 2022; O'Connor and Ono, 2013). Further evidence indicates that sharks and scombrids were part of the diet of other fisher-gatherer communities throughout the Pacific. For example, while inshore resources, such as coral reef fish, often dominated Southeast Asian Pleistocene and early Holocene assemblages, the archaeological record indicates that pelagic and outer-reef species were also exploited,

Table 1
Listing of sharks (seven families of the Selachii) and Scombridae (Teleostei) currently reported (or potentially present) on the coasts of Micronesia (NOAA Fisheries, 2025,; Mull et al., 2022; ,IUCN, 2025; Froese and Pauly, 2025), as along with relevant taxonomic and ecological information based on Carpenter and Niem (1998), Collette and Nauen, 1983 and Compagno (1984). In bold: available modern specimens (derived from fingerprints or sequences) for this study.

Division	Family	Species	Citation	Common name	Habitat/behaviour	Average size (cm)
Selachii	Alopiidae	*Alopias pelagicus*	Nakamura,1935	Pelagic thresher	Pelagic-oceanic; solitary or in small groups	300–350
Selachii	Carcharhinidae	*Carcharhinus albimarginatus*	Rüppell, 1837	Silvertip shark	Pelagic-oceanic; solitary or in small groups	200–250
Selachii	**Carcharhinidae**	***Carcharhinus amblyrhynchos***	**Bleeker, 1856**	**Blacktail reef shark**	**Reef-associated; can form schools**	**150–200**
Selachii	Carcharhinidae	*Carcharhinus amboinensis*	Müller &Henle, 1839	Pigeye shark	Inshore (occasionally brackish); solitary	200–220
Selachii	**Carcharhinidae**	***Carcharhinus falciformis***	**Bibron, 1839**	**Silky shark**	**Reef-associated but can be found offshore; can form schools; highly migratory**	**200–250**
Selachii	**Carcharhinidae**	***Carcharhinus galapagensis***	**Snodgrass and Heller, 1905**	**Galapagos shark**	**Reef-associated; solitary or in loose groups**	**250–300**
Selachii	**Carcharhinidae**	***Carcharhinus leucas***	**Valenciennes, 1839**	**Bull shark**	**Inshore (occasionally brackish); solitary or in loose groups; migratory**	**230–240**
Selachii	**Carcharhinidae**	***Carcharhinus limbatus***	**Valenciennes, 1839**	**Blacktip shark**	**Inshore and offshore; schooling; migratory**	**160–180**
Selachii	**Carcharhinidae**	***Carcharhinus longimanus***	**Poey, 1861**	**Oceanic whitetip shark**	**Pelagic-oceanic; solitary or in loose groups**	**250–300**
Selachii	**Carcharhinidae**	***Carcharhinus melanopterus***	**Quoy and Gaimard, 1824**	**Blacktip reef shark**	**Reef-associated; solitary or in loose groups**	**150 to 180**
Selachii	**Carcharhinidae**	***Carcharhinus obscurus***	**Lesueur, 1818**	**Dusky shark**	**Inshore and offshore; solitary or in loose groups; highly migratory**	**270–300**
Selachii	**Carcharhinidae**	***Galeocerdo cuvier***	**Péron & Lesueur, 1822**	**Tiger shark**	**Inshore and offshore; solitary; highly migratory**	**300–350**
Selachii	**Carcharhinidae**	***Prionace glauca***	**Linnaeus, 1758**	**Blue shark**	**Pelagic-oceanic; schooling; highly migratory**	**180 to 220**
Selachii	**Carcharhinidae**	***Triaenodon obesus***	**Rüppell, 1837**	**Whitetip reef shark**	**Reef-associated; in loose groups**	**140–160**
Selachii	Ginglymostomatidae	*Nebrius ferrugineus*	Lesson, 1831	Tawny nurse shark	Reef-associated; in loose groups	250–300
Selachii	**Lamnidae**	***Isurus paucus***	**Guitart, 1966**	**Longfin mako**	**Pelagic-oceanic; solitary; highly migratory**	**250–300**
Selachii	**Lamnidae**	***Isurus oxyrinchus***	**Rafinesque, 1810**	**Shortfin mako**	**Pelagic-oceanic; solitary**	**180–250**
Selachii	**Rhincodontidae**	***Rhincodon typus***	**Smith, 1828**	**Whale shark**	**Pelagic-oceanic; solitary or in loose groups**	**900–1200**
Selachii	**Sphyrnidae**	***Sphyrna lewini***	**Griffith and Smith, 1834**	**Scalloped hammerhead**	**Inshore and offshore; schooling; highly migratory**	**200–270**
Selachii	**Sphyrnidae**	***Sphyrna mokarran***	**Rüppell, 1837**	**Great hammerhead**	**Inshore and offshore; solitary or in loose groups**	**300–380**
Selachii	**Stegostomatidae**	***Stegostoma tigrinum***	**Forster, 1781**	**Zebra shark**	**Reef-associated; solitary or in loose groups**	**250–300**
Teleostei	**Scombridae**	***Acanthocybium solandri***	**Cuvier, 1832**	**Wahoo**	**Pelagic-oceanic; solitary or in small groups**	**100–150**
Teleostei	Scombridae	*Auxis thazard*	Lacepède, 1800	Frigate tuna	Pelagic-neritic; schooling	30–50
Teleostei	Scombridae	*Auxis rochei*	Risso, 1810	Bullet tuna	Pelagic-neritic; schooling, highly migratory	30–50
Teleostei	**Scombridae**	***Euthynnus affinis***	**Cantor, 1849**	**Kawakawa**	**Pelagic-neritic; schooling, highly migratory**	**40–60**
Teleostei	Scombridae	*Grammatorcynus bilineatus*	Rüppell, 1836	Doublelined mackerel	Epipelagic; reef-associated; schooling	70–90
Teleostei	**Scombridae**	***Gymnosarda unicolor***	**Rüppell, 1836**	**Dogtooth tuna**	**Epipelagic; reef-associated; solitary or in small groups**	**100–150**
Teleostei	**Scombridae**	***Katsuwonus pelamis***	**Linnaeus, 1758**	**Skipjack tuna**	**Pelagic-oceanic; schooling, highly migratory**	**40–60**
Teleostei	Scombridae	*Rastrelliger brachysoma*	Bleeker, 1851	Short mackerel	Pelagic-neritic; schooling	15–20
Teleostei	Scombridae	*Rastrelliger kanagurta*	Cuvier, 1816	Indian mackerel	Pelagic-neritic; schooling	25–30
Teleostei	Scombridae	*Scomberomorus cavalla*	Cuvier,1829	King mackerel	Pelagic-neritic; schooling; highly migratory	85–120
Teleostei	Scombridae	*Scomber australasicus*	Cuvier,1832	Blue mackerel	Pelagic-neritic; schooling; seasonally migratory	30–40
Teleostei	**Scombridae**	***Thunnus alalunga***	**Bonnaterre, 1788**	**Albacore**	**Pelagic-oceanic; schooling, highly migratory**	**80–100**
Teleostei	**Scombridae**	***Thunnus albacares***	**Bonnaterre, 1788**	**Yellowfin tuna**	**Pelagic-oceanic; schooling, highly migratory**	**100–150**
Teleostei	**Scombridae**	***Thunnus obesus***	**Lowe, 1839**	**Bigeye tuna**	**Pelagic-oceanic; schooling, highly migratory**	**100–150**

albeit less frequently (e. g., Boulanger, 2023b; O'Connor et al., 2017; Boulanger et al., 2019; Kealy et al., 2020; Samper Carro et al., 2016). Austronesian-speaking populations, emerging around 4000 BP, brought advanced fishing techniques as part of a broader suite of cultural and technological advancements, including pottery-making and long-distance trade. Their rapid expansion across the Pacific—from Taiwan to the distant islands of Fiji and Tonga—was marked by significant maritime adaptations (Skoglund et al., 2016; Bellwood, 1997, 2017); archaeological sites across Oceania, including those in the Batanes Islands in the Philippines (Campos, 2009, 2013), the Mariana Islands and Palau in Micronesia (e.g., Ono and Clark, 2012; Amesbury, 2013; Leach and Davidson, 1988), and the Marquesas (e.g., Buckley et al., 2021; Nims et al., 2024; Leach et al., 1997) and Society Islands (Leach et al., 1984; Ohman and Kahn, 2024) in Polynesia, have yielded fish bone assemblages that reveal both inshore and offshore fishing practices. These findings underscore the significant role of advanced fishing techniques and marine resource exploitation in shaping the subsistence strategies and expansive maritime adaptations of prehistoric island communities across the Pacific.

Today, many shark and scombrid species continue to constitute a significant portion of worldwide fisheries—both large-scale industrial and smaller traditional—reflecting their enduring importance as key marine resources. However, their intensive exploitation has driven many shark species to the brink of endangerment, while scombrids face significant overfishing pressures (Dulvy et al., 2024; Heithaus et al., 2008; Majkowski, 2007; Pacoureau et al., 2021; Pauly and Christensen, 1995). Despite their modern significance, our understanding of the role of marine predators such as sharks and scombrids, especially in the prehistoric diets of the first hunter-gatherers and Neolithic islanders of the Pacific, remains relatively incomplete.

The western Pacific region, known for its rich marine biodiversity, hosts numerous species of both groups—14 species of Scombridae and 21 species of sharks (Selachii), including 13 species of Carcharhinidae, have been reported or potentially present along the coasts of Micronesia (Table 1). Although these species occupy diverse habitats and exhibit varied life histories, challenges in archaeological analysis persist. Elasmobranch remains are underrepresented in archaeological assemblages due to challenges in preservation, identification, and quantification, constraining our understanding of the relative importance of sharks and other cartilaginous fish, compared to bony fish, in prehistoric subsistence strategies (Gilson and Lessa, 2021; Kozuch and Fitzgerald, 1989; Ono and Intoh, 2011; Rick et al., 2002). On the other hand, scombrids—often represented by postcranial remains and vertebrae that are to some extent characteristic of the family—are notoriously difficult to identify beyond the family level using comparative anatomy alone (Boulanger, 2023; Boulanger et al., 2022; Lambrides and Weisler, 2013, 2018; O'Connor et al., 2011; Samper Carro et al., 2018).

To address these gaps, methods such as Zooarchaeology by Mass Spectrometry (ZooMS) offer promising advances in genus- and in some cases species-level identification (Buckley et al., 2009). When applied to elasmobranch and scombrid remains, ZooMS can overcome long-standing challenges in the taxonomic identification of their remains, while enhancing our understanding of past biodiversity, exploitation patterns, and human interactions with marine ecosystems (Boulanger, 2021; Buckley et al., 2021, 2024; Rick et al., 2019). In this study, ZooMS was used to analyse shark and scombrid bone samples from the Powa archaeological site in Fais, Micronesia, where a substantial number of specimens from these taxa have been recovered. The aim was to identify the range of shark and scombrid species exploited over the last two millennia by testing and refining previous tentative identifications and 'type' classifications based on comparative anatomy.

1.2. Background and previous analysis of the fish remains

Previous excavations and analyses at the Powa site (site code FSPO, standing for 'FaiS' and 'POwa') clearly highlighted that fishing and marine exploitation were central to subsistence activities in prehistoric Fais, Micronesia. Fish bones from layers 3, 4, 8, and 9 were identified by Ono and Intoh (2011) and quantified using NISP (Number of Identified Specimens) and MNI (Minimum Number of Individuals). According to the authors, this analysis revealed a total of 13,277 fish bones, including representatives from 13 'inshore fish families', such as Epinephelidae (formerly Serranidae, groupers), Scarinae (formerly Scaridae, parrotfish), Balistidae (triggerfish), Lutjanidae (snappers), Acanthuridae (surgeonfish, tangs, unicornfish), Lethrinidae (emperors, emperor breams, pigface breams), Diodontidae (porcupinefish), Labridae (wrasses), Haemulidae (grunts, sweetlips), Siganidae (rabbitfish), Muraenidae (moray eels), Holocentridae (squirrelfish, soldierfish), Ostraciidae (boxfish), as well as five 'pelagic-outer reef fish families', such as Carcharhinidae (requiem sharks), Lamnidae (mackerel sharks), Scombridae (tunas, bonitos, mackerels), Carangidae (jacks, pompanos) and Sphyraenidae (barracudas) (Table 2). Among offshore fish, sharks and Scombridae dominated both MNI and NISP counts. Their NISP values were much higher than those of other taxa (including inshore taxa), likely because vertebrae and caudal peduncles were more often recovered for these groups. Tuna identification in particular relied on vertebrae, as cranial bones for this family were sparse compared to those of other bony fishes (Ono and Intoh, 2011). This may be due to taphonomic processes and the relative fragility of Scombridae cranial bones compared to those of other taxa, rather than to anthropogenic factors, since heads are typically consumed and thus usually transported back to the sites intact. Tuna vertebrae ranged in diameter from 8 to 22 mm, with morphological features resembling those of *Katsuwonus pelamis* (skipjack tuna) and *Thunnus albacares* (yellowfin tuna) (Ono and Intoh, 2011). These vertebrae suggest the presence of middle-to large-sized tuna, measuring at least 40 cm in total length, based on comparisons with reference data (Ono and Intoh, 2011). Within the sharks, five taxa were tentatively distinguished based on vertebral characteristics and

Table 2
NISP values for pelagic/outer reef fish (previously referred to as "offshore," with associated types) and inshore fish (see previous publications for family-level counts), following Ono and Intoh (2011).

	Phase IV	Phase III	Phase IIB	Phase IIA	Phase I	Total
Type A – *C. plumbeus*	16	41	105	30	8	**200**
Type B – *C. falciformis*	9	13	61	17	6	**106**
Type C – *C. galapagensis*	20	12	55	16	4	**107**
Type D – *P. glauca*	11	9	39	10	0	**69**
Type E – *Galeocerdo* sp.	4	0	0	0	0	**4**
Unknown (Selachii) A	58	47	241	69	17	**432**
Unknown (Selachii) B	0	1	2	0	1	**4**
Unknown (Selachii) C	0	0	1	2	0	**3**
Lamnidae	0	0	2	0	0	**2**
Selachii (unidentified)	38	40	185	46	21	**330**
Scombridae	22	30	802	186	1	**1041**
Carangidae	7	2	4	7	0	**20**
Sphyraenidae	0	0	1	1	0	**2**
Total 'pelagic/ outer reef fish' (above)	**185**	**195**	**1498**	**384**	**58**	**2320**
Total 'inshore fish' (see previous publication for details)	**229**	**139**	**301**	**254**	**69**	**992**
Total NISP	**414**	**334**	**1799**	**638**	**127**	**3312**

categorized into 'types' (Ono and Intoh, 2011). Although species-level identification was limited by the reference collection (e.g., only eight species of sharks from the western Pacific, including six Carcharinidae species), tentative classifications included *Carcharhinus plumbeus* (Nardo, 1827) (sandbar shark) or *Carcharhinus longimanus* (oceanic whitetip shark; Type A), *Carcharhinus falciformis* (silky shark; Type B), *Carcharhinus galapagensis* (Galapagos shark; Type C), *Prionace glauca* (blue shark; Type D), and genus *Galeocerdo* (ground shark; Type E); one vertebra type was assigned to the Lamnidae family, while three other unidentified taxa were categorized as Unknown Types A, B, and C due to distinctive morphology but insufficient reference materials (Table 2) (Ono and Intoh, 2011). The size distribution of shark vertebrae, with most specimens identified as Carcharhinidae and Lamnidae, exceeded 20 mm in diameter. Morphological comparisons possibly indicated that medium-to large-sized sharks, around 200 cm in length, were perhaps exploited at the Powa site (Ono and Intoh, 2011).

On Fais Island, today, the traditional method for shark fishing involves using a thick coconut fiber string (sennit cord) to encircle sharks on the water's surface or employing large bait hooks with a sturdy coconut fiber line whereas tuna, on the other hand, are primarily caught through trolling with lures or hooks (Anell, 1955; Ono and Intoh, 2011). The archaeological evidence from the Powa site provides valuable insights into fishing techniques. While the production and use of fishhooks may have contributed to the notable increase in scombrid exploitation during Phase II, the absence of trolling lures—typically associated with pelagic fishing—suggests that other methods, possibly opportunistic, played a significant role (Ono and Intoh, 2011). Interestingly, lure shanks, which are directly linked to specialized pelagic fishing, were only recovered in larger quantities from Phase III, despite the fish bone analysis showing a marked decline in tuna remains after this phase. The discovery of a single *Pinctada maxima* pearl shell lure shank from Phase II suggests that such tools may have been in limited use earlier, potentially indicating an experimental or opportunistic approach to pelagic fishing during this period (Anell, 1955; Ono and Intoh, 2011). However, the increase in lure shanks during Phase III raises questions about their primary function. Some shanks appear to have holes in their head, suggesting they could have perhaps served dual purposes as ornaments or curated items from earlier phases, rather than being exclusively used for fishing (Ono and Intoh, 2011). This evidence points to a dynamic fishing strategy, where specialized techniques such as lure fishing may have emerged alongside or in response to environmental conditions and resource availability. In earlier phases, opportunistic methods likely dominated, reflecting a flexible exploitation of marine resources. Over time, the shift towards more specialized tools and techniques highlights an evolving adaptation to the richness of the environment and the increasing reliance on specific taxa, such as Scombridae, until their decline in later phases prompted further changes in subsistence practices.

2. Material and methods

2.1. The Powa site, Fais, Micronesia

Fais is a raised coral island in Micronesia, located at 9°46′N and 140°3′E, and is politically part of the Yap State within the Federated States of Micronesia (Fig. 1). Situated ~80 km east of Ulithi Atoll, the island spans 2.7 km in length and 1.1 km in width, covering an area of 2.8 km^2. Its geography is marked by a narrow fringing reef, steep cliffs on the northeast end and west side, and the absence of a lagoon or safe anchorage, making landing challenging, particularly during rough weather. Fishing is limited by the reef's structure, steep drop-offs, and the scarcity of reef fish, with activities focusing on angling or cast netting and targeting pelagic species such as sharks, although sharks are not commonly consumed nowadays (Ono and Intoh, 2011). Archaeological investigations led by Intoh in the 1990s revealed continuous habitation on Fais for the past 1800 years (Intoh, 1993, 1995, 1996a, 1996b, 1997), evidenced by artifacts such as potsherds, as well as a predominance of marine faunal remains—including fish, marine turtles (Chelonidae), mollusks, and crustaceans—alongside terrestrial remains such as mammals and birds (Intoh, 1996a, 1997; Intoh and Shigehara, 2004). The findings highlight the island's reliance on both local resources and pottery trade with nearby high islands of volcanic origin (as opposed to atolls), such as Yap (Fig. 1), to address resource limitations (Intoh and Dickinson, 2002).

Archaeological excavations at Powa, located on the southern coast near the central part of the present village, uncovered a deep cultural deposit during excavations in 2005, comprising of the oldest layers of occupation on Fais. Four 1 × 1 m units (FSPO-3, 4, 8, and 9) were

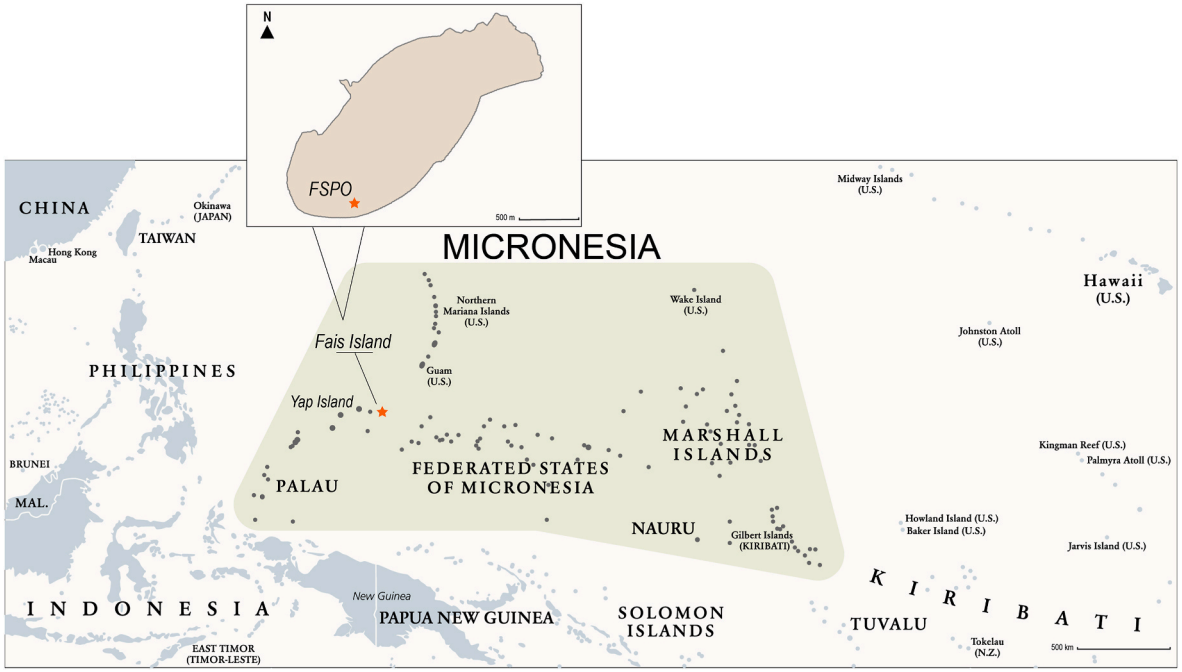

Fig. 1. Map of Micronesia and the Pacific with the position of Fais Island, the Powa (FSPO) archaeological site and Yap Island. (Base map: iStock.)

excavated, revealing twelve stratigraphic layers, with the lowest layer (Layer 12) reaching a depth of 3.3 m (Supplementary Fig. S1). Excavations followed natural stratigraphy, with deposits dry-screened through 3 mm mesh or recovered *in situ*. Charcoal samples collected from nearly all excavation layers at the Powa site provided dating information, with nine samples submitted to Beta Analytic, Inc. for AMS dating (Ono and Intoh, 2011) (Table 3). Combined with artifact analysis, five cultural phases were defined: Phase I, IIA, IIB, III and IV. The earliest phase, marked by Layer 12, was dated to approximately CE 230–420, representing Phase I. Subsequent phases spanned periods from CE 400 to post-CE 1400. Layers from Phase IV, particularly Layer 6 and above, though lacking direct radiocarbon dates from the 2005 excavation, are inferred to date to around CE 1200 (Ono and Intoh, 2011) (Table 3). The site yielded a rich collection of artifacts, including items crafted from marine shell, coral and bone, alongside significant faunal remains. Pig (*Sus scrofa*), dog (*Canis familiaris*) and dolphin (Delphinidae) remains were found as deep as Layer 10, while rat (*Rattus rattus*), marine turtle (Chelonidae) and fish remains appeared consistently across all layers, extending to the lowest depths of Layer 12 (Ono and Intoh, 2011) (Table 3).

2.2. Collagenous composition of fish remains

Teleost bone shares the same fundamental components as mammalian bone tissue, comprising three main constituents: a mineral matrix, an organic phase and water (Meunier et al., 2008). The organic phase of bone is primarily composed of type I collagen, a structural protein that makes up about 95 % of the organic material in bone (Henriksen and Karsdal, 2019; Meunier et al., 2008). It is highly conserved across vertebrates and survives relatively well in warm and tropical environments compared to DNA (Harvey et al., 2022). It features a helical structure formed by three polypeptide chains, each consisting of approximately 1000 amino acid residues. Most vertebrates possess two genes, COL1A1 and COL1A2, that code for the two α1 chains and one α2 chain that form the triple helix (Henriksen and Karsdal, 2019). However, in teleost fish, a gene duplication of COL1A1 has resulted in a unique collagen composition. The teleost triple helix consists of three distinct chains: one α1, one α2, and one α3 chain, the latter being a product of the COL1A1 gene duplication (Meyer and Van de Peer, 2005; Morvan-Dubois et al., 2003; Piez, 1965). This genetic divergence has made teleosts the group with the greatest within-species variation in collagen sequences among vertebrates (Buckley, 2018). However, this distinct third chain does not appear to exist in sharks and lampreys (Kimura and Ohno, 1987), given the timing of the duplication event that gave rise to this (Harvey et al., 2021). As their name suggests, the skeletal structure of cartilaginous fishes remains primarily composed of cartilage, as they do not develop osseous skeletons, having secondarily lost this ability to produce endoskeletal bone (Coates and Sequeira, 1998). Elasmobranchs do develop a relatively thin outer layer of cortical mineralisation over most of their skeleton (Dean et al., 2015; Seidel et al., 2016), which is typically characterised by type II collagen, a triple helical molecule made up of three identical alpha chains (COL2A1). This diversity in collagen structure likely contributes to the adaptability of both teleost and elasmobranchs to various environmental and physiological conditions, further distinguishing their bone composition from that of mammals, and enhancing their taxonomic resolution through ZooMS analyses (Buckley, 2018). Such analyses have already been applied to a range of specific groups, such as flatfish (Dierickx et al., 2022), groupers (Winter et al., 2023), and collectively both salmon and whitefish (Guiry et al., 2020) as well as studies spanning wider ranges of taxa within a given geographical region (e.g., Harvey et al., 2022, 2018).

2.3. Sampling archaeological material for ZooMS

A subset of 131 archaeological bones was randomly selected based on stratigraphic layer, taking into account the limited availability of material per layer (e.g., Type E has only one identified specimen), the inherently destructive nature of the method and the collection curator's requests. No specific identified types, element, or size, were targeted, as prior identifications were not always indicated on the storage bag labels. Nonetheless, this information, when available, has been reported in Tables S1 and S2 to ensure greater transparency. The samples typically weighed less than 0.2 g each. These were then either subsampled (i.e., scombrid remains; $n = 77$) or analysed intact where possible (e.g., shark remains; $n = 54$), so that at least ~25–50 mg per archaeological sample was processed for collagen peptide mass fingerprinting following Buckley (2013) at the Manchester Institute of Biotechnology (University of Manchester).

2.4. Sampling modern material for ZooMS

To add to reference data published in Buckley et al. (2024), several additional teeth specimens from the blacktip reef shark (*Carcharhinus melanopterus*), the blacktail/grey reef shark (*Carcharhinus*

Table 3
Overview of stratigraphy, material culture, and faunal assemblages from the 2005 excavation according to Ono and Intoh (2011).

Layer	Cultural phase	Radiocarbon calibrated age (Lab. #)	Artifacts	Faunal remains	Stratigraphic notes
1	Phase IV (CE 1400–historic)		Only laminated potsherds	Rat, fish	
2			Only laminated potsherds	Rat, fish	
3		CE 1264–1389 (Wk-3567)		Rat, fish	
4			Only laminated potsherds; trolling lure	Pig, dog, dolphin, rat, fish	Dark packed sandy soil
5	Phase III (CE 1200–1400)		Laminated potsherds continue; CST & Plain potsherds fade; trolling lure	Pig, dog, dolphin, rat, fish	
6		CE 1037–1409 (NZ7886); CE 1200–1290 (Beta-213064)	Laminated potsherds appear; CST & Plain potsherds decrease; shell tools	Pig, dog, dolphin, rat, fish	Dark packed sandy soil
7	Phase IIB (CE 600–800)	CE 895–1205 (Beta-79259)	Plain potsherds dominant; CST still present; turtle shell tools; fishhooks; shell tools	Pig, dog, dolphin, rat, fish	
8		CE 630–710 (Beta-213063)	Increase in Plain potsherds, CST still present; turtle shell tools; shell tools	Pig, dog, dolphin, rat, fish	
9		CE 553–777 (NZA2137)	Plain and CST potsherds; shell tools	Pig, dog, dolphin, rat, fish	Dark packed sandy soil
10	Phase IIA (CE 400–600)	CE 420–610 (Beta-221149); CE 440–640 (Beta-221150)	Yapese CST & Plain potsherds; Tridacna shell adzes, bracelets, beads	Apparition of pig and dog	
11		CE 420–610 (Beta-237516); CE 410–600 (Beta-213062)	Yapese CST & Plain potsherds; Tridacna shell adzes, bracelets, beads	Rat, fish	
12	Phase I (CE 1–400)	CE 468–687 (NUTA2347); CE 230–410 (Beta-213060); CE 240–420 (Beta-213061); CE 260–280 Beta-237515	Tridacna shell adzes, bracelets, beads; Yapese CST & Plain potsherds	Rat, fish	

amblyrhynchos) and the whitetip reef shark (*Triaenodon obesus*) were acquired from SeaLife, UK, were sampled following the acid-soak approach described below, as was that of the silky shark (*Carcharhinus falciformis*; UF30119). However, due to potential misidentifications given the presence of other species within the tanks, these were supported by polishing film wipe surface abrasions of the latter two (catalogue numbers USNM51215 & USNM110310 respectively) as well as those of blackspot shark (*Carcharhinus sealei;* USNM151233) and spot-tail shark (*Carcharhinus sorrah;* USNM170488) from well-identified museum material. Additionally, taxa from the surface abrasion of modern reference material housed at the National Museum of Ethnology, Japan, of repeat samples (i.e., replicates of taxa presented in Buckley et al., 2024) including tiger shark (*Galeocerdo cuvier*; FO235), sandbar shark (*Carcharhinus plumbeus;* FO 228) and Galapagos shark (*Carcharhinus galapagensis*; FO 233), were obtained using locally purchased polishing film wipes (see below for details). Carcharhinus falciformis vertebrae was sampled from Florida Museum of Natural History.....

2.5. ZooMS analysis of acid-soluble collagen

In brief, 1 mL 0.6 M hydrochloric acid (Sigma-Aldrich, UK) was added to each intact sample for decalcification overnight. Then half of the acid-soluble fraction was ultrafiltered into 50 mM ammonium bicarbonate (ABC; Sigma-Aldrich, UK), with two centrifugation steps (20 min; 12,400 rpm) and recovered in 0.1 mL solution for digestion with 0.4 μg sequencing grade trypsin (Promega, UK) overnight. Initially this was then diluted 1/20 and 1 μL co-crystalised with 1 μL 10 mg/mL alpha-cyano hydroxycinnamic acid (Sigma-Aldrich, UK) in 50 % acetonitrile (ACN)/0.1 % trifluoroacetic acid (TFA) onto a stainless-steel Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-ToF) mass spectrometric target plate. Using a Bruker Rapiflex MALDI-ToF instrument, up to 20,000 laser shots were acquired over the mass/charge (m/z) range 700–3700 and resultant spectra of archaeological samples were manually categorized into 'groups' that each were composed of their own set of peptide markers which were then compared to those for modern shark collagen fingerprint reference spectra mentioned above (Table 4; Table 5).

2.6. ZooMS analysis of polishing-film wipe extracts

In addition to additional modern reference material in Japan, extractions using these wipes were also tested on a select few ($n = 6$) archaeological samples. In brief, wipes ~5 × 20 mm in size (2000 grit, Sankyo Fujistar, Japan), after abrasion against the sample surface, were submersed in 100 μL 50 mM ABC and digested with trypsin for subsequent MALDI analysis as above. To improve confidence in homology of peptide biomarkers, as well as attempt to improve peptide concentration for samples yielding poor fingerprints, peptide fractionation was carried out using OMIX C18 ZipTips into 10 % and 50 % ACN/0.1 % TFA elutions following Buckley et al. (2009) and analysed by MALDI as above.

Table 4
Selected collagen peptide biomarkers for scombrids considered within this study; lack of number indicates that homologous markers were not inferred because they were not required to make the distinction. *[a]Other peptides exist in LC-ESI-MS/MS data that have close (within 2 Da) m/z value or [b]Ala-Ser masked by Pro-Hyp transitions. Adapted from Buckley et al. (2021). Column headings indicate α (A) chain followed by amino acid start number (conversion to the Brown et al., 2021system achieved by subtraction of 17 and 10 for α1(I) and α2 (I) respectively).

Taxon/Peptide location	A3(I) 524	A3(I) 704	A1(I) 674	A1(I) 161	A2(I) 670	A1(I) 602
Dogtooth tuna (*G. unicolor*)	1040	1455			2552	2857
Wahoo (*A. solandri*)	1052	1455	2273	2593	2540	2895
Skipjack (*K. pelamis*)	1082	1445	2288	2476	2484	2927
Kawakawa (*E. affinis*)	1056	1445	2330		2482	2885
Albacore tuna (*T. alalunga*)	1038	1445			2510	2867
Yellowfin tuna (*T. albacares*)	1052	1445	2314	2518*	2544	2867
Bigeye tuna (*T. obesus*)	1052	1445	2288*a	2534b	2544	2867

Table 5
Selected collagen peptide biomarkers for the five groups of sharks (see Supplementary Fig. S16 for spectra of fractionated *Carcharhinus falciformis* vs *Carcharhinus galapagensis*) in comparison to other related taxa from the region; lack of number indicates that homologous markers were not inferred because they were not required to make the distinction. Column headings indicate α (A) chain followed by amino acid start number (conversion to the Brown et al. (2021) system achieved by subtraction of 17, 10 and speculatively 19 for α1(I), α2(I) and α1(II) respectively). *location not confirmed by LC-MS/MS, only inferred by m/z range to exclusion of others within region; locations inferred by comparison with Buckley et al. (2024) from ([a]) Figs. S24 and 25, ([b]) Figs. S39–41, and ([c]) Figs. S42 and 44.

Taxon/Peptide location	A1(I) 621[a]	A1(I) 452[b]	A2(I) 715[c]	(A1(I)438/A2(I) 20/A1(I)603/A2 (I)767)
Group 1	1305	1663	2462	2867/2883, 2939/2955, 2992
Group 2	1305	1677	2462	2871
Group 3	1305	1677	2434/ 50	2857, 2939/ 2955, 2965
Group 4	1291	1663	2434/ 50	2841, 2925/ 2941, 2981
Group 5	1291	1693	2434/ 50	2827, 2925/ 2941, 2971
Bull shark (*C. leucas*)	1291	1663	2462	2867/83, 2925/ 2941, 2992
Galapagos shark (*C. galapagensis*)	1291	1693	2434/ 50	2811/2827, 2955/2971
Silky shark (*C. falciformis*)	1305	1677	2434/ 50	2857
Blacktip shark (*C. limbatus*)	1321	1649	2462	2873, 2939
Oceanic whitetip shark (*C. longimanus*)[DNA]	(1291)	(1693)	(2450)	(A1(I)438: 2867/ 2883)
Grey reef shark (*C. amblyrhynchos*)	1305	1693	2450	2827, 2955/ 2971, 2981
Blacktip reef shark (*C. melanopterus*)	1305	1663	2448	2821/37, 2939/ 2955, 2965
Whitetip reef shark (*T. obesus*)	1305	1677	2462	2871, 2939/ 2955, 2992
Dusky shark (*C. obscurus*)	1291	1693	2450	2827, 2925/2941
Tiger shark (*G. cuvier*)	1277	1663	2424	2853/69, 2927
Blue shark (*P. glauca*)	1327	1651	2434/ 50	2787, 2955, 2987/3003
Blackspot shark (*C. sealei*)	1291	1693	2462	2867/2883, 2992
Spot-tail shark (*C. sorrah*)	1291	1609*	2434/ 50	2867/2883, 2992
Sandbar shark (*C. plumbeus*)	1291	1629*	2434/ 50	2841, 2981
Whale shark (*R. typus*)	1293	1693	2473/ 89	
Zebra shark (*S. tigrinum*)	1303	1663	2531/ 47	

2.7. LC-MS/MS sequencing

LC-MS/MS was carried out at the Biological Mass Spectrometry facility of the University of Manchester. Digested samples were analysed using an UltiMate® 3000 Rapid Separation LC (RSLC, Dionex Corporation, Sunnyvale, CA) coupled to a QE HF (Thermo Fisher Scientific, Waltham, MA) mass spectrometer. Mobile phase A was 0.1 % formic acid in water and mobile phase B was 0.1 % formic acid in acetonitrile and the column used was a 75 mm × 250 μm internal diameter 1.7 μM

CSH C18, analytical column (Waters, UK). A 1 μL aliquot of the sample was transferred to a 5 μL loop and loaded on to the column at a flow of 300 nL/min for 5 min at 5 % B. The loop was then taken out of line and the flow was reduced from 300 nL/min to 200 nL/min in 0.5 min. Peptides were separated using a gradient that went from 5 % to 18 % B in 34.5 min, then from 18 % to 27 % B in 8 min and finally from 27 % B to 60 % B in 1 min. The column was washed at 60 % B for 3 min before re-equilibration to 5 % B in 1 min. At 55 min the flow is increased to 300 nL/min until the end of the run at 60 min. Mass spectrometry data were acquired in a data-directed manner for 60 min in positive mode. Peptides were selected for fragmentation automatically by data-dependent analysis on a basis of the top 12 peptides with *m/z* between 300 and 1750 Th and a charge state of 2, 3 or 4 with a dynamic exclusion set at 15 s. The MS Resolution was set at 120,000 with an AGC target of 3×10^6 and a maximum fill time set at 20 ms. The MS2 Resolution was set to 30,000, with an AGC target of 2×10^5, a maximum fill time of 45 ms, isolation window of 1.3 Th and a collision energy of 28. All data were collected in centroid mode. Raw files were then converted to mascot generic format (MGF) files, which were searched against a locally curated database (see Supplementary Material).

2.8. Database searching of proteomic sequencing (LC-MS/MS) data

The local database for scombrid bone collagen sequences was created from the protein BLAST search of collagen sequences from three-spined stickleback (*Gasterosteus aculeatus*) against 'Scombridae' (taxid: 8224), including *Thunnus albacares* (XP_044186193.1, XP_044191232.1 and XP_044193815.1), *T. thynnus* (XP_067472083.1, XP_067467313.1 and XP_067431685.1), and *Thunnus maccoyii* (XP_042249887.1, XP_042291469.1 and XP_042253453.1) with XP codes representing sequence IDs of COL1A1, COL1A2 and COL1A3 respectively (Supplementary Material). Additionally, sequences directly retrieved from BLAST searches of the genomes of *Thunnus obesus* (GCA_964033675.1), *Acanthocybium solandri* (GCA_964033665.1), *Euthynnus affinis*

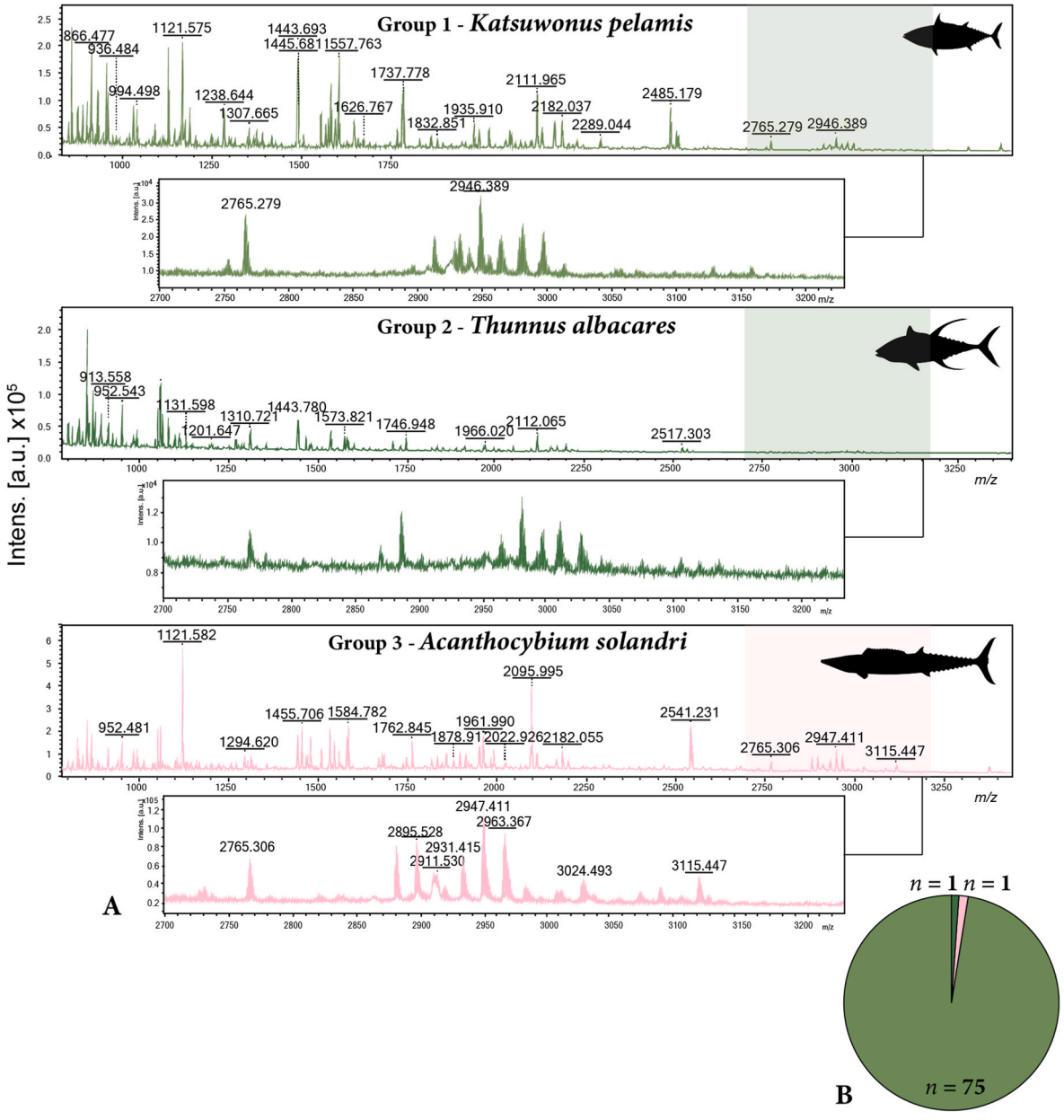

Fig. 2. A- MALDI-ToF mass spectra of collagen digests from the three archaeological scombrid taxa discriminated in the study. (Silhouettes: Phylopic.) B- Pie chart with NISP for each identified scombrid species. From top to bottom showing CB10, CB2, and CB1.

(GCA_029490765.1), *Katsuwonus pelamis* (GCA_041053085.1) and *Thunnus orientalis* (GCA_021601225.2) are also presented in the Supplementary Material. Due to the depauperate number of shark collagen sequences publicly available, particularly with respect to those relevant to this study, LC-MS/MS analyses were not attempted for the archaeological specimens of this study. However, for MALDI peaks manually determined as potentially useful taxonomic biomarkers with identifiable sequence information (Buckley et al., 2024), the peptide sequences were also compared with those retrievable from the translated genomic search of *Carcharhinus longimanus* (Supplementary Material; GCA_030264375.1). The MALDI *m/z* values were manually predicted via their amino acid substitutions while maintaining their relative number of likely post-translational modifications (i.e., oxidations and deamidations; Lawrence and Buckley, 2025); each of the three peptide markers of clearly identifiable sequence (Buckley et al., 2024 Supplementary Fig. S24/25, S39-41 & S42/44) appear to possess one oxidation PTM, the latter both one and two.

3. Results

3.1. Scombrid ZooMS and LC-MS/MS

All of the 77 archaeological scombrids sampled from the Powa site, yielded mass spectra clearly indicative of collagen peptide mass fingerprints, with many peaks of familiar *m/z* (Fig. 2; Table 4). The near-complete majority of spectra ($n = 74$ of 77; 75 including one identification from the 'shark' batch) appear to be indicative of one taxon, *Katsuwonus pelamis* (skipjack tuna). Each of the remaining two spectra represented two distinct species (Fig. 2), *Thunnusalbacares* (yellowfin tuna) (CB2; 9–16 FSP09 from Layer 7), and *Acanthocybium solandri* (wahoo) (CB1; 8–490 FSP08 from Layer 9B).

LC-MS/MS sequencing was carried out for each of the apparently distinct scombrid groups based on the collagen peptide mass fingerprint. This included the *Acanthocybium solandri* sample (CB1; Supplementary Table S3), the *Thunnus* sample (CB2; Supplementary Table S4), and two of the *Katsuwonus pelamis* samples (CB10 & CB9, the latter of which showing some additional peaks to the majority; Supplementary Fig. S2 & Supplementary Tables S5–6). Together these allowed for improved

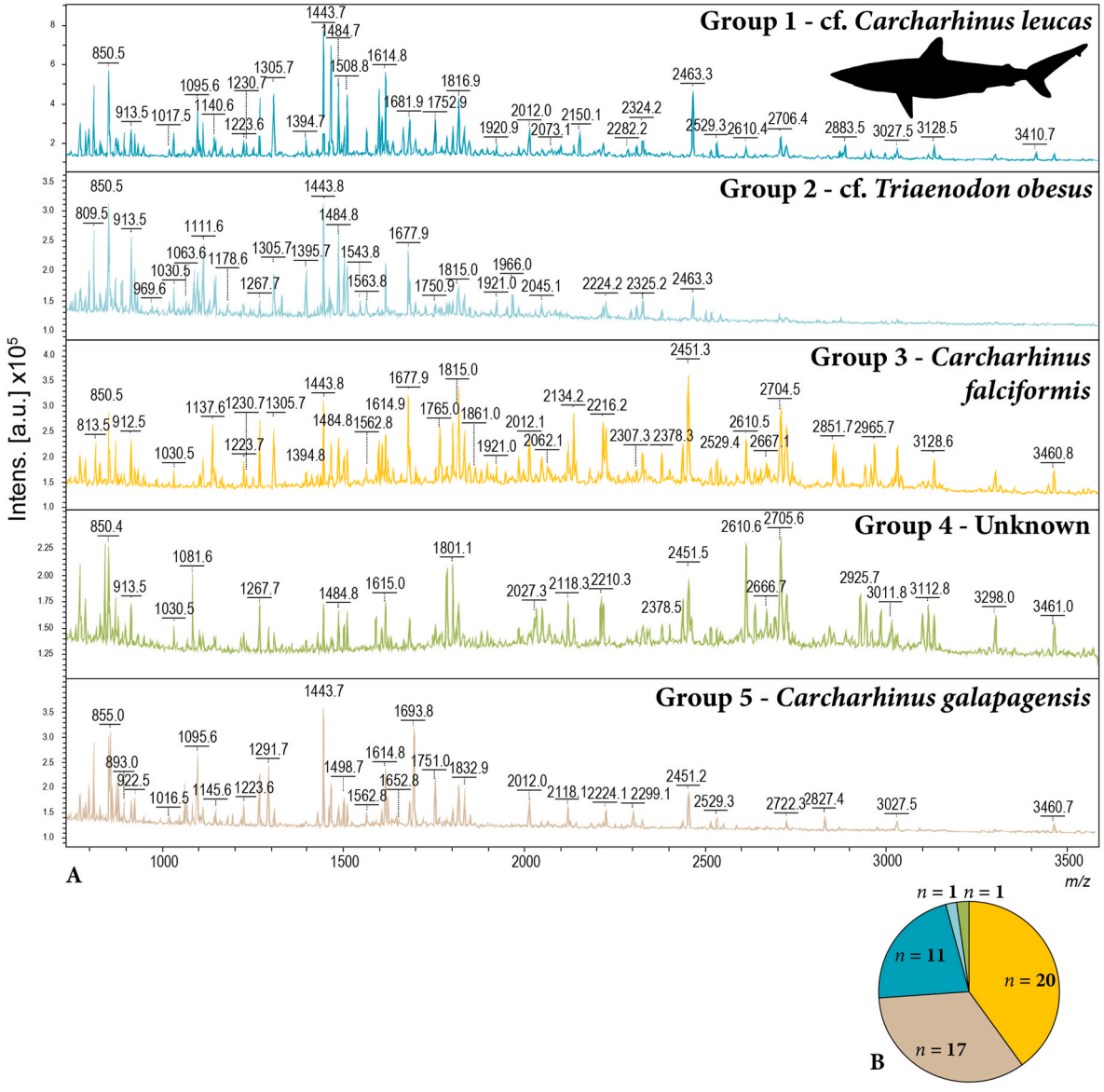

Fig. 3. MALDI-ToF mass spectra of collagen digests from the five archaeological shark taxa discriminated in the study. (Silhouette: Phylopic.) B- Pie chart with NISP for each identified shark species/group. From top to bottom, CB105, CB118, CB100, CB148 & CB98.

confirmation of scombrid biomarkers (see Supplementary Figs. S3–S10). This was particularly relevant for adding further confidence in the assignment of the *Thunnus* specimen to *Thunnus albacares* (Supplementary Table S4) as opposed to its closest relative (in this study, based on those known in the region), *Thunnus obesus* (Supplementary Figs. S7–S10). It also enabled a confident assessment of the peaks present in the archaeological *Acanthocybium solandri* (Supplementary Table S3) and *Katsuwonus pelamis* (Supplementary Table S5) but also helped confirm the identification of one *Katsuwonus pelamis*-like collagen peptide mass fingerprint spectrum (sample CB9; 9–376~378 FSP09 from Layer 9B) that yielded additional peaks derived from missed cleavage (incomplete tryptic digestion; Supplementary Table S6).

3.2. Shark ZooMS

Although the polishing film wipes yielded collagen peptide mass fingerprints for the modern reference samples in this study (Fig. 3; Supplementary Figs. S11–14), they proved successful for only one of the six archaeological samples tested (Supplementary Fig. S15). Therefore, the main analysis focused on the use of the more traditional acid-soluble ZooMS approach (Buckley, 2013; van der Sluis et al., 2014).

Of the 54 archaeological shark remains sampled through the standard approach, all except four (CB112, 121, 126 & 149) yielded a successful collagen peptide mass fingerprint (i.e., with at least 10 peaks > S/N 3 detected at > *m/z* 2000 (Harvey et al., 2016), though the majority of the 'poor' samples clearly yielded collagen peaks in their spectra). Six clearly distinct fingerprints could be confidently identified. However, one of these fingerprints derived from a tuna (*Thunnus albacares* – CB_123; FSP04 Layer 8 1/5) (Supplementary Table S2). Of the remaining five groups, three could be matched to analysed reference taxa via their proposed biomarkers with some confidence: Group 3 ($n = 20$), here identified as *Carcharhinus falciformis* (silky shark); Group 2 ($n = 1$) matched to *Triaenodon obesus* (whitetip reef shark), and Group 5 ($n = 17$, including the single successful archaeological wipe result (FSP4_L4)), strongly matched as *Carcharhinus galapagensis* (Galapagos shark). Furthermore, the previous morphological study proposed (Ono and Intoh, 2011), with appropriate caution, that their Type A was potentially either *Carcharhinus plumbeus* or *Carcharhinus longimanus,* Type B as *Carcharhinus falciformis*, Type C as *Carcharhinus galapagensis*, Type D as *Prionace glauca* and Type E as *Galeocerdo* sp., reinforcing the confidence that in our Group 3 and Group 5 identifications, *Carcharhinus falciformis* and *Carcharhinus galapagensis,* respectively. However, we struggled to link our third most abundant taxon (Group 1; $n = 11$) with any of the other suggested taxa (*Prionace glauca, Galeocerdo cuvier, Carcharhinus plumbeus* or *Carcharhinus longimanus*; Supplementary Fig. S11 & Table 5) despite including comparisons to previously published spectral data as well as genome-derived sequence information (Supplementary Material). This spectrum type had a notable number of markers matching to *Carcharhinus leucas* (bull shark), but with enough apparent differences be most likely another taxon – potentially the dominant taxon known in Micronesia missing from our reference material being *Carcharhinus albimarginatus* (silvertip shark) though acknowledge that we cannot rely on modern species distributions to make inferences of taxa present in the past. The single sample constituting Group 4 (CB148) yielded the majority of marker peaks common with *Carcharhinus plumbeus* (e.g., *m/z* 2841 & 2981) though the peak at *m/z* 1663 could indicate that it derives from another taxon, such as the far less common *Carcharhinus amboinensis* (pigeye shark) or from a species with current distributions further afield such as the Indo-West Pacific. We also note that Ono and Intoh, 2011 had three 'unknown' groups, one (Unknown A) with a dominant amount, the other two (B & C) with negligible amounts.

One of the key results identified here is that where almost one third of the randomly sampled shark assemblage appeared to give fingerprints of a particular (single) taxon (Group 1), this ZooMS-categorized group notably matches all three previously described 'Types' A, B and C (Ono and Intoh, 2011) (Table 2). Similarly, twenty specimens appeared to yield a fingerprint matching one group (Group 3), *Carcharhinus falciformis,* also spanning morphological Types A, B and C of previous identifications. Furthermore, eleven samples yielded collagen peptide mass spectra consistent with *Carcharhinus galapagensis* (Group 5) appear to span morphological Types A and B.

4. Discussion

The study successfully demonstrated the utility of ZooMS (collagen peptide mass fingerprinting) in identifying both teleost and elasmobranch remains from archaeological samples. For the scombrid remains, the near-complete identification rate (74 out of 77 specimens) as *Katsuwonus pelamis* highlights the robustness of the method for well-represented taxa. In contrast to this, while 50 out of 54 shark samples yielded useable collagen fingerprints, there remains a substantial challenge in identifying their fingerprints to a particular species with confidence, until either the reference databases (MALDI spectra or genome-derived sequences) are complete enough to include the majority of likely taxa. For example, clearly dominant (in terms of their signal-to-noise ratio) markers could be observed that likely reflect viable/homologous taxonomic discriminators (e.g., *m/z* 1663-1677-1693, and *m/z* 2424-2450-2462; see Buckey et al. 2024), have allowed for the exclusion of potential taxa as the source of our unknowns (Groups 1 and 4); the identification of such markers can also be enhanced with greater confidence through C18-based fractionation (Supplementary Figure S11, S13 & S16) in the absence of known sequence information, as used in the early development of ZooMS collagen peptide mass fingerprinting (Buckley et al., 2009). The ever-increasing number of genomes available offers a constantly increasing resource that is valuable to ZooMS collagen fingerprinting, offering the means to predict expected peptide biomarkers in particular taxa without the need to have access to the appropriate biological tissues. Taking the abovementioned example peptide at *m/z* 1663 (DGDVGAPGAAGPAGPPGER), shown in Buckley et al. (2024; Fig. S40) for some shark taxa, we retrieve a sequence of DGDIGAPGPAGPAGPSGER, so would expect a peak at *m/z* 1693 (assuming one oxidation), which we do observe in our reference spectra also.

Although the collagens are complicated more so than most proteins by their relatively high number of post-translational modifications, particularly the oxidations of proline and lysine residues, incorporating these can be achieved through the inclusion of LC-MS/MS-based sequencing approaches (Lawrence and Buckley, 2025). The inclusion of tandem mass spectrometry, whether by LC-MS/MS or MALDI-MS/MS also more readily allows for the discovery of novel peptide sequences, useful contributions as taxonomic biomarkers. Yet we acknowledge that despite this extensive resource, due to the vast nature of evolutionary diversity among fish, much further work is needed to consider the entire spectra with reference to currently incomplete suite of appropriate species.

The use of polishing film wipes represents a promising non-destructive approach for collagen peptide mass fingerprinting (Coutu et al., 2021; Ebel et al., 2024; Kirby et al., 2020). Although the approach was not successful for most of the tested archaeological shark remains in this study (Supplementary Fig. S15; see also Buckley et al., 2021), its use with modern reference samples as exemplified here, highlights its potential, particularly when working with specimens of high conservation or cultural value. The speed at which such reference materials can be processed, i.e., from sampling through digestion to spectrum acquisition within 1–2 h, is perhaps one of the greatest benefits to this approach, though access to accurately identified reference materials remains the most time-consuming component. As there is also the risk of mis-identified reference materials, for example one of the *Pristis* specimens by Buckley et al. (2024), it would also be appropriate to collect at least 3–5 specimens per claimed species (e.g., similar to the requirements for machine-learning approaches to ZooMS analyses (see Gu and Buckley, 2018). By enabling collagen extraction without causing

substantial damage to the specimen, the polishing film wipes allow for the preservation of the physical integrity of culturally and scientifically significant materials. This is especially beneficial for specimens housed in museums or heritage collections, where destructive sampling is either undesirable or prohibited. Further development of such non-destructive techniques could enhance their applicability across diverse contexts, ensuring ethical and legal considerations are upheld while advancing scientific research.

The findings of this study corroborate and build upon the taxonomic identifications reported by Ono and Intoh (2011), highlighting notable advancements by refining species assignments and clarifying overlaps in morphological characteristics. The identification of scombrid species, particularly *Katsuwonus pelamis* and *Thunnus albacares* (Ono and Intoh, 2011), was corroborated and extended by this study; the dominant presence of *Katsuwonus pelamis* was confirmed, while one other scombrid species, *Acanthocybium solandri* was also detected. While the earlier study identified five taxa within Carcharhinidae using vertebral morphology—constrained by the limited reference collections available at the time—including *Carcharhinus plumbeus* or *Carcharhinus longimanus* (Type A), *Carcharhinus falciformis* (Type B), *Carcharhinus galapagensis* (Type C), *Prionace glauca* (Type D), and *Galeocerdo* sp. (Type E), this analysis allowed for a more nuanced understanding of shark species diversity.

A discrepancy between the new identifications and those of the earlier study was observed (Table 2; Fig. 2; Fig. 3; Supplementary Table 1; Supplementary Table 2). This discrepancy concerns both species-level identifications and the categorization of morphological types, highlighting that tentative identifications of Carcharhinidae based solely on comparative anatomy reflect not only limitations in the reference collections but also a lack of diagnostic features in the bones themselves. Although both approaches, i.e., morphology-based or ZooMS-based exhibit limitations in their ability to identify the correct taxon, the latter is an inability only limited by current reference materials, whereas the former suffers the more formidable challenge of limitations in morphologically diagnostic features notwithstanding locally available reference materials and expertise (Hawkins et al., 2022). Therefore, further identification beyond the family level based on comparative anatomy should only be undertaken with caution, as clearly exemplified by the attempted categorisations by Ono and Intoh (2011).

Interestingly, the taxa identified in this study reflects a wide range of ecological niches, highlighting the strategic exploitation of both pelagic and reef-associated resources by the inhabitants of Fais. This pattern aligns with previous findings by Ono and Intoh (2011), who documented an abundance of inshore species such as groupers and parrotfish, alongside sharks and scombrids, at the site. Among the sharks, the silky shark (*Carcharhinus falciformis*) stands out for their broad habitat range; they are highly migratory and often associated with tuna schools, highlighting the connectivity between pelagic ecosystems and demonstrating the interdependence of different fishing practices at the site (Pérez San Juan et al., 2024). Additionally, the presence of the Galapagos shark (*Carcharhinus galapagensis*) emphasizes the significance of reef ecosystems, as this species often inhabits rugged reef environments and deeper slopes (Carrier et al., 2012; Compagno, 1984). The scombrid species identified, including skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and wahoo (*Acanthocybium solandri*), are key components of pelagic food webs. Skipjack tunas are fast-swimming, highly migratory species that form large schools and occupy surface waters, whereas the yellowfin tuna, like skipjack, are highly migratory but often found in deep offshore waters. However, wahoo, though pelagic, are typically solitary or found in small groups; they often inhabit coastal waters and hunt along the edges of deep reefs (Collette and Nauen, 1983). At the site more broadly, the ecological roles of these taxa suggest a balanced exploitation strategy—targeting apex predators, mid-level predators and schooling fish—leveraging the island's proximity to both rich pelagic waters and productive reef ecosystems. This diversity of habitats emphasizes the limitations of overly broad ecological categorizations based solely on family-level identifications. For example, among the Carcharhinidae, we observe both inshore and offshore species, complicating simplistic classifications. The same holds true for the Scombridae, where ecological roles vary significantly across species. Therefore, we must nuance discussions of "pelagic" fishing in archaeological contexts, recognizing that species often occupy a wider ecological range than implied by general labels. Species-level identification is essential to accurately interpret patterns of marine exploitation and to distinguish between inshore and offshore fishing activities. In contrast, family-level identification, while useful at other sites, may be insufficient to support detailed ecological and cultural interpretations.

While species-level shifts cannot be fully understood due to the limited sample size in this study, the analysis of sharks and scombrids at the Powa site reveals significant changes in their exploitation over time (Ono and Intoh, 2011), reinforcing the complementarity of traditional morphological methods and biomolecular approaches. Shark NISP shows a gradual increase from Phase I (CE 200–400), peaks during Phase IIB (CE 600–800), and then declines in Phase IV (post- CE 1200) (Table 2) (Ono and Intoh, 2011). Composite Chi-square tests, using Monte Carlo simulations with 10,000 sample tables, performed by Ono and Intoh (2011) indicated significant increases in shark abundance from Phase IIA to IIB, and from Phase IIB to Phase III, followed by a notable decrease in Phase IV. This suggests that shark exploitation was not static but rather subject to changing cultural or environmental factors (Ono and Intoh, 2011). Scombrids and, in particular, skipjack tuna, which were confidently identified in this study (Fig. 2), show a distinct pattern of exploitation; their NISP is minimal in Phase I but increases substantially in Phases IIA and IIB before sharply declining in Phases III and IV (Table 2). This rise during Phase II aligns with increased artifact counts, including fishhooks introduced in Phase IIB, which likely facilitated the capture of large-bodied offshore species like tuna (Ono and Intoh, 2011). Pelagic and outer-reef species may have contributed a higher protein yield, likely due to their generally larger size (see Table 1). The surge in shark and scombrid exploitation during Phase II coincides with broader trends, such as population growth and possible technological advancements, such as improved fishing techniques or new navigation skills. However, the subsequent decline in both taxa's NISP in later phases suggests changes in resource availability, highly related to seasonality, environmental conditions, or cultural preferences (Ono and Intoh, 2011).

From the early phases of occupation, prehistoric human impacts on marine resources appear to have been limited. However, by the middle phases, evidence suggests that fishing pressure had begun to develop in the area (Table 2). Clarifying this trajectory requires further investigation and more species-level identifications to provide a broader picture, while climatic variability—particularly El Niño-Southern Oscillation (ENSO) events—may also have been a significant factor. ENSO-driven hydroclimate fluctuations are known to influence the distribution and abundance of skipjack tuna and other pelagic species across the western and central Pacific Ocean. Indeed, Lambrides and Weisler (2018), based on the analysis of archaeological datasets—such as that from Ebon Atoll—and a review of literature from other regional, demonstrated correlations between ENSO variability and shifts in tuna fisheries over the past 2000 years. These patterns suggest that periods of climatic instability may have reduced the availability of tuna in certain areas, impacting subsistence practices. Regional hydroclimate changes associated with ENSO could have driven skipjack and other tuna species to adjust their range or abundance, reducing their accessibility to local fishers. Similarly, shark populations, primarily comprising carcharhinid species, may also have experienced significant declines over time. However, limited comparative data from both archaeological and modern records hinders the ability to assess long-term trends in shark abundance accurately (e.g., Martin et al., 2016). While sharks were consistently exploited as a major marine resource at Fais, changes in

their relative abundance could reflect a combination of human exploitation pressures and/or selective hunting strategies, and ecological shifts, potentially tied to the same climatic variability potentially affecting tuna stocks. However, to accurately assess the impact of ENSO on Fais and the surrounding region, further investigations are necessary.

5. Conclusion

In light of these uncertainties and the lack of robust baseline data, new analytical approaches are essential for clarifying long-term patterns of marine resource use. Ultimately, ZooMS represents an indispensable tool for addressing these gaps, enabling researchers to document millennial-scale patterns of marine resource exploitation in a region where recent material has been historically difficult to identify due to the lack of comprehensive reference collections. In this study, the ability of sequencing-supported ZooMS to provide confident species-level identifications for scombrid remains that include both yellowfin tuna and bigeye tuna offers insights that were previously unattainable with traditional morphological analyses. Furthermore, we emphasize that genome-derived sequence data are important for clarifying the taxonomic resolution of ZooMS results, as notable differences in ZooMS spectra can arise from enzymatic missed cleavages.

More significantly, the comparison of previously-published morphological analyses and ZooMS groupings of archaeological shark remains reveal discrepancies in categorizations, confirming the need for multidisciplinary approaches to obtain insights into past species exploitation at large scales, perhaps through the use of high-throughput ZooMS (e.g., Buckley et al., 2016; Oldfield et al., 2024). Nonetheless, despite our attempts to expand upon shark ZooMS reference markers to include the silky, blackspot, spot-tail, oceanic, whitetip reef, blacktip reef and grey reef sharks, this aspect of ZooMS analyses remains a substantial challenge, particularly when exploring taxa taxonomically distinct from those that have already been well characterised and/or offering limited DNA-based sequence information.

Once met, advancement in ZooMS analyses to include relatively larger components of any particular marine assemblage not only enhances our ability to understand past subsistence practices but can also shed light on the long-term sustainability of fishing practices and their ecological impacts. For example, the lack of robust baseline data for sharks emphasizes the importance of expanding datasets to better evaluate historical population dynamics and their ecological and cultural implications, highlighting the need for further research integrating high-resolution local climate reconstructions, comprehensive species-level identification of fish remains, and modern ecological data. Such studies would enhance our understanding of how climatic variability, fishing practices, and ecological factors have shaped the availability of key marine resources in the Pacific, informing sustainable fisheries management for future food security and economic resilience (Andrews et al., 2022, 2023; Lambrides and Weisler, 2018; Pauly, 1995).

CRediT authorship contribution statement

Clara Boulanger: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Rintaro Ono:** Project administration, Formal analysis, Funding acquisition. **Michiko Intoh:** Formal analysis, Project administration, Writing – review & editing. **Michael Buckley:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Funding acquisition.

Data availability statement

The original contributions presented in the study are available on FigShare at 10.6084/m9.figshare.30359044 and presented as additional figures in the Supplementary Material. Further inquiries can be directed to the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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After excavation, all faunal remains and artifacts were loaned under a permit issued by the Historic Preservation Office (HPO) in Yap and transported to the National Museum of Ethnology at Osaka, Japan, for sampling. The samples for ZooMS were selected in Japan, brought to the University of Manchester, and then sent back to the National Museum of Ethnology in Japan. Modern reference materials of aquarium-based reef sharks were donated by SeaLife, UK but supported by polishing film wipes of reference material from the Smithsonian Institution with assistance for which we thank Torben Rick. We also thank Nicole Fuller, Michelle LeFebvre and Coleman Sheehy of the Florida Museum of Natural History as well as Andrew Kitchener of the National Museums Scotland for reference sample access. This research is funded by a Japan Society for the Promotion of Science (JSPS) Research Grant awarded to Rintaro Ono (Grant Numbers: 16H06409, 18KK0019, 20K20504, and 21H04368) and jointly to Rintaro Ono and Clara Boulanger (Grant Number: 22KF0373).

Appendix A. Supplementary data

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