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Collagen peptide markers for three extinct Australian megafauna species

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Recent advancements in biomolecular archaeology, such as stable isotope and ancient DNA research, have expanded our understanding of megafauna extinction processes and dynamics. The rise of palaeoproteomics, specifically Zooarchaeology by Mass Spectrometry (ZooMS), has added yet another method to this toolkit, as it can be used to taxonomically identify megafauna remains amongst highly fragmented bone assemblages. However, taxonomic identifications with ZooMS are reliant on the availability of collagen peptide markers for the regional fauna of interest. In the absence of a global reference database, most studies to date have been restricted to Eurasian contexts. Here, we report ZooMS peptide markers for three extinct Australian megafauna species: *Zygomaturus trilobus*, *Palorchestes azael*, and *Protemnodon mamkurra*. We show that these taxa can be differentiated from extant Australian fauna with these peptide markers. This foundational work represents an important step in establishing ZooMS as a method that can be used to identify new megafauna specimens in Australia's highly fragmented fossil record and ultimately help resolve fundamental questions related to human–fauna–environment interactions.

KEYWORDS

Zooarchaeology by Mass Spectrometry, Diprotodontidae, Palorchestidae, Macropodidae, late Quaternary

1 Introduction

The emergence of novel analytical methodologies in archaeology and palaeontology has helped shed new light on long-standing research questions within the discipline. Amongst the topics such methods have helped to address is the timing and nature of the global megafauna extinctions in the late Quaternary. In most cases, the application of

chronometric dating and modelling approaches are seen as key to understanding the timing and demise of megafauna species at a global, continental, and local level (e.g., Brook and Bowman, 2002; Stuart and Lister, 2012; Prescott et al., 2012; Stuart, 2014). More recently, the application of biomolecular methods has led to a deeper understanding of extinction processes and dynamics (see also Swift et al., 2019). The application of stable isotope analysis, for example, has allowed for improved reconstruction of megafauna diet (e.g., Bocherens et al., 2017; Ma et al., 2019; Koutamanis et al., 2023; Varela et al., 2023), mobility (e.g., Price et al., 2017; Wooller et al., 2021; Heddell-Stevens et al., 2024), and ecology (e.g., Trayler et al., 2015; González-Guarda et al., 2017; Rabanus-Wallace et al., 2017). Similarly, the application of ancient DNA has revealed new insights into the demography and population dynamics of megafauna species (e.g., Llamas et al., 2014; Fellows Yates et al., 2017; Pečnerová et al., 2017), as well as their migration and geographic range shifts (e.g., Haile et al., 2009; Lorenzen et al., 2011; Seersholm et al., 2020; Canteri et al., 2022).

One biomolecular technique that has not yet been extensively applied to research questions related to late Quaternary megafaunal extinctions is palaeoproteomics, and specifically Zooarchaeology by Mass Spectrometry (ZooMS). ZooMS is a type of peptide mass fingerprinting in which differences in collagen type I between (sub)families, genera, and sometimes species are used to taxonomically identify collagen-bearing materials, such as bone and ivory (Buckley et al., 2009). The method offers several key advantages, such as its ability to provide taxonomic information from fragmentary and otherwise unidentifiable zooarchaeological and paleontological remains (Brown et al., 2021b; Sinet-Mathiot et al., 2023), its scalability to screen large fragmentary assemblages for a targeted species of interest (Douka et al., 2019), and its applicability to material coming from a wide range of environments, including sub-tropical and tropical ranges (Peters et al., 2023; Wang et al., 2023). Yet, an important prerequisite for the successful application of ZooMS is the presence of a comprehensive reference database of collagen peptide markers to make these taxonomic identifications possible. Thus far, studies that have used ZooMS to identify megafauna remains have mostly been restricted to Eurasia (e.g., Buckley et al., 2017b; Brown et al., 2021b; Smith et al., 2024; Xia et al., 2024) and North America (e.g., Kubiak et al., 2023; Antonosyan et al., 2024). This geographical bias can largely be attributed to the absence of collagen peptide markers for extinct megafauna from other continents.

To address this lacuna and build upon reference libraries recently created for extant Australian fauna (Buckley et al., 2017a; Peters et al., 2021), we sought to begin to develop peptide markers for extinct megafauna species in Australia, a country for which peptide markers are currently only available for a single extinct megafaunal taxon, *Simosthenurus occidentalis* (Buckley et al., 2017a). While the age of megafaunal reference specimens poses challenges to collagen preservation, especially in the warmer climates found in much of Australia, recent research suggests that collagen preservation in the continent extend back to over 50,000 years ago, even in warmer and more humid regions of Australia (Peters et al., 2023). Nonetheless, the poorly delineated age of many megafauna reference specimens, often attributed only to broad geological periods, poses further challenges to the selection of suitable material for peptide marker development.

Here, we report ZooMS peptide markers for three megafauna species from southern Australia and Tasmania that went extinct in the Late Pleistocene, namely, *Zygomaturus trilobus*, *Palorchestes azael*, and *Protemnodon mamkurra*. The targeted specimens were all directly dated previously using AMS radiocarbon dating (Gillespie et al., 2012, 2015). The *Protemnodon mamkurra* specimen was accurately dated to 42.2–43.1 ka cal BP, the other two specimens extend beyond the limits of radiocarbon dating (Gillespie et al., 2012, 2015). Species of *Zygomaturus* and *Palorchestes* were amongst the largest-bodied mammalian megafauna of Australia during the Pleistocene (Figure 1; Johnson, 2006), and both represent families (Diprotodontidae and Palorchestidae, respectively) that went completely extinct in the late Quaternary (Koch and Barnosky, 2006). Remains of *Zygomaturus trilobus* have been recovered from fossil sites across mainland Australia (Long et al., 2002; Webb, 2008). It was adapted to forested environments (Black et al., 2012), feeding on both C_3 and C_4 plants (DeSantis et al., 2017). *Palorchestes azael*, on the other hand, was a highly specialized browser with distinct, powerful forelimbs and sharp claws (Richards et al., 2019) that was widely distributed across eastern Australia and Tasmania (Pledge, 1991; Long et al., 2002). *Protemnodon* represents a clade of extinct giant kangaroos, of which all species are now extinct. *Protemnodon mamkurra* was widespread across the forested environments of southern Australia and Tasmania (Kerr et al., 2024). The development of collagen peptide markers for these species will enable future ZooMS research into the extinction of megafauna in Australia.

2 Materials and methods

2.1 Material

The samples that were analyzed for this study consist of three megafauna specimens from which collagen was previously extracted for radiocarbon and stable isotope analysis (Figure 2; Table 1). This includes a rib fragment of *Zygomaturus trilobus* from Mowbray Swamp, Tasmania (MSZ-1), a humerus of *Palorchestes azael* from Spring Creek, Victoria (SCPal-1), and a femur of *Protemnodon mamkurra* sp. nov. from Mt. Cripps, Tasmania (MCP-2) (Gillespie et al., 2012, 2015). The six dates reported for the highly contaminated *Zygomaturus trilobus* form a curve approaching an asymptote of >50,500 BP, while the three dates of the less-contaminated Spring Creek *Palorchestes azael* reach an asymptote of >53,500 BP (Gillespie et al., 2012, 2015). Both results are near the maximum age possible using the applied chemistry and radiocarbon method, and, as also suggested by the geology (Gill and Banks, 1956; Banks et al., 1976), they should be treated as minimum ages for these two specimens (Chappell et al., 1996). The *Protemnodon mamkurra* specimen from Mt. Cripps yielded an age of 42.4–43.1 cal BP (Gillespie et al., 2012, 2015). This specimen is

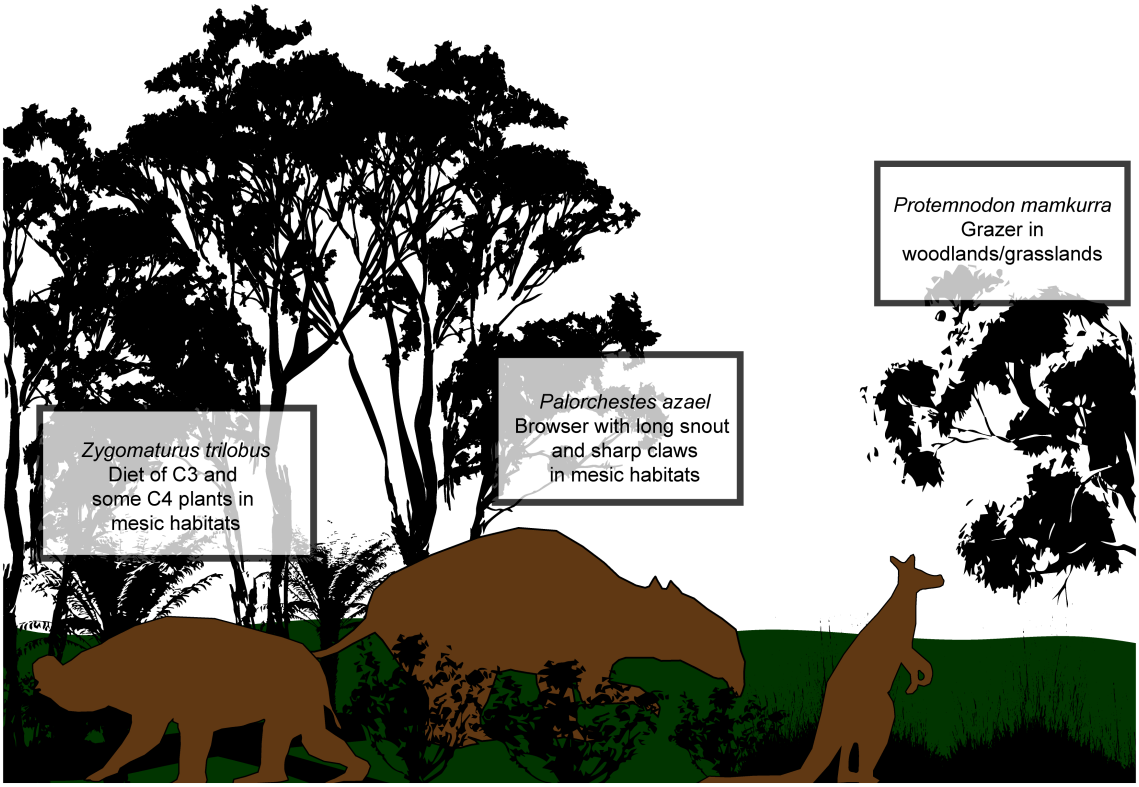

FIGURE 1
Diagram showing the three extinct megafauna species studied with imagined reconstruction and associated habitats, based on Prideaux et al. (2009), Gillespie et al. (2012, 2015), and Richards et al. (2019). *Zygomaturus trilobus* probably weighed ~500–700 kg, was quadrupedal, and was found in mesic habitats near the continental margin. *Palorchestes azael* was likely ~1000 kg, a mostly quadrupedal browser but able to stand on two legs to browse higher bushes and trees, while *Protemnodon mamkurra* was a browser/mixed-feeder of ~100–150 kg (Kerr et al., 2024). All three species were found in southern Australia and Tasmania.

one of the youngest extinct marsupial megafauna reported from Australia to date, and covers a small period of overlap with the first humans in Tasmania, who could have entered Tasmania via the earliest pedestrian land crossing available at ~43 ± 4 ka (Lambeck and Chappell, 2001). All megafauna reference specimens were originally sampled by RG from collections at the Queen Victoria Museum and Art Gallery (Launceston, Tasmania) and Museums Victoria (Melbourne, Victoria).

2.2 Zooarchaeology by Mass Spectrometry

An acid insoluble protocol was used for the *Protemnodon mamkurra* and *Palorchestes azael* bones to extract collagen (Buckley et al., 2009; Welker et al., 2015). Approximately 20 mg of bone was demineralized in 500µl of 0.6 M hydrochloric acid (HCl) for 3 days and washed 3 × with 200 µl of 50 mM ammonium bicarbonate (AmBic). 100 µl of AmBic was added and the sample gelatinized at 65°C for 1 hour. 50 µl of the resulting supernatant was removed and 1 µl of 0.4 µg/µl trypsin solution (Pierce™ Trypsin Protease, Thermo Scientific) was added for digestion at 37°C for ~18 h. The following day, 1 µl of 5% trifluoracetic acid (TFA) was added to the supernatant, which was then purified and concentrated using C18 ZipTips (Pierce™ C18 Tips, Thermo Scientific), spotted in triplicate with matrix solution (α-cyano-4-hydroxycinnamic acid of 10 mg/ml in 50% acetonitrile/0.1% TFA) and analyzed with a MALDI-TOF-MS (Autoflex, Bruker Daltonics). The *Z. trilobus* sample consisted of ultrafiltered gelatin that was previously prepared for radiocarbon dating using the Oxford protocol (Higham et al., 2006). About 2 mg of this pretreated gelatin was separated and 50 µl AmBic was added. Then, the same steps of digestion, purification, and spotting were undertaken as described for the other two samples. The resulting MALDI spectra were processed in mMass v5.5.0 (Strohalm et al., 2010) with smoothing (Method = Savitzky-Golay, Window size = 0.3, Cycles = 2), baseline correction (Precision = 15, Relative offset = 25), and peak picking (S/N = 3.0, Picking height = 75%).

2.3 Liquid chromatography tandem mass spectrometry

Following ZooMS analysis, 20 µl of the collagen extract was dried down for further LC-MS/MS analysis at the Functional Genomics Center Zurich using a Q-Exactive HF mass spectrometer (Thermo Scientific) coupled with an ACQUITY

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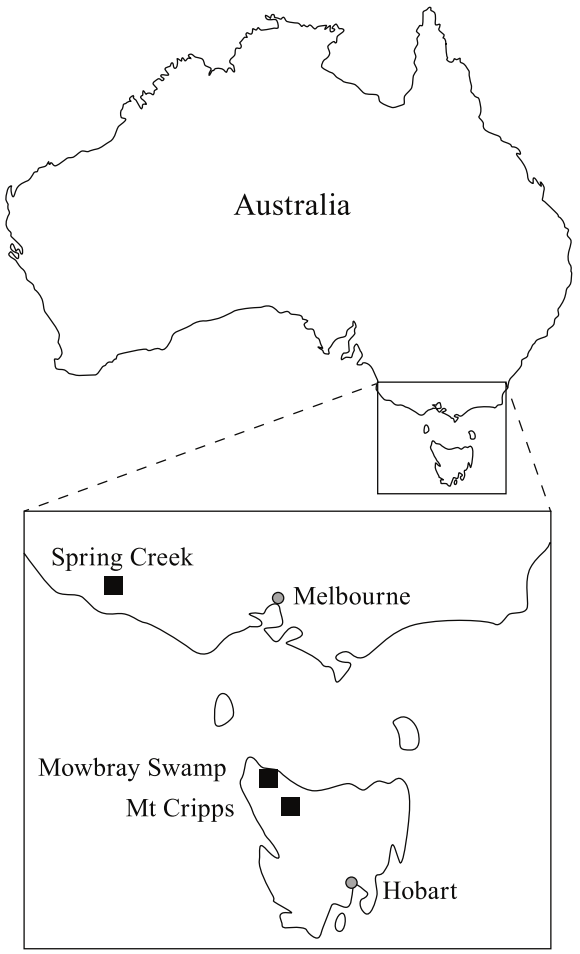

FIGURE 2
Location of sites containing megafauna reference specimens included in this study (modified after Gillespie et al., 2015).

UPLC M-Class system (Waters, AG). Solvent composition was 0.1% formic acid for channel A and 0.1% formic acid in 99.9% ACN for channel B. The column temperature was 50°C. For every sample, 4 µl of peptides were loaded on a commercial MZ Symmetry C18 Trap Columns (Å, 5 µm, 180 µm × 20 mm, Waters) followed by a nanoEase MZ C18 HSS T3 Column (100 Å, 1.8 µm, 75 µm × 250 mm, Waters). The peptides were eluted at a flow rate of 300 nl min^{-1} by a gradient from 5 to 40% B in 120 min and 98% B in 5 min. After each run, the column was cleaned with 98% solvent B for 5 min and holding 98% B for 8 min prior to re-establishing loading condition. The mass spectrometers were operated in data-dependent mode (DDA) performing higher energy collision dissociation (HCD) fragmentation on the 12 most intense signals per cycle. Full-scan MS spectra (300–1500 m/z) were acquired at a resolution of 120,000 at 200 m/z after accumulation to a target value (AGC) of 3,000,000, while HCD spectra were acquired at a resolution of 30,000 using a normalized collision energy of 28 (maximum injection time: 50 ms; AGC: 10,000 ions). Unassigned singly charged ions were excluded. Precursor masses previously selected for MS/MS measurement were excluded from further selection for 30 s, and the exclusion window was set at 10 ppm. The samples were acquired using internal lock mass calibration on m/z 371.1012 and 445.1200.

2.4 Peptide marker development

The identification and confirmation of collagen peptide markers followed multiple steps. First, candidate collagen peptide markers were identified. For this, MALDI spectra were visually inspected using mMass v. 5.5.0. (Strohalm et al., 2010) and compared to a list of published collagen markers (Buckley et al., 2017a; Peters et al., 2021). To confirm candidate peptide markers, the MS/MS data was analyzed using Byonic v. 3.2.0. (Protein Metrics Inc., Bern et al., 2012), following a multi-stage approach first introduced by Richter et al. (2020).

Initially, the MS/MS spectra were searched against a reference database including all collagen type I (COL1α1 and COL1α2) sequences available for marsupials from NCBI and UniProt, collagen peptide sequences for marsupials reported in Peters et al. (2021), and common contaminants. The taxa for which complete collagen sequences were available are koala (*Phascolarctos cinereus*, XP_020853290.1 & XP_020855640.1), common wombat (*Vombatus ursinus*, A0A4X2KF99 & A0A4X2M815), Tasmanian devil (*Sarcophilus harrisii*, G3WK23 & G3VSR0), and kangaroo (*Macropus* sp., Buckley et al., 2017b). The search parameters were set to: cleavage sites fully specific on C-term arginine (R) and lysine (K); 3 missed cleavages allowed; 6 common mass changes and no rare mass changes allowed; common mass changes: oxidation of lysine (K), methionine (M) and proline (P), deamidation of asparagine (N) and glutamine (Q); protein FDR 2%. Peptide sequences (with PEP2D scores <0.01) corresponding to candidate peptide markers were recorded.

Candidate peptide markers for which the peptide sequence could not be identified in the initial search were re-analyzed using an error-tolerant search strategy. Here, the same database was used, but with different search parameters to allow for the identification of novel sequence variants. Parameter settings that were altered are: 2 missed cleavages allowed; 5 common and 1 rare mass change allowed; rare mass changes: all sequence variants allowed. All other

TABLE 1 Specimens used to develop ZooMS peptide markers.

Site	Species	Element	Museum No.	Sample ID
Mt Cripps, Tasmania	*Protemnodon mamkurra*	Femur	2001GFV:40	MCP-2
Mowbray Swamp, Tasmania	*Zygomaturus trilobus*	Rib fragment	1992 GFV:148	MSZ-1
Spring Creek, Victoria	*Palorchestes azael*	Humerus	P177944	SCPal-1

parameter settings were identical to those listed for the initial search. All possible sequence variants were noted down and their corresponding masses recorded.

The samples were then searched against a database with the proteomes of *V. ursinus* (UP000314987) and *S. harrisii* (UP000007648), as well as all sequence data available in Swissprot. The parameter settings for this search were: cleavage sites fully specific on C-term arginine (R) and lysine (K); 3 missed cleavages allowed; 2 common and 1 rare mass change allowed; common mass changes: oxidation of lysine (K), methionine (M) and proline (P), deamidation of asparagine (N) and glutamine (Q); rare mass changes: pyro-Glu on N-term glutamic acid (E) and glutamine (Q), ammonia-loss on N-term cysteine (C); protein FDR 2%. The results were checked for other identified bone proteins and (common) contaminants to confirm the authenticity of the samples.

A new database was created using the output of the first three searches. This database includes the collagen type I sequences of the original reference database, all sequence variants identified in the error tolerant search, the bone proteins identified in the proteome-wide search, and common contaminants. The MS/MS spectra were searched once more against this database, using the same parameter setting as in the first database search. Only peptides recurring at least three times and with a PEP2D score <0.01 were considered confirmed.

TABLE 2 Collagen peptide markers for Australian megafauna developed in this study.

	Zygomaturus trilobus	*Palorchestes azael*	*Protemnodon mamkurra*
COL1α1 508–519	1162	1162	1162
COL1α2 978–990[1]	***1159 (1175)***	x	*1150 (1166)*
COL1α2 484–498	1453	1453	1453
COL1α2 502–519	*1598*	1598	1598
COL1α2 889–906	1624[2]	1624[2]	1652
COL1α2 292–309	x	x	1680
COL1α2 793–816	**2177**	**2177**	*2145*
COL1α2 454–483[1]	*2335 (2351)*	2335 (2351)	*2335 (2351)*
COL1α1 586–618[1]	**2869 (2885)**	**2869 (2885)**	2897 (2913)
COL1α2 757–789[1]	**2959 (2975)**	**2959 (2975)**	*2943 (2959)*
COL1α2 10–42	2975	2975	**3008**

Naming of peptide markers follows Brown et al. (2021a). Markers in italics have been observed in LC-MS/MS data but are not visible in MALDI-TOF-MS spectra. Bolded markers can be used to differentiate between megafauna species.
[1]Masses indicated in brackets represent the same peptide marker with an additional oxidation. This results in a mass shift of +16 Da.
[2]A peak at *m/z* 1652 is also visible in MALDI-TOF-MS spectra. This peptide marker should thus be used with caution.

3 Results

The three megafauna specimens all showed good collagen preservation, enabling the development of collagen peptide markers for all three species. An overview of the identified peptide markers can be found in Table 2, and associated sequence data is reported in Table 3 (see also Supplementary Figures S1-S8).

ZooMS allows for the unique identification of *Protemnodon* compared to extant kangaroo genera (*Macropus*, *Notamacropus*, *Osphranter*, *Lagorchestes* and *Lagostrophus*) for which peptide markers were previously developed (Peters et al., 2021) through the identification of peptide marker COL1α2 10–42 (*m/z* 3008 in *Protemnodon*). Similarly, *Protemnodon* can be differentiated from another genus of extinct kangaroo, *Simosthenurus* (Buckley et al., 2017a), using peptide markers COL1α1 586–618 (*m/z* 2897/2913 and *m/z* 2881/2897, respectively) and COL1α2 10–42 (*m/z* 3008 and *m/z* 2975, respectively).

Zygomaturus trilobus and *Palorchestes azael* can be differentiated from other extant and extinct large-bodied marsupials using a combination of peptide markers, most notably COL1α2 793–816 (Figure 3, *m/z* 2177), COL1α1 586–618 (*m/z* 2869/2885), and COL1α2 757–789 (*m/z* 2959/2975). It should be noted, however, that it is not possible to distinguish between the two species using ZooMS. The only observed difference between them is at peptide marker COL1α2 978–990, but since no peptide sequence could be confirmed for *P. azael* at this location, this peptide marker should not be used to make identifications. The absence of collagen peptide markers to differentiate between the two species does not necessarily reflect a phylogenetic signal. COL1 is a highly constrained protein with sequence mutations accumulating at a slow rate (Stover and Verrelli, 2011). As such, ZooMS can in many cases only be used to make genus- or family-level identifications (Richter et al., 2022).

4 Discussion and conclusion

We report collagen peptide markers for three extinct Australian marsupial megafauna taxa, *Zygomaturus trilobus, Palorchestes azael*, and *Protemnodon mamkurra*. The samples used in this study also further showcase the value of using leftover collagen or gelatin from radiocarbon dating and stable isotope analysis for palaeoproteomic analysis (e.g. Charlton et al., 2016; Mylopotamitaki et al., 2024; Smith et al., 2024). Here, this leftover gelatin was specifically used as reference samples for ZooMS peptide marker development, and, in doing so, minimizing the need for additional destructive sampling of valuable reference specimens of extinct taxa (Pálsdóttir et al., 2019).

With the addition of reference data for *Zygomaturus trilobus*, *Palorchestes azael*, and *Protemnodon mamkurra*, ZooMS can now be used to support the identification of four extinct Australian megafauna taxa. All of these taxa can be differentiated from extant

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TABLE 3 Peptide sequences corresponding to collagen peptide markers reporting in this study.

Marker		Sequence	Mass
COL1α1 508–519	**P1**	GVQGPPGPQGPR	1162
COL1α2 978–990	**A**	PG**Q**AGAVGPAGLR	1150 (1166)
		PG**H**AGAVGPAGLR	1159 (1175)
COL1α2 484–498	**B**	GLPGEFGLPGPAGPR	1453
COL1α2 502–519	**C**	GPPGESGAVGPTGSIGSR	1598
COL1α2 889–906		GEPGP**A**GSVGPVGPFGAR	1624
		GEPGP**V**GSVGPVGPFGAR	1652
COL1α2 292–309	**P2**	GPNGEPGSTGPTGPPGLR	1680
COL1α2 793–816	**D**	GLPGVSG**A**LGEPGPLGI**A**GPPGAR	2145
		GLPGVSG**S**LGEPGPLGI**S**GPPGAR	2177
COL1α2 454–483	**E**	GEQGPAGPPGFQGLPGPSGPAGE**G**GK	2335 (2351)
COL1α1 586–618	**F**	GLTGPIGPPGPAGPSGDKGESGPSGP**A**GPTGAR	2869 (2885)
		GLTGPIGPPGPAGPSGDKGESGPSGP**V**GPTGAR	2897 (2913)
COL1α2 757–789	**G**	GPPGE**A**GATGPPGSSGPQGLLGAPGILGLPGSR	2943 (2959)
		GPPGE**S**GATGPPGSSGPQGLLGAPGILGLPGSR	2959 (2975)
COL1α2 10–42		GPPGASGPPGAQGFQGPAGEPGEPGQTGPAG**A**R	2975
		GPPGASGPPGAQGFQGPAGEPGEPGQTGPAG**S**R	3008

Naming of peptide markers follows Brown et al. (2021a). Differences between sequences are bolded and underlined.

marsupial species. However, collagen peptide markers were only developed for a single species per genus. This means that there is a reasonable possibility that other species within these genera will have identical peptide marker sets. For example, *P. anak*, another species of *Protemnodon* with a geographic range spanning eastern Australia (Kerr et al., 2024), and *P. tumbuna*, a species specific to New Guinea (Prideaux et al., 2022), are likely to have an identical set of peptide markers to *Protemnodon mamkurra*. Therefore, the peptide markers reported in this study can optimally be used to make genus- rather than species-level identifications.

Importantly, all of the specimens analyzed as part of this study, as well as those from Buckley et al. (2017a), originated from

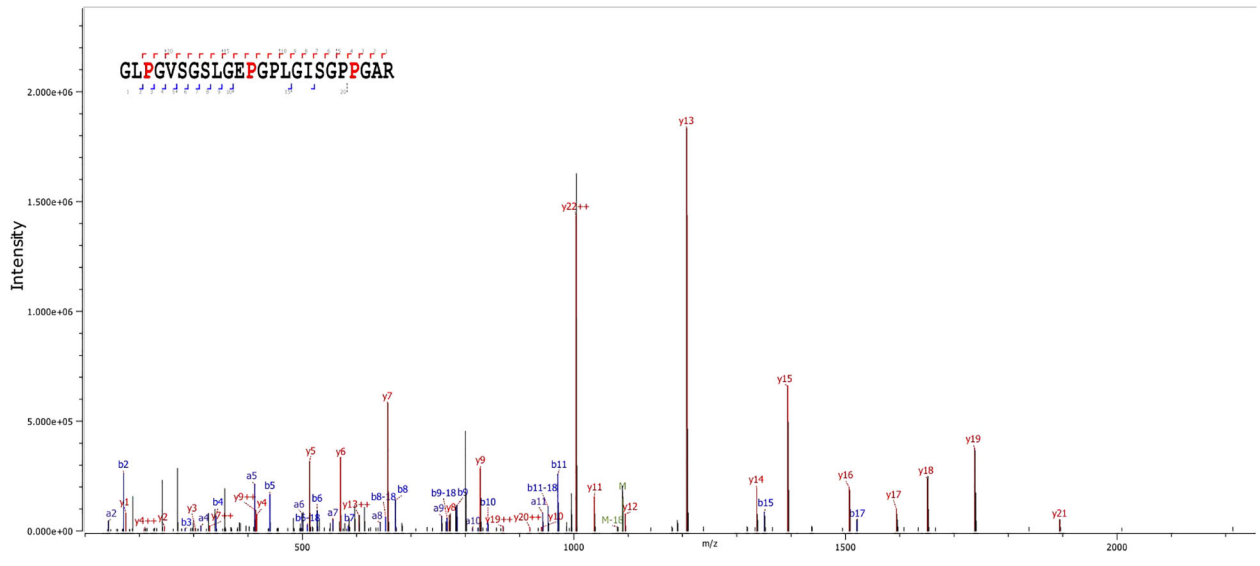

FIGURE 3
Example of MS/MS spectrum of peptide marker COL1α2 793–816 at *m/z* 2177 for *Zygomaturus trilobus* (MSB01; LC-MS/MS analysis code: DA1075).

Tasmania or southern Australia, reflecting regions with cooler temperatures. This means that there is a regional bias in extinct megafauna species for which collagen peptide markers have been developed, as markers are currently only available for species from these more temperate regions of Australia. Regardless of this geographical bias, the fact that ZooMS peptide markers are genus- or (sub)family-specific in most instances (Janzen et al., 2021; Peters et al., 2021; Richter et al., 2022) means that the newly reported megafauna peptide markers can still be applied to identify possible megafauna specimens in fragmented assemblages across the country, even expanding into Papua New Guinea, which was formerly part of the palaeocontinent of Sahul prior to sea level rise at the end of the Last Glacial Maximum (Lambeck and Chappell, 2001) and accordingly closely related megafaunal taxa can be found there. For example, as mentioned previously, *P. tumbuna* can likely be identified with the *Protemnodon* peptide markers reported in this study. In addition, extinct Papua New Guinean species in the subfamily Zygomaturinae (e.g., *Hulitherium*, *Kolopsoides*, and *Kolopsis*) may have similar peptide markers to *Zygomaturus*. However, as is the case for Australia, issues with collagen preservation in the tropical and humid environment of Papua New Guinea may impact the success of ZooMS as a method for the identification of these extinct megafauna species. Nevertheless, these new ZooMS markers can be used to explore the possible presence of late surviving megafauna in the New Guinea Highlands where climatic conditions are more amenable to collagen preservation (Prideaux et al., 2022).

The development of collagen peptide markers for extinct Australian megafauna species represents a significant step in the establishment of ZooMS as a useful technique in addressing archaeological and paleontological research questions on the continent. Future work will be critical in expanding this reference library, as well as in applying the markers to the identification of bone assemblages from archaeological and paleontological sites. By expanding the body of identifiable megafauna specimens for Australia, these markers have the potential to play a key role in improving understanding of megafauna palaeobiology and palaeodemography, and in identifying megafauna specimens with collagen preservation suitable for subsequent stable isotope analysis and radiocarbon dating. Ultimately, we expect that the palaeoproteomic identification and analysis of megafauna specimens from localities across Sahul will provide important new insights into the long-debated extinction of megafauna in the late Quaternary.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: http://www.proteomexchange.org/, PXD053101; http://doi.org/10.52891/zenodo.14418148, Zenodo record 14418148; http://doi.org/10.25345/C5XW4872S, MSV000095033.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

CP: Conceptualization, Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. AO: Investigation, Visualization, Writing – original draft, Writing – review & editing. RG: Resources, Writing – review & editing. NB: Conceptualization, Funding acquisition, Resources, Writing – review & editing. KD: Conceptualization, Funding acquisition, Resources, Writing – review & editing.

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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.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmamm.2025.1564287/full#supplementary-material

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