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Pursuing pademelon provenance: a pilot study using portable XRF to trace field-collection of museum mammal specimens

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Abstract
Internationally, the value and usefulness of museum zoological specimens are compromised when supporting contextual data are lost or disconnected from the specimen. In this pilot study, twelve *Macropodidae Thylogale* (pademelon) skins with known provenance from the Australian Museum (Sydney) were analysed using portable X-ray fluorescence spectroscopy and principal component analysis. Elemental composition of preservative residues was assessed to establish if common patterns existed and could be associated with particular field collectors. Specimens were differentiated, and the field collector deduced, based on elemental analysis of preservative residues on skins. Each of the nineteenth century field collectors, in this study, were found to have applied the same or similar preservatives to zoological specimens over a number of years, which showed a consistent pattern of practice. Additionally, the specimens obtained by each of the field collectors could be distinguished from one another based on the preservative residues. These discoveries provide exciting prospects for the use of X-ray fluorescence spectroscopy to couple museum specimens with unknown contextual data via their field collector and associated archival evidence, and hence, enable a considerable enhancement of their value as museum and research objects.

Keywords Natural history museum, Portable X-ray fluorescence spectroscopy, Provenance, Zoological specimens, Taxidermy, Principal component analysis

Introduction

During the nineteenth century, innumerable mammal skins were collected, traded, and incorporated into natural history collections around the world [1–5]. Each skin was typically associated with information about the context from which the animal was removed. Such data, including geographic location and collection date, are essential to enable global research into biodiversity, systematics, health, and historical studies [6–14]. Through the cycles of exhibition and changes in data storage mechanisms, many older specimens have become disassociated from their contextual data [15]. Without this critical data, specimens are left "historically unreliable" [16] and regardless of their rarity or unique characteristics, these specimens have a greatly diminished value in furthering scientific research [17–19].

Zoological specimens with missing data are widely acknowledged as a problem when using natural history museum collections in research [20–22]. Several studies have attempted to quantify the extent of this problem with the use of publicly available biological collection data aggregators, but the results are often interwoven with database filtering and retrieval complexities [23, 24]. However, a handful of surveys have documented the number of records found that have a complete absence

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of data for specific fields, and these may provide a rough indication of the number of specimens that are affected by data loss. Using the Global Biodiversity Information Facility (GBIF), Gaiji, et al. found 26.8% of specimens globally were without data for year of collection [25]. Malaney and Cook, using Vertnet, found 6% of mammal specimens in USA museums were missing a year of collection [18]. Peterson, et al., with a selected data set from Vertnet, found 4.6% of ornithological specimens had no location information [26], and the Australian Environmental Resources Network reported 18% of specimens in Australian collections had erroneous or missing location data [27]. Based on these studies and using the estimate of 2.1 billion specimens in the world's museums [28], 6% represents as many as 120 million specimens that cannot be used in research where time is an important factor because of missing date data.

The 'Merchants and Museums' project investigated pathways to reconnect 'no data' museum specimens with archival and historical data [29]. Within this project, it was proposed that the chemical residues from preservatives used on zoological specimens may lead to the identification of the field collector and, thereby, provide the necessary viable links to archival and other contextual data to confirm or re-establish provenance.

Numerous analytical methods have been applied to zoological museum specimens, contributing to individual object histories by determining pesticide and preservative use, including Fourier transform infrared spectroscopy [30, 31], inductively coupled plasma mass spectrometry [32, 33], and atomic absorption spectroscopy [5, 34]. Mass spectrometry and subsequent stable isotope analysis has been successfully applied to determination of the geographic origin of zoological material by focussing on chemical residues acquired by an animal during life [35–37]. The biggest limitation to the use of these analytical techniques is the necessity to remove a sample from the specimen, and often destruction of the sample via the analysis. Such destructive sampling progressively diminishes the irreplaceable zoological resource that museums provide [38, 39]. In contrast, non-invasive spectroscopic analytical techniques enable the characterisation of zoological specimens without physical sampling thus supporting museums' aim of in-perpetuity preservation for future research and education [40].

The use of portable X-ray fluorescence (pXRF) spectroscopy is well established in museum practice due to its capacity to yield compositional data on multiple elements at the same time, without physical sampling, or the relocation of heritage objects to an external site for analysis, at relatively low cost [41]. In air, it is sensitive to elements with atomic numbers above magnesium and provides immediate elemental information in the form of counts (of fluorescent X-ray photons), that are linearly dependent on the concentration of each element in the sample [42, 43]. It is particularly well suited to comparative studies [44] and its application has successfully revealed histories of manufacture, and the provenance of inorganic materials used in historic objects [45–48]. However, the technique has been underused in the study of museum zoological skins for exploring historical methods of specimen manufacture and has not previously been successful in establishing provenance including the 'when' and 'where' a live animal was made into a zoological specimen. In museum specimens, pXRF has been used almost exclusively for qualitative and semi-quantitative measurement of elements toxic to humans for the purpose of managing the exposure of workers and visitors to poisonous chemicals [31, 49–52]. This limited use may be attributed to the difficulty in applying proven pXRF methodologies for establishing provenance of inorganic materials, which compare elemental concentrations in parts per million, to biological material [49, 53–56]. Obtaining accurate quantitative pXRF data from biological material is complicated by inherently high concentration of low atomic number elements which cannot be detected by pXRF in normal atmosphere, as well as heterogeneity in skin porosity and density, particle size, surface geometry, and absorption or enhancement interactions between elements [57–63]. For zoological museum specimens, this is further complicated by variations in the distribution of hand applied preservatives, and in the difficulty in separating preservative residues applied in the field, from undocumented pesticides applied via routine treatments in the museum [64–66]. Thus, the full potential of pXRF to aid research on the histories and provenance of museum zoological specimens is yet to be realised.

Over 250 instruction manuals and pamphlets were published during the eighteenth and nineteenth centuries each including at least one preservative recipe [67–69]. By the mid nineteenth century, instruction manuals offered several preservative mixtures for each zoological class indicating that practitioners selected the recipe that best suited their situation. However, it was not always clear which mixture should have been or was used under which circumstances. Changes in the nomenclature of ingredients added further ambiguity to our interpretation [67, 70]. Literature on the history of specimen preservation has inferred that nineteenth century field collectors followed the trends and innovations documented in these contemporary instruction manuals [71, 72]. In contrast, Merle Patchett's and Adrian van Allen's work on the craft nature of specimen preparation, hypothesised that specimen preparators were less influenced by innovations and

trends, but were likely to have maintained the unique actions passed down from their teachers [73–76]. However, field records documenting the preservative mixtures collectors applied in the field, are rare both in Australia and the rest of the world [34, 77–79]. It is not clear if field collectors always used the same recipe over several years, used different preservative mixtures for specific climatic conditions, or updated their practices in response to published innovations. For field preservation, neither position has been supported by archival field records nor by physical evidence from the specimens themselves.

In this proof-of-concept investigation, a zoological specimen is recognised as a man-made object, created with the application of preservatives at the time of the animal's death [73, 75]. By focusing on the residues applied at this moment, this study explores that capability of pXRF spectroscopy to provide evidence of nineteenth century field collection practices, and to determine when and where a zoological specimen was extracted from the wild. To achieve this, analytical methodologies previously applied to inorganic materials in artistic and archaeological objects, and in geological science [45, 53, 56, 80, 81], were adapted to the semi-quantitative nature of XRF data collected from biological material [55].

Principal component analysis (PCA) was used to investigate the similarities and differences amongst the specimens. This multivariate statistical analysis method addresses trace and bulk elements alike and simultaneously, allowing for the identification of difference between specimens regardless of concentration of those elements. PCA extracts the latent relationships within and between data sets and condenses the most important information in the data into a series of principal components (PCs). PCs are ordered and numbered based on the quantity of information within the data set that they describe, with PC1 describing the most information and subsequent PCs describing less. The information held within each PCs is presented as a Loadings plot. Data from individual pXRF measurements are plotted as a point within a Scores plot. Points that cluster together are similar, and separation between clusters indicate dissimilarity. Scores and Loadings plots are interpreted together to describe the similarities and differences between specimens [82, 83].

The aim of this pilot study was three-fold:

1. To test the capacity of the experimental protocol to identify and/or differentiate between individual specimens
2. To determine if collectors followed a consistent pattern of practice over different expeditions that may be used to produce a characteristic elemental profile or fingerprint; and
3. To investigate if specimens acquired by two or more collectors can be differentiated using these elemental profiles.

Experimental

Portable XRF spectroscopy

Analysis was performed using a Bruker Tracer 5i portable XRF spectrometer (Karlsruhe, Germany; heretofore referred to as Tracer), equipped with a rhodium (Rh) thin-window X-ray tube; X-ray generator 6–50 kV with 4.5–195 μA, maximum 4 W output) and a 10 mm^2 silicon drift detector (Proprietary 20 mm^2 silicon drift detector with 140 eV @ 250,000 cps Mn Kα; resolution for optimum light element analysis.). Specimens were analysed in air at 40 kV and 30 μA for 40 s with a Ti 25 Al 300 filter and using an 8 mm collimator.

XRF spectra were collected in situ at the Australian Museum in accordance with the conditions defined in the NSW Radiation Control Act 1990 [84], and by a certified portable pXRF operator. The Tracer was mounted on its stand beneath a custom-built specimen stage that enabled the pademelon skin to rest, supported, above the nose of the instrument. No preparation was undertaken prior to analysis of the skins. Skins were positioned so that a relatively flat surface was presented to the nose of the instrument. Fresh nitrile gloves were stuffed with kitchen paper and manipulated into shape to support the specimen in position and prevent movement during spectral collection. The instrument was controlled via a laptop using Bruker ARTAX software (v8.0.0.476).

Appropriate protocols were followed to ensure the safety of researchers and museum staff including the use of required personal protection equipment when handling specimens. Analysis was conducted in a well-ventilated room with restricted access to visitors. Specimens were handled by trained museum object handlers. Gloves were replaced between each specimen to minimise the risk of cross-contamination.

A minimum of twenty sites were analysed for each *Thylogale* in order to provide data that represented the whole object, and to account for variations in skin and preservative application, as well as cross-contamination and pesticide exposure from within the museum environment. A minimum of two spectra were collected from the belly, back, left and right sides, and the head. Additionally, all specimens had at least one area where no fur remained on the skin, typically where the legs rested against the shelf in storage. At least one spectrum was collected from the bald area for each specimen. The specimen stage was cleaned with 70% ethanol and water, and disposable

paper towel after each specimen then fresh supports were assembled.

Data analysis

Spectra with dead times above 10%, or low signal, were excluded as they were considered unsuitable for reliable elemental analysis. ARTAX software (v7.8.2.0 Bruker) was used to conduct Bayesian deconvolution on the remaining 218 XRF spectra, with escape and pileup peak, and background correction enabled. Peak area data were then normalised to the Compton peak area (18.5-19.5 keV) of each spectrum in Microsoft© Excel. No further pre-processing was undertaken. Resulting data were imported into VEKTOR DIREKTOR (v1.1 KAX Group, Australia) and principal component analysis (PCA) was performed using random cross-validation, and mean centring for 1000 iterations over five components.

Materials

Unlike taxidermy mounted specimens, research study skins typically undergo few preservation treatments after their arrival at a museum [85]. This investigation analysed only study skins to minimise the potentially confounding effect of chemicals and contaminates arising from subsequent preservation and pesticide treatment to the skin. To further reduce possible confounds from the museum's environment, specimens were selected from a single museum, The Australian Museum. Specimens were selected from a single genus to maintain comparable thickness and density of the skin and fur, and to reduce possible impact of endogenous elemental residues associated with significantly different animal diets.

In many cases, a museum will have only a few representative specimens of each species making the selection of a large group of specimens challenging [59, 78]. However, The Australian Museum holds over thirty-five *Thylogale (Marsupialia: Macropodidae,* known as pademelon) study skins (Fig. 1). Twenty-one of these are attributed to professional museum collectors, Kendall Broadbent (1837–1911), Edwin James Cairn (unknown-1939) and Robert Grant (c.1854–1923). From this group, three species from three distinct regions were chosen to assess the impact of the animal's habitat on elemental differences and specifically on preservative choice. The coastal New South Wales (NSW) habitat of *T.thetis* is typified by temperate dry forests; the Far Northern Queensland habitat of *T.stigmatica* is characterised by tropical rain forests; and the climate in central Tasmania, the habitat of T.*billardierii,* may be described as cool temperate forests and grasslands. The field notes held at the Australian Museum archives for all three of these collectors did not include information on the preservatives applied in the field, and no further documentary evidence on their practices was identified at the outset of this study.

A total of twelve *Thylogale* study skins representing three species (*T.thetis*, *T.stigmatica*, and T.*billardierii*) were selected (Table 1). Six pademelon skins represented two collecting expeditions by Kendall Broadbent: Two T.*billardierii* were collected during an 1897 expedition to Tasmania [86] and four *T.stigmatica* were collected during an 1880 expedition to Queensland [87]. Three *T.stigmatica* skins represented two collecting expeditions by Robert Grant working with E.J. Cairn: an 1887 expedition to the Bellenden Ker Ranges [88] and an 1889 expedition to Herberton [89] both in Northern Queensland. Three *T.thetis* skins represented one collecting expedition undertaken by Grant alone: an 1892 expedition to Seal Rocks and the Bellinger River NSW [90]. All the specimens selected were stable, with no significant

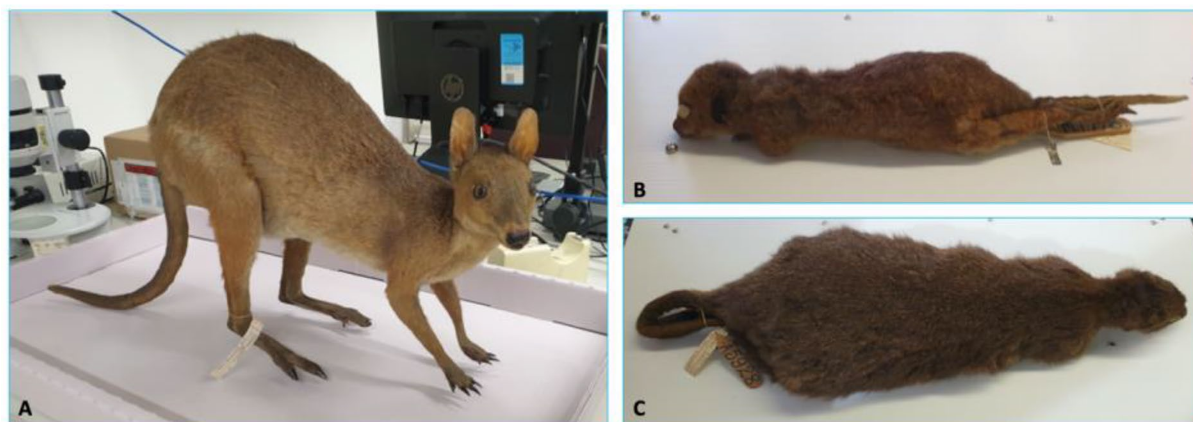

Fig. 1 Examples of *Thylogale* specimens at the Australian Museum. **A** *Thylogale stigmatica* adult mounted skin A. 1370 **B** *Thylogale stigmatica* juvenile study skin M.128. **C** *Thylogale billardierii* adult study skin A. 5928

Table 1 *Thylogale* study skins included in this study

Field collector	Expedition year	*Thylogale* species	Collection location	Accession number
Broadbent	1879	*billardierii*	Tasmania	A5339 A5928
Broadbent	1880	*thetis*	Darling Downs, QLD	A9721 A9722 A9725 A9726
Grant and Cairn	1888	*stigmatica*	Bellenden Ker, QLD	M128 M129
Grant and Cairn[a]	1889	*stigmatica*	Herberton district, QLD	M494
Grant	1892	*thetis*	Bellinger river, NSW	M793 M796 M798

[a] Although registered as collected by Grant and Cairn, the report from the collecting trip shows Grant was not with the expedition when Cairn collected M494 [91]

differences in skin or fur condition to suggest any differences in preservation, and all were stuffed with cotton and/or plant fibre.

Results

Figure 2A shows the co-added XRF spectra for each specimen analysed in this study, which are plotted on a log scale of peak intensity in order to accentuate the peaks due to trace elements at low concentration. Specimens acquired by Broadbent (green), Grant working alone (orange), and Grant with Cairn (dark purple) were clearly differentiated by bulk elemental composition, specimens attributed to the same field collector had the same bulk elements present in similar concentrations This consistency was observed in skins collected during different expeditions with minor variation. This comparison required only the relative concentrations provided inherently by pXRF spectroscopy, therefore data were collected and presented as counts rather than absolute concentrations. These characteristics provided strong evidence that each of the collectors that are relevant to this study repeatedly used the same preservative mixture over time, *and* that the mixtures used by specific collectors differed from one another.

To further investigate, the Bayesian deconvoluted and Compton normalised XRF peak area data for all twelve pademelon specimens were compared using PCA. Elemental differences between the group collected by Broadbent, and those collected by Grant, and Grant with Cairn were revealed (Fig. 3). A total of 97.73% of the variance was described by principal component 1 (PC1) and PC2. Data from each location sampled on the specimens collected by Broadbent clustered along principal PC2 (6.29% of variance, Fig. 3, green). Neither expedition location, species, nor date had an impact in this analysis. Zinc (Zn), lead (Pb), and copper (Cu) distinguished specimens collected by Broadbent from other skins in the study (PC2 Loadings, Fig. 3B). Pademelon specimens collected by Grant with Cairn during both expeditions clustered along PC1(91.44% of variance, Fig. 3, purple) and were separated from the other specimens due to the presence of high relative concentrations of arsenic (As), as shown in PC1 Loadings Fig. 3B. Pademelon specimens collected by Grant alone (Fig. 3, yellow) during the expedition to Bellinger River NSW, clustered at the junction of the other groups due to a lower relative concentration of elements (ie the average of the elements). However, this observation is confounded because the pademelon species collected by Grant during the 1892 Bellinger River expedition (*T. thetis*) was different to those collected during the other expeditions examined in this study.

Broadbent

Within the group of specimens collected by Broadbent, Hg and Cu are correlated (Pearson's R^2 correlation is 0.72), which provided strong evidence that they were applied to the skins together (Fig. 4A). Both elements correlated to a lesser degree with Zn (Hg and Zn, $R^2=0.5$, Zn and Cu, $R^2=0.45$). While Pb was a distinguishing element for specimens collected by Broadbent when compared to spectra from other specimens, it was not correlated with Hg, Cu or Zn in the Broadbent specimens.

A further PCA of specimens collected by Broadbent only, illustrated that elemental variation in the skins formed three distinct groups observed via a 3D correlation loadings plot (Fig. 5A). The major variance described by PC1was dominated by Zn (75.05%), PC2 by As (12.72%), and PC3 by Pb (7.29%) as shown in Fig. 5B, C and D. Zn is an endogenous element in animals however, the relationship of Zn with Hg (shown above) demonstrated that the majority of Zn detected

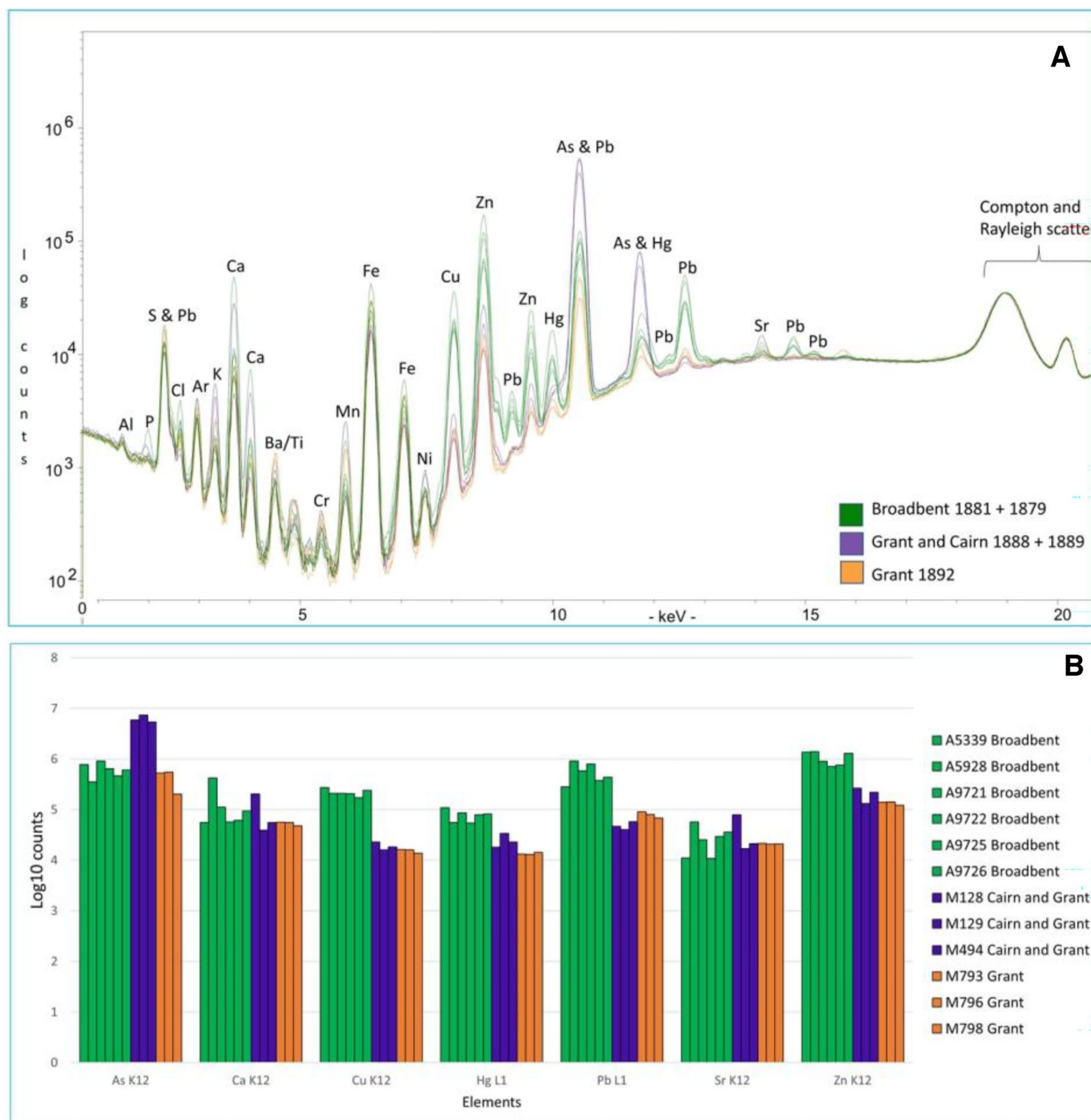

Fig. 2 **A** Comparison of co-added XRF spectra (log scaled) collected from twelve pademelons (*Thylogale*) showed that skins collected by each of: Broadbent, Grant, and Grant with Cairn formed distinct elemental patterns with copper, zinc, mercury, arsenic and lead immediately recognised as significant bulk elements. **B** Bar graph of significant bulk elements, derived from Compton normalised accumulated XRF spectra, showing that each field collector repeatedly used similar mixture on numerous specimens.

was exogenous and artificially applied with Hg. These three distinct sources of variation (in PC1, PC2 and PC3) were interpreted as residues from three different applications of chemicals. These applications may have all occurred at the time of field collection, or be a combination of field collection preservatives and pesticide treatments since the acquisition of the animal from the wild.

Grant working with Cairn and alone

The initial PCA that compared XRF data showed that pademelons acquired by Grant working with Cairn in both 1888 and 1889 expeditions had a higher relative concentration of As than those collected by Broadbent, and those collected by Grant alone (Fig. 3A). Additionally, a correlation between the As Kα peak (at 10.54 keV) and a Lα peak for thallium (Tl) (at 10.27 keV) was

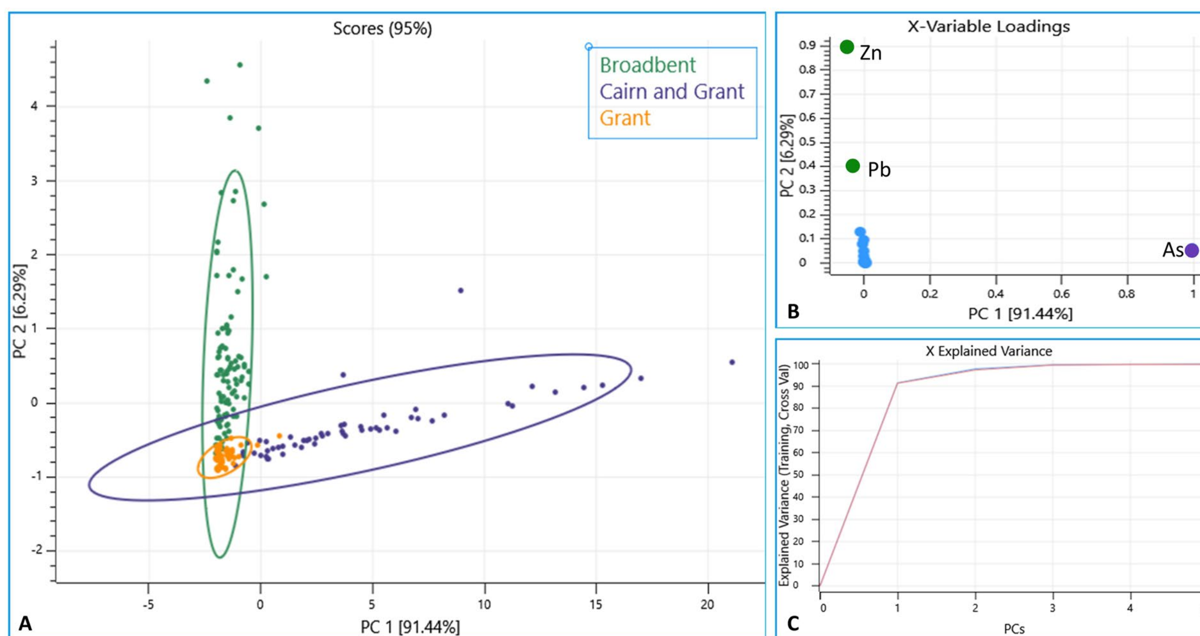

Fig. 3 Principal component analysis overview **A** PC1 versus PC2 scores plot shows clear differentiation between specimens collected by Grant, Grant with Cairn, from those collected by Broadbent. **B** The variable loadings plot shows the elements Zn and Pb describe Broadbent's specimens long PC2, while a high relative concentration of As describes specimens collected by Grant with Cairn along PC1. **C** The X-Explained Variance plot shows that two PCs explain over 97% of the XRF data for both calibration and cross-validation sets

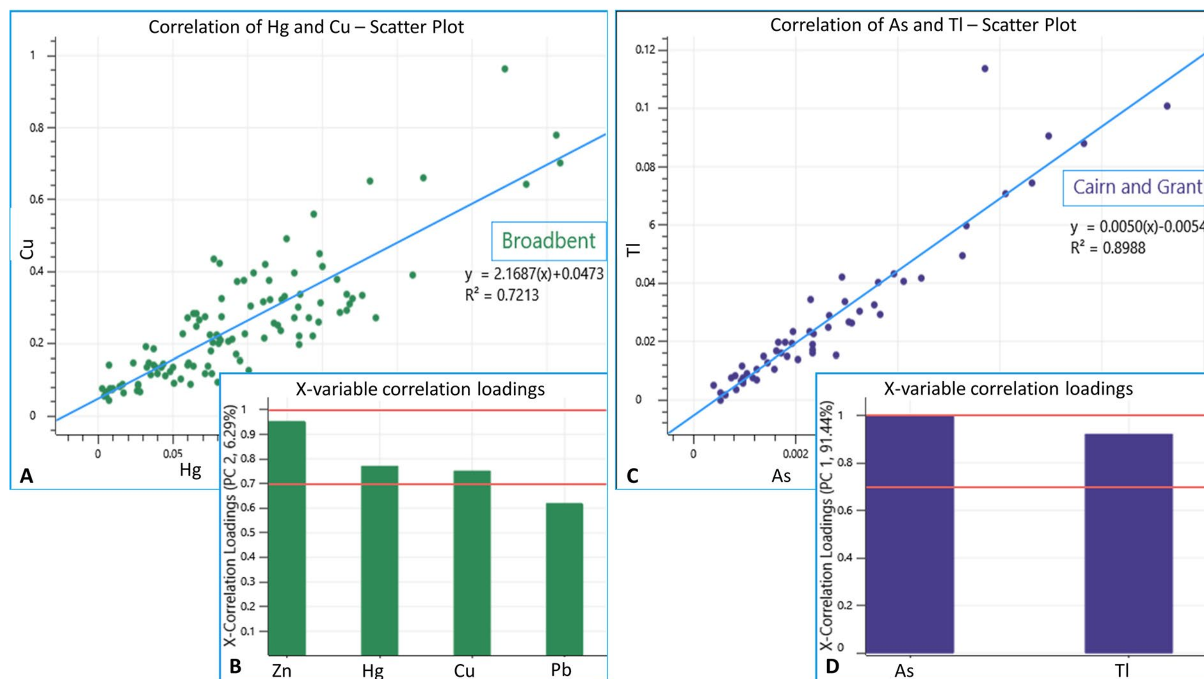

Fig. 4 Correlation loadings plot for PC2 showed that Zn, Hg, and Cu are correlated in specimens acquired by Broadbent (**B**), this is supported by Pearson's correlations (**A**). Correlation loadings plot for PC1 showed a correlation between As and Tl in specimens acquired by Grant with Cairn (**D**) (supported by Pearson's correlations **C**)

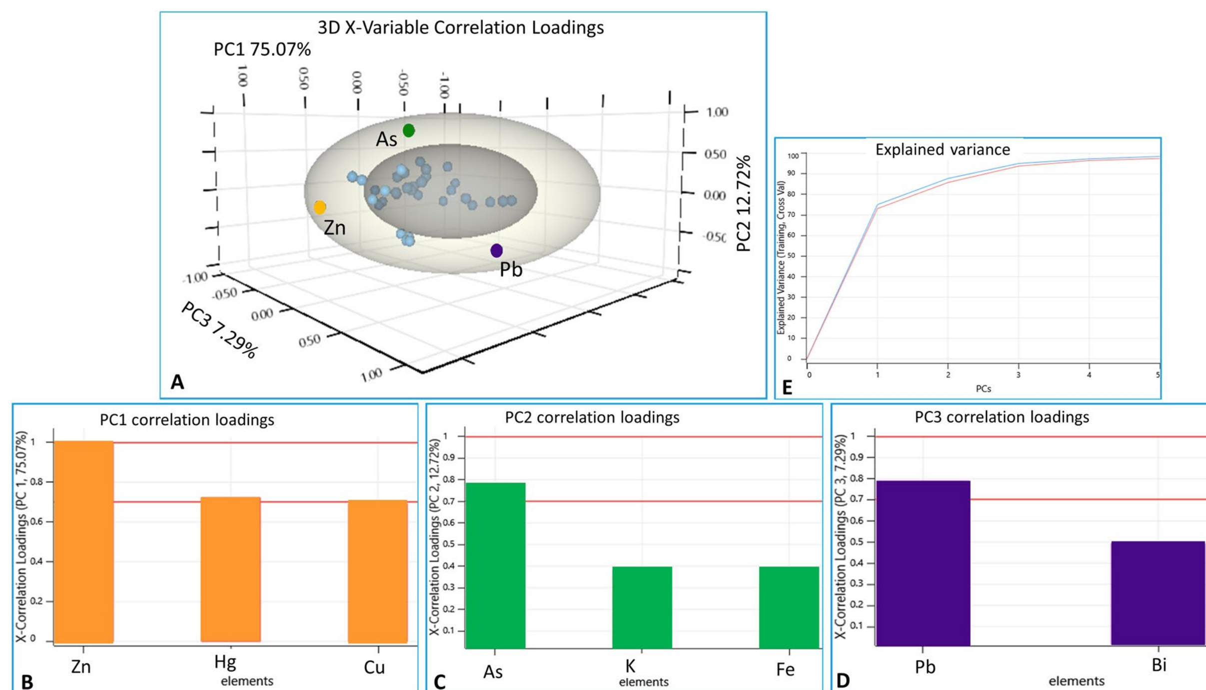

Fig. 5 PCA 3D correlation loadings plot showed three distinct groups of variation: **A** described by PC1, PC2, and PC3 (**B**-**D**) in the Broadbent specimens. (**E**) shows explained variance of the PCA of only the Broadbent specimens

identified in the Bayesian deconvoluted data for specimens collected by Grant working with Cairn ($R^2=0.89$) (Fig. 4C, D). Re-examination of the raw XRF data showed that Tl Lβ peaks did not correspond with the Tl Lα peaks in these samples indicating that Tl was not, in fact, present. Rather, a spectral overlap had been created around 10.3 keV by the very high As concentration in these specimens. Overlaps of elemental peaks occur in XRF spectroscopy in the presence of high concentrations of particular elements, causing misinterpretation during software run deconvolutions of the XRF data. This highlights that full reliance on software generated results, without human intervention to cross-check unexpected results, can lead to significant misinterpretations of complex data sets in chemical analysis [43, 92]. A cross-check to determine whether all of the required Tl peaks were present within the original data set, showed that the presence of Tl in the software generated assignment of elemental peaks as an artefact and hence, was removed in the interpretation of elemental differences.

A further PCA was therefore conducted to investigate the differences between pademelons acquired during Grant's expedition to the Bellinger River in 1892 and those from the two expeditions he undertook with Cairn. Variation between these two groups was explained by a single principal component (Fig. 6C). PC1 (99.82%) exclusively described variations in specimens collected by Grant with Cairn and was strongly dominated by As (Fig. 6A, B). Pademelons collected by Grant working alone in 1892 (Fig. 6A, B) were described by PC2 (0.08% of the variation). While Grant did use As in the preservation of *T. thetis* in 1892, the significantly higher concentration of As (95% confidence) in the *T. stigmatica* specimens collected by Grant and Cairn formed the greatest difference between the two groups. It was noted that this result was confounded with species variation therefore, a final analysis was undertaken to determine whether species independent information could be obtained.

When As was removed from this final analysis, more information was gained from the comparison of these two groups. Five of the six specimens analysed clustered mainly along PC1 (41.85% of variance) showing that aside from differences in As concentration, the elemental characteristics of the preserved specimens were similar (Fig. 7A, D, E). However, this was not the case for M.128, (collected by Grant and Cairn) in which relatively high concentrations of Ca and Sr were detected. This was described by PC2 with 25.3% of variance (Fig. 7A, B, C).

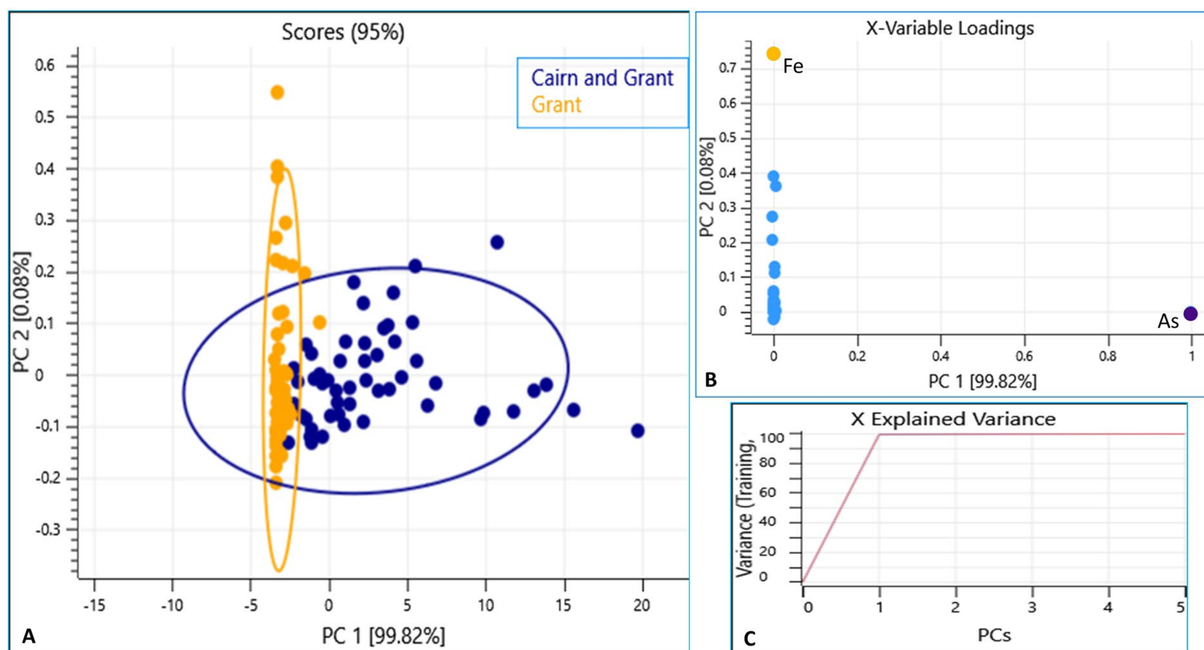

Fig. 6 PC1 versus PC 2 scores plot (**A**) showed specimens collected by Grant compared with those acquired by Grant when working with Cairn. **B** Specimens collected by Grant and Cairn were described predominantly by As (PC1) while specimens acquired by Grant working alone were mainly described by Fe (PC2). The X-Explained Variance plot (**C**) showed that a single principal component, PC1, explained the majority of differences in elemental content between the groups

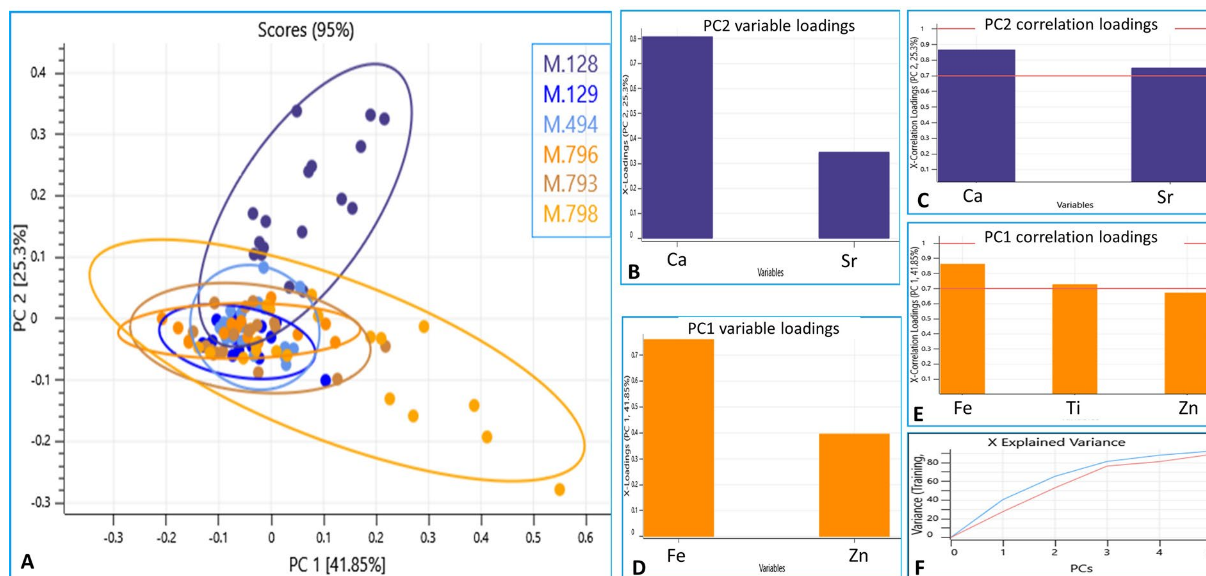

Fig. 7 When As was removed from the PCA, little difference was found between specimens collected by Grant with Cairn and those collected by Grant working alone in the PC1 versus PC2 scores plot (**A**). PC1 was dominated by Fe (**D**, **E**) and describes five of the six specimens. PC2 described only M.128 with Ca and Sr being important elements (**B**, **C**). Explained variance plot (**F**)

Discussion

Analysis of the XRF spectroscopic peak area data collected from the specimens investigated in this study revealed that each of the nineteenth century naturalists repeatedly used the same preservative chemicals, in similar proportions, over multiple expeditions, and over time. These patterns of practice were sufficiently different from each other, that groups of specimens acquired from the

field by each naturalist were differentiated (Fig. 3). In all specimens studied, neither the application of pesticides at unknown intervals in the history of the specimen, nor the contribution of elements from endogenous sources, such as Ca, Cu, Fe and Zn, confound the capacity to differentiate between collectors.

The data acquired from specimens collected by Broadbent showed that he used a similar mixture of chemicals as skin preservatives in Tasmania's cool climate (1879) and Queensland's hot tropical climate (1881) though the expeditions were in distinctly different climatic regions and with different access to local chemical supplies (Fig. 6). PCA of correlations within the XRF data provided strong evidence that the Broadbent specimens were chemically treated three times, once with lead, once with arsenic, and once with zinc in combination with mercury and copper (Fig. 5). These correlations suggest multiple chemical application "events" which may originate from the field-preservation process, or from preventive pesticide treatments undertaken in museums. However, the data, alone, was unable to describe a timeframe for these application events. With the information from this analysis, the archival documents were searched again and two new observations were made. Correspondence between Broadbent and his employer, Edward Ramsay, showed that both men went to some effort to ensure that Broadbent's preferred chemicals and materials were sent to him including "corrosive" [sublimate] (mercuric chloride) [93, 94]. Correlations (Fig. 5B) also provided evidence that copper was combined with mercuric chloride at application which indicated that these elements were ingredients in a preservative or pesticide applied to Broadbent's specimens. This was the first indication that our protocol may contribute specific information of Broadbent's field preservation practice. Additionally, the search of archival material related to Broadbent revealed a receipt for the purchase of chemicals [94] "arsenic alba", "salt tartar" (potassium carbonate), "camphor", and "rock lime", a list of ingredients matching to Ramsay's published arsenical soap recipe [95] but with quite different proportions. It was proposed that the chemical application event shown in Fig. 5C, in which arsenic and potassium are identified, may be related to the application of this arsenical soap. However, the configuration of the pXRF in this case limited detection of potassium and therefore the full relationship between arsenic and potassium may be masked – this will be investigated in a follow up study.

PCA showed that specimens collected by Grant with Cairn had a higher relative concentration of arsenic than the specimens collected by Grant working alone (Fig. 6). This may be due to the use of a different preservative mixture in the field. However, because the two *Thylogale* species in this comparison are not the same (*T. thetis* and *T. stigmatica*), there is a possibility each species may have been exposed to different pesticides in the museum. Further PCA was undertaken to resolve this potential confound. The removal of arsenic from the subsequent PCA showed a strong similarity between the elemental characteristics of the pademelons collected by Grant and those collected by Cairn and Grant together regardless of the different pademelon species represented. Thus, other than arsenic, the preservatives applied by Grant were very similar whether working with Cairn or alone.

At the same time, the pademelon juvenile specimen M.128, while visually similar to the other dry study skins attributed to Cairn and Grant's 1888 Bellenden Ker expedition, was revealed to be unique. Elevated levels of Ca and Sr detected in M.128 were attributed to elemental leaching from bone and tissue, and subsequent salt redeposition onto soft tissues, which was consistent with the use of formalin in preservation [96–98]. This observation, coupled with a general report of the Bellenden Ker expedition that showed only one juvenile mammal was collected [88], led to the conclusion that M.128 was initially wet preserved in 'spirits'. Although M.128 had a different history or preservative application, it exhibited the same high arsenic that was characteristic of other specimens collected by Grant and Cairn. This indicated that high concentration arsenic was applied to M.128 after it was removed from spirits, prepared as a dry specimen, and stored with the other *T. stigmatica* collected by Cairn and Grant. It was concluded the variation observed between specimens collected by Grant working alone and those collected by Cairn and Grant (which was characterised by very high As) was the result of pesticides applied at the museum and not a different preservative applied in the field. Thus, Grant's preservation practice did not change significantly when working with Cairn. This shows that the developed methodology when applied in this case study, can discriminate between specimens collected by different collectors *and* can clarify field-collection preservation practices over an individual collector's career. Equally important is the ability to determine the nature of variations in the sequence of preservation as a subset of the general pattern of preservation practice.

Study limitations

Three limitations should be considered when interpreting this study in the context of zoological museum collections. Firstly, a small number of specimens and field collectors were used which inherently limits the potential to apply the observations made here to other field collectors or global collection practices. Secondly, the instrument settings used in the collection of XRF data were set to optimise collection of fluorescence from elements with higher weights than manganese, therefore, reducing the

sensitivity to detection of light weight elements. Finally, the correlation analysis used for the Broadbent specimens in this study cannot provide the order of sequence for different preservative applications.

Conclusions

As specimen collecting decreases through climate change, and social, moral, and legal constraints on field collecting from the wild, museum zoological specimens are recognised as increasingly important, non-renewable resources [59, 99]. Hand in hand with this increasing importance, is a need to return museum specimens with limited use due to lack of contextual data, to the museum's collection of research ready material. To achieve this without physically sampling, non-destructive methods to establish provenance and object histories must be found. Based on the results presented in this proof-of-concept study, a protocol for applying pXRF and PCA has been designed and verified, which has several exciting potential applications for museum zoological collections.

By applying pXRF spectroscopy to twelve *Thylogale* study skins from the Australian Museum, and analysing all elements simultaneously via PCA, it has been demonstrated that the nineteenth century field collectors in this study, followed a consistent pattern of practice in specimen preparation, *and* that the specimens acquired by different field collectors were discriminated from one another. The capacity to recognise specimens as part of a group, and identify those that are unique, demonstrated the potential for this protocol to be applied to reunite unlabelled or mislabelled specimens with their legacy data, and to authenticate the provenance of culturally significant specimens, and other skin-based objects.

Using correlations within the data, this study has shown that separate applications of preservatives and pesticides, or contaminates, were distinguished as unique 'events' in the "afterlife" [100] of a specimen. Knowledge acquired through the application of this protocol, was used to identify specific animals within generalised reports, and to recognise the significant documents within the archives. Furthermore, the capacity of the protocol to identify an individual skin that was once "wet" preserved in formalin from within a group of similar dry study skins was demonstrated. Thus, illustrating the value of this type of study to bridge gaps in the archival records of museum specimens, and a potential for application in determining the recipes applied to zoological specimens both in the field and in the museum.

This major advance in the application of pXRF spectroscopy in combination with PCA to differentiate preservation methods and materials used in the fieldwork of nineteenth century Australian naturalists opens new doors for augmenting geographical and temporal specimen data. This, in turn, broadens opportunities for investigating potentially important natural history specimens (e.g., extinct or locally extinct species) as well as enhancing the use of museum specimens in research including environmental, medical, and historical studies. The future application of this protocol may shine light on the often undocumented, methods and materials used in the preparation of zoological skins in the nineteenth century.

Abbreviations

As	Arsenic
Ca	Calcium
Cu	Copper
Fe	Iron
Hg	Mercury
PCA	Principal component analysis
PC	Principal component
Pb	Lead
pXRF	Portable X-ray fluorescence
Sr	Strontium
Ti	Titanium
Tl	Thallium
XRF	X-ray fluorescence
Zn	Zinc

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Author contributions

PXRF in-situ analysis was undertaken by CC. PCA of XRF peak area data was undertaken by CC and BS and interpreted by CC. JP and EC contributed to the conception of this work. JP facilitated access to museum specimens at the Australian Museum. EC, PL and JP provided academic supervision. CC wrote the first draft of the manuscript, all authors read, provided input to subsequent drafts and approved the final manuscript.

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Availability of data and materials

The data set created and analysed during this study is available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests. Brad Swabrick owns the software used for the PCA, Vektor Direktor v1.1 and thus has a commercial interest in this study.

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