DART mass spectrometry as a potential tool for the differentiation of captive-bred and wild lion bones

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Abstract
In recent years lion bones have been legally traded internationally to Asian markets from captive-bred sources in South Africa. There are also indications of increasing instances of illegal international trade in wild lion bones. The existence of parallel captive and wild supplies of lion bone are a cause of law enforcement concern regarding the potential for the laundering of illegally sourced bones through legal trade, and present a problem for the assessment of the conservation impact of wild lion bone trade due to the difficulty of determining what market-share wild and captive-bred lion bones account for. Captive-bred and wild lion bone are visually indistinguishable and no reliable method currently exists for distinguishing them. We present a preliminary study that explores the use of DART mass spectrometry as a method to differentiate between captive-bred and wild lion bones. We find that DART is able to differentiate between a batch of captive-bred South African lion bone and a batch of wild lion bone and suggest that DART mass spectrometry shows strong potential as a tool for the regulation and investigation of lion bone trade. Further testing is needed to prove the suitability of this technique. Therefore, we suggest that further research focuses on testing the capability of DART to differentiate between contemporary wild and captive-bred lion bone originating from South Africa, and attempts to identify chemical markers in bone that can be used as indicators of captive-bred origin.

Keywords Conservation · Wildlife trade · IWT · Regulation · Law enforcement · Forensics · Panthera leo
Introduction

Wildlife trade is a multi-billion dollar industry that encompasses a great variety of organisms across a broad range of uses, and occurs in both legal and illegal guises (Broad et al. 2014). The overexploitation of wildlife for trade purposes can have severe impacts on species conservation (Hemley 1994) and the unregulated, illegal trade in wildlife is believed to be amongst the largest illegal industries in the world with significant links to corruption, violence, subversion of development and social stability, and the spread of zoonotic diseases of global health concern (Zimmerman 2003; Warchol 2004; Macdonald and Laurenson 2006; Rosen and Smith 2010; UN 2018; Gore et al. 2019; Volpato et al. 2020).

Sustainable wildlife trade, typically from farmed or ranched sources, has been suggested as a means to reduce the prevalence and negative impacts of illegal wildlife trade—through the reduction of financial incentives for illegal trade, whilst maintaining positive livelihood contributions of legal trade (Bulte and Damania 2005; Damania and Bulte 2007). However, the simultaneous existence of both legal and illegal supplies of wildlife products has led to concern regarding the potential for laundering of products from illegal sources into legal markets and thus negatively impacting wild populations (Fischer 2004; Lyons and Natusch 2011; Jimenez-Bustamante and Rentería 2018). In addition to conservation-based concerns there are also wider societal concerns regarding the facilitation of transnational organised crime by wildlife markets (Warchol 2004; van Uhm 2018). Supply-side approaches to reduction of wildlife exploitation thus require means to verify authenticity and thereby reduce possibilities for criminal activity (i.e. smuggling and laundering) in order to be successful (Damania and Bulte 2007). The trade in lion (Panthera leo) bones and body parts exemplifies such concerns.

In the wild, lions are classified as vulnerable, having disappeared from ~92% of their historic range and declined by ~43% during the two decades between 1993 and 2014, leaving an estimated 23,000–39,000 individuals living in the wild (Bauer et al. 2016). In addition to established threats (see Bauer et al. 2015) the illegal trade in wild lion parts is believed to be increasing and concerns have been raised that associated poaching may become a threat to wild lion populations (Williams et al. 2017a; Everatt et al. 2019). Increasingly, instances of seizures of illegal lion products linked to intercontinental trade are reported, especially to East and Southeast Asia (e.g. EAGLE 2017, 2018; Everatt et al. 2019).

In South Africa there are more than 300 registered facilities for breeding and rearing at least 7800 lions, as indicated on Threatened or Protected Species (ToPS) permits for South Africa, a number which excludes keeping-only facilities—the inclusion of which is likely to increase the estimated number of facilities to over 400, however absolute numbers are not known (Williams et al. 2015; Williams and ‘t Sas-Rolfes 2019). The international commercial trade in the skeletons (bones, teeth, and claws) of these captive-bred lions is permitted from South Africa under CITES Appendix II. Although in August 2019 export was placed on-hold following a domestic law judgement whereby export quotas for lion skeletons set in 2017 and 2018 were deemed to have been unlawful and unconstitutional due to an insufficient consideration of animal welfare (Republic of South Africa 2019). Nevertheless, at present there still remains the potential for future legal trade in lion skeletons. Commercial international trade in the bones and skeletal parts of wild lions is not permitted by CITES (CITES 2016). In total, over 6000 skeletons have been legally permitted for export from South Africa to Southeast Asia since
2008, where they are believed to be used in traditional Asian medicine, health tonics, and ornaments (Williams et al. 2015).

A number of conservation, political, and socio-economic concerns have been raised surrounding the trade in lion bones and skeletons, including the influence of transnational crime (Coals et al. 2019). Organised crime groups have been linked to the legal trade in lion bones from South Africa (Williams et al. 2015) and illegal sales of lion bone and attempts to smuggle lion bones and bone products out of South Africa have been reported (e.g. Outhwaite 2018; De Telegraaf 2019). Such reports feed concern regarding the potential for laundering wild-origin lion bone through captive-bred lion bone trade both in South Africa and further down the supply chain in Asia (EIA 2017; EMS 2018).

Captive-bred and wild lion bones are indistinguishable to the naked-eye and thus present a challenge to enforcement authorities regarding the detection and prevention of laundering. In recent years South African exports of lion bone have been subject to DNA-profiling tests and physical inspections at various stages of the export process from farm to airport as required under a quota implementation protocol (DEA 2017). The purpose of these tests are primarily to detect laundering of the bones of other felid species, specifically tiger (*Panthera tigris*) (Dalton et al. 2018), and to ensure that multiple lion skeletons are not declared as one for export (Williams et al. 2021) but not whether skeletons initially sampled on farms came from captive-bred or wild sources. Genetic techniques can prove useful and effective for the detection of laundering of wild products into captive-bred trade (Ogden et al. 2009; Hogg et al. 2018). However, no genetic test can specifically distinguish captive-bred from wild lions (Miller et al. 2014).

Chemical elemental techniques, predominantly stable isotope and X-ray fluorescence analyses, have been used to differentiate between captive-bred or wild specimens of wildlife (Hinsley et al. 2016; Sugiyama et al. 2018; Brandis et al. 2018; He et al. 2018) but recent attempts to differentiate captive-bred and wild lion hair through carbon and nitrogen stable isotope analyses have not been conclusive (Hutchinson and Roberts 2020). However, advances in technology have significantly increased the speed and convenience of multi-compound mass spectrometry analyses through use of Direct Analysis in Real Time (DART) mass spectrometry (Cody et al. 2005a, b). DART is an ambient atmospheric ion source which produces analyte ions by reaction of ionised components of the air with the sample which may be solid, liquid, or gas. Analyte ions then pass into a mass spectrometer; the spectra produced are suitable for determination of multiple analytes and assigning tentative chemical formulas to mass peaks (Kim et al. 2010; Smoluch et al. 2016). DART mass spectrometry has proven effective for the identification of illegal substances, notably explosives and drugs (e.g. Nilles et al. 2010; Grange and Sovocool 2011), and is particularly useful in forensic and security applications where the generation of results are time-sensitive (Pavlovich et al. 2018). DART is increasingly employed in law enforcement, however, usage is not yet prevalent in wildlife forensics and wildlife crime detection. Nevertheless, it has been used to identify and differentiate between timber species in trade (Cody et al. 2012; Lancaster et al. 2012; Espinoza et al. 2014) and to identify rhinoceros horn (Price et al. 2018).

Bone incorporates metabolised chemical inputs accumulated throughout an animal’s life (Meier-Augenstein 2017). Therefore, the chemical composition of bones can be used to determine animals’ captive or wild provenance based on differences in chemical input (e.g. Kays and Feranec 2011). It would be expected that observable differences between captive and wild lion bones might be due primarily to diet, but could also include other inputs such as veterinary drugs and environmental chemicals such as pesticides. Wild lions have a varying diet, predominantly comprised of medium to large sized ungulates, with specific prey...
species eaten depending on the regional ungulate composition (Hayward and Kerley 2005; Davidson et al. 2013). Commercially captive-bred lions in South Africa are typically fed livestock; often mortalities from cattle farms, or other domestic animals such as chickens and donkeys, often supplemented with ‘predator powder’ vitamin and mineral additives (www.vtech.co.za) (pers. obs.). Commercial lion breeding and keeping facilities are often associated with agricultural and livestock-producing land, particularly in the Free State Province where the majority of commercial lion captive-breeding facilities are located (pers. obs.; Williams and ‘t Sas-Rolfes 2019). Although the majority of captive-bred lions’ food is livestock they may also be fed commercially raised wild ungulate species—which form a large part of South Africa’s commercial game meat and game hunting industries (pers. obs.). Nevertheless, in general it is assumed that predators raised in captivity tend to have more restricted diets than their wild counterparts (e.g. Kays and Feranec 2011).

We conducted a preliminary study to test whether DART mass spectrometry could potentially be used to differentiate between a batch of captive-bred and a batch of wild sourced lion bones. We expected differences in the chemical composition of lion bone batches to be identifiable with the multiple-compound spectra approach of DART mass spectrometry. We also intended to tentatively identify diagnostic compounds for any observable difference between captive-bred and wild lion bone.

**Method**

**Material**

One phalangeal bone was taken from each of 29 verified captive-bred lion skeletons from commercial breeding facilities in South Africa. Captive-bred bone samples were sourced from a commercial trader in captive-bred lion skeletons and were accessioned with the National Zoological Gardens of South Africa (Table 3 in Appendix 1). The trader sources material from a range of legal lion breeding and keeping facilities in South Africa. We understand that the material used in this study came from at least 8 different facilities. We therefore believe the captive-bred samples used in this study are representative of the type of material present in commercial lion skeleton exports. For this proof of concept study we used bone samples from the skulls of six historical museum specimens recorded to have been collected from wild lions in Anglo-Egyptian Sudan in the early twentieth century (Table 4 in Appendix 2). The choice of markedly different geographical regions, ages, and bone types between the captive and wild samples was thought to be appropriate for this test of concept because if differences were not evident from such seemingly different sample types then they were less likely to be distinguishable in contemporary wild lions from Southern Africa. A randomly selected captive-bred sample was withheld from analysis and was used as an ‘unknown’ sample to test validity of the classification.

**Sample preparation**

In order to present material of sufficient thinness and homogenous structure to the DART ion source each bone was drilled to a depth of 10 mm at low-medium speed (to avoid friction burning the bone) with a 3 mm drill-bit to create fine bone powder. The drill-bit was cleaned in between taking each sample through immersion in 99% ethanol and wiping with paper towel. Powders from each bone sample were individually suspended in ~10 ml of
99% ethanol which was pipette-dropped (two drops per sample) onto DART Quickstrip fine mesh grids balanced over free space so that the ethanol did not come into contact with any surface apart from the DART Quickstrip mesh. The ethanol was then allowed to evaporate from the DART Quickstrip mesh before the resulting powder, which adhered to the Quickstrip mesh, was introduced to the DART ion source using an automated rail. The 29 captive-bred samples were tested singly whilst the six wild samples were analysed in duplicate (total bones = 6; total spectra generated = 12). All samples (captive-bred and wild) were analysed at the Proteomics & Metabolomics Facility, The Roslin Institute, University of Edinburgh, Scotland, UK on the 21st of January 2020. Following calibration of the mass spectrometer, background spectra were recorded using blank DART Quickstrip mesh directly before introduction of lion bone samples. Captive-bred samples were analysed first (using 3 DART Quickstrips consecutively) directly followed by wild samples (using 1 DART Quickstrip).

DART-MS analyses were performed on a DART-SVP ion source (IonSense Inc, Sangus, MS, USA) interfaced to a micrOTOF QII Quadrupole Time-of-Flight mass spectrometer (Bruker Daltonics, Bremen, Germany). The mass spectrometer was calibrated by infusing ESI low concentration tune mix (Agilent) prior to DART analysis. The reference tune mix included, betaine, hexamethoxyphosphazene, hexakis(2,2-difluoroethoxy)phosphazene, hexakis(1H, 1H, 3H-tetrafluoroproxy)phosphazene, hexakis(1H, 1H, 5H-octafluoropentoxy)phosphazene, hexakis(1H, 1H, 7H-dodecafluorohpehtoxy)phosphazene, hexakis(1H, 1H, 9H-perfluoronomonyloxy)phosphazene, hexakis(1H, 1H, 4H-hexafluorobutylphoxy)phosphazene, hexakis(1H, 1H, 6H-decafluoroheptyloxy)phosphazene, hexakis(1H, 1H, 8H-tetradecafluorooctyloxy)phosphazene, ranging from m/z 118.0862 to 2721.8948. Additionally, on DART source, quinine ([M+H]⁺ = 325.1911) was loaded on the DART Quickstrip and spectra were collected in positive ion mode between sample data acquisition.

The optimised parameters ranges for DART were the following; helium ionization gas (99.997%) at a flow rate of 3 L/min with nitrogen (99.998%) as standby gas, DART grid voltage of 600 V, ionisation temperature of 500 °C and linear rail speed of 1 mm/min. The MS system was operated in positive-ion mode, acquiring profile MS data in a mass range of 100–1000 m/z, using oTOF control software. Collision cell energy was set at 10 eV and quadrupole energy at 5 eV with a PrePulse Ion storage of 10 µs. Data preview and integration was achieved by Compass DataAnalysis 4.2 (Bruker Daltonics, Bremen, Germany) and centroided peakslits were exported for further analysis in Mass Mountaineer.

Analysis

Analysis of DART-generated spectra was carried out using the mass spectrum elemental composition and classification software Mass Mountaineer (Mass Mountaineer Version 5.0, 2018 https://diabloanalytical.com/ms-software/mass-mountaineer/). As DART is an ambient ionisation source a background ambient spectrum was taken using DART Quickstrip mesh without bone samples. The background spectrum was subtracted from the sample spectra and the spectra were searched for common contaminants, including cleaning solutions, plastics, and products of recent decomposition (e.g. putrescine compounds), which were also removed from sample spectra. Kernel Discriminant Analysis (KDA) was used to test for separation between the sample classes of captive and wild.

KDA is an extension of Principal Component Analysis (PCA) that maps features into a higher-dimensional space using a non-linear function, and thus allows separation of points that may not be linearly separated in two-dimensions (Souza 2010). KDA is a supervised
learning technique which uses class membership of samples in the training set in order to maximise class separation (Mass Mountaineer 2018). The ions (i.e. mass peaks) selected for the classification of captive and wild bone samples by KDA had an abundance threshold of at least 10%. Classification accuracy was assessed by leave-one-out cross-validation (LOOCV) which successively omits each sample from the training set and thus compares each sample in turn against the whole of the training set (Cody et al. 2012).

Fisher’s ratios were calculated to determine the discriminating power of mass peaks (i.e. features), of over 10% abundance, for the difference between captive and wild classes. Higher Fisher’s ratios for a class relative to other classes indicate that the feature has a higher discriminating value for that class. Fisher’s ratios are calculated as follows:

$$Fisher's\ Ratio = \frac{(m_1 - m_2)^2}{v_1 + v_2}$$

where $m_1$ and $m_2$ are the means for a feature in class 1 and class 2, and $v_1$ and $v_2$ are the variances for class 1 and class 2 respectively. $m_1$ and $v_1$ represent the in-class mean and variance for the feature, and $m_2$ and $v_2$ represent the between-class mean and variance for the feature (Mass Mountaineer 2018).

Potential compound compositions were calculated for selected mass peaks with higher discriminating value (i.e. greater differences in Fisher’s ratios) using the ‘Composition’ function of Mass Mountaineer. We chose mass peaks for which the Fisher’s ratio for the captive class was approximately double-or-greater than the wild class (Table 5 in Appendix 3). Potential compound formulae generated by Mass Mountaineer were searched for in the online databases Chemspider (http://www.chemspider.com) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) and, where possible, tentative compound names were assigned.

We also searched spectra for matches to masses of commonly used veterinary tranquillizers and sedatives (Table 2) provided to us by a member of the lion bone trade industry.

**Results**

When wild samples from each individual lion, taken in duplicate, were considered as discrete classes duplicates largely overlaid each other in the KDA; indicating that the method produced replicable spectra (99.32% variance covered on 3 Principal Components and 66.67% classification accuracy using leave-one-out cross-validation LOOCV) (Fig. 1). Replicates showed close matching of spectra (Fig. 2) and major features in those spectra were replicated.

Kernel Discriminant Analysis (KDA) showed clear separation between the spectra of captive-bred and wild lion bone samples (Fig. 3) with 97.50% classification accuracy using LOOCV. Three principal components accounted for 100% of the variance. A randomly selected captive-bred spectrum was excluded from the training set and was used as an unknown; it was successfully classified with KDA (Fig. 3).

Identification of the composition of mass peaks is not required for classification. However, we tentatively attempted to identify potential compounds that may be responsible for the key diagnostic mass peaks for the difference between captive-bred and wild lion bone spectra. At a level of 10% relative abundance peaks were selected based upon their Fisher’s ratios (Table 5 in Appendix 3) and were tentatively assigned compound names (Table 1).
We found no matches within the captive-bred spectra mass peaks for the masses of commonly used veterinary tranquilizers and sedatives (Table 2).

**Discussion**

The visual similarity of captive-bred lion bone to wild lion bone creates complications for the regulation and investigation of potentially illegal lion bone trade. To date, there has been no analytical method developed for accurately determining captive-bred lion bone from wild lion bone. We therefore conducted a preliminary investigation to determine whether Direct Analysis in Real Time (DART) mass spectrometry has potential to be used as a tool to distinguish between lion bone from captive-bred and wild sources. We found that DART analysis was able to reveal clear separation between a batch of captive-bred and a batch of wild lion bone mass spectra. This finding has relevance for discussions about the lion bone trade as the indistinguishability of captive from wild sources has been suggested to present a significant barrier to regulating lion bone trade and generating accurate intelligence concerning captive-bred or wild origins of lion products in the global market. We suggest that, with suitable techniques and resources, captive-bred and wild lion bones could be chemically distinguishable.

A well-acknowledged (but not sole) condition for farmed sources of wildlife to be considered non-detrimental is that its system of trade should not be vulnerable to the laundering of wild products (Dutton et al. 2013). In order to prevent laundering attempts, physical inspections, including verified ‘chain of custody’ for skeletons from farm to airport, have been undertaken for lion skeletons exported from South Africa as part of the 2017 and
Fig. 2  Wild lion bone spectra duplicate matching (spectra = blue, top; duplicate = red, below). a Wild 1; b Wild 2; c Wild 3; d Wild 4; e Wild 5; f Wild 6
2018 export quotas (Williams et al. 2021). Although such measures largely prohibit any substitutions of skeletons after the first instance of inspection they do not provide surety of the captive-bred origin of skeletons (Williams et al. 2021). Our preliminary results, and others (e.g. Hutchinson and Roberts 2020), indicate that chemical methods, such as DART mass spectrometry, could provide such a measure. In addition, the relative contributions of wild and captive-bred lion parts in seizures and global end-markets are unknown and concerns have been raised that a conservation-significant contribution to overall trade may be made from wild animals (EIA 2017; Everatt et al. 2019). DART analysis shows potential to be able to provide information about the captive-bred origins of such lion products in illegal trade markets and seizures.

As a proof of concept this study used commercially captive-bred lion bone from South Africa and museum samples of wild-collected lion bone from Anglo-Egyptian Sudan (historical) in the early twentieth century. We were certain of the wild and captive origins of all samples in the study. Although DART analysis revealed clear separation between wild and captive-bred samples we cannot be certain that this separation is solely due to the wild or captive origin of the lions as additional variables could influence the result. Amongst these is the geographical area from which the samples originated. Indeed, bone, and other tissues, have been used to geo-locate biological samples through geographically variable chemical composition (although differences used in this way are often O and H isotope ratios e.g. Juarez 2008; Sugiyama et al. 2018). We searched spectra for, and removed, peaks generated by common contaminant compounds, it is thus unlikely that treatments such as cleaning solutions or plastic packaging lead to the observed differences. An additional, not-inconsiderable factor is the age of the bone sample and subsequent state of decomposition which may have influenced the observable difference between the two sample types. Although we treated common products of recent decomposition as contaminants and

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**Fig. 3** Kernel Discriminant Analysis for separation between captive-bred and wild lion bone. Captive \( n = 28 \); Wild \( n = 12 \) (6 samples in duplicate). 100% variance covered on 3 Principal Components; 97.50% classification accuracy using LOOCV. ‘Unknown’ captive-bred sample (black) correctly grouped with other captive-bred samples.
**Table 1** Tentative assignments of mass peaks for differentiation of captive-bred lion bone from wild lion bone, identified through Fisher’s ratios (Table 5 in Appendix 3)

| Mass (m/z) | Formula       | Name                                                                 |
|-----------|---------------|----------------------------------------------------------------------|
| 199.176*  | C₁₁H₂₃N₂O    | 1-Ethoxyimino-2,2,6,6-tetramethylpiperidin-1-ium                      |
| 257.267   | C₁₅H₃₃N₂O    | 2-(Decanoylamino)-N,N,N-trimethylethanaminium                       |
| 279.237   | C₁₅H₃₁O₂     | Linoleate                                                            |
| 280.258   | C₁₈H₃₄NO     | 2-(Adamantan-2-ylxyo)-N,N,N-triethylethanaminium                   |
| 281.254*  | C₁₇H₃₃N₂O    | 1-[(Decyloxy)methyl]-2-ethyl-3-methyl-1H-imidazol-3-ium              |
| 283.286   | C₁₈H₃₅      | 3-4-[[2-[Ethyl(dimethyl)ammonio]ethyl](dimethyl)ammonio]butyl]-1-methyl-1H-imidazol-3-ium |
| 299.260*  | C₁₅H₃₅O₃     | 3-Hydroxyoctadecanoate                                              |
| 399.335*  | C₂₀H₄₃N₂O    | 3-Hexadecyl-1-(2-oxo-propyl)-3H-benzoimidazol-1-ium                  |
| 519.138   | C₂₄H₄₀N₆O₈  | 1-Benzyl-4-[(E)-2-hydroxy-2-(4-methoxyphenyl)-1-(2,4,6-trinitrophenyl)vinyl]-1H-1,2,4-triazol-4-i um              |
| 523.472   | C₂₅H₄₀N₆O   | 3-(12-Hydroxydocyl)-1-[13-(3-pyridinyl)tridecyl]pyridinium           |
| 536.166   | C₂₂H₂₂N₄O₅  | 1-Methyl-4-[4-(2-nitro-4-[6-nitro-4-quinolinyl]amino]benzoyl]amino]phenyl]amino]pyridinium |  |
| 551.502   | C₂₅H₃₆N₂O   | 3-(14-Hydroxytetradecyl)-1-[13-(3-pyridinyl)tridecyl]pyridinium       |
| 552.503   | C₂₅H₄₂N₂O   | 3-(13-Aminotridecyl)-1-[13-(1-oxido-3-pyridinyl)tridecyl]pyridinium  |
| 565.515   | C₂₈H₄₅N₂O   | 3-(14-Hydroxytetradecyl)-1-[14-(3-pyridinyl)tetradecyl]pyridinium     |
| 580.536   |               | Unidentified compound                                                 |
| 684.203*  |               | Unidentified compound                                                 |

Potential elemental compositions were limited to Mass Mountaineer default settings for calculating possible formulae of organic compounds: C (0 to 50), H (0 to 100), O (0 to 10), and N (0 to 10).

*Absent (0 abundance) in captive-bred bone
removed them from analysis we cannot be certain of the influence of longer-term decomposition on captive-bred-wild sample differences.

The peaks identified from their Fisher’s ratios as diagnostic for the separation between captive-bred and wild lion bone were large, complex compounds (Table 1). Of those tentatively assigned compounds several, containing imidazolium, pyridinium, and triazolium (Table 1), were of interest due to the use of imidazole, pyridine, and triazole derivatives in pharmaceutical drugs (including antibiotics and tranquilizers), and pesticides (e.g. Borgers 1980; Ross et al. 1980; Shimizu et al. 2000; Kharb et al. 2011). However, such derivatives are also naturally occurring biological molecules (Shimizu et al. 2000). In addition, a number of diagnostic compounds were found to occur in wild but not captive bones (Table 1). Therefore, we do not think it likely that man-made chemical additives are the main reason for the differing chemical compositions of captive-bred and wild lion bones. Nevertheless, we expected the different diets of captive-bred and wild lions to have an influence on body tissue chemical composition, and dietary studies across a range of organisms demonstrate the use of body tissue chemical composition to reflect dietary differences (e.g. Ambrose 1991; Cerling and Harris 1999; Katzenberg 2008). Our preliminary results suggest that amongst selected compounds with highly different Fisher’s ratios there are absences in captive-bred lion bone that were found in wild lion bone. We cannot explain these differences. However, it is possible that the more restricted diet of captive-bred lions had an effect. Future work could beneficially focus on detailed examination of dietary specifics in the differentiation of wild and captive-bred origin lion bone.

The development and use of chemical profiling analyses in the monitoring of CITES-associated wildlife trade generally lags behind DNA-based techniques (Ogden and Mailley 2016). Nevertheless, we suggest that with further development DART mass spectrometry is likely to prove a useful tool in the regulation and investigation of lion bone trade. Although there is a long way to go before the use of DART analysis as a forensic method for lion bone trade, this study demonstrated that DART analysis can differentiate between a batch of captive-bred South African lion bones and bones of separate, wild origin. The development of robust forensic methods is difficult and often lengthy (Ogden and Mailley 2016) but for now DART analysis appears to be able to provide useful intelligence and research information for the lion bone trade. Future implementation of DART analysis into export regulation protocol as a screening method could be used to identify skeletons with chemical compositions that may be worthy of further investigation and verification of captive-bred origin. We searched captive-bred spectra for mass peaks of commonly used tranquilizers and veterinary sedatives (Table 2), we found no matches. This is unsurprising as we did not consider the metabolic products of these veterinary chemicals in detail, which can be complex and poorly understood (Tranquilli et al. 2013). Mass spectrometry is a key technique in the identification of drug metabolites (Zhu et al. 2011) and DART has been
successfully used in the identification of trace amounts of drug metabolites (Sisco et al. 2016). However, our primary research objective was not to identify specific chemical markers, rather to broadly differentiate between captive-bred and wild lion bone spectra. The identification of specific chemical markers in captive-bred lion bone, of which we believe veterinary drugs are likely candidates, would greatly aid in the detection of captive-bred lion bone. In addition, the mandatory introduction of suitable markers by lion breeders and keepers should also be considered. We thus suggest that refining knowledge of chemical markers in captive-bred lion bone is an important area upon which future research effort could focus.

DART analysis provides significant operational benefit over other chemical techniques (such as stable isotope analysis) due to the speed with which mass spectra can be generated (in this study a DART Quickstrip of 12 samples was analysed in approximately two minutes). Time was added to our method by the powdering of bone. Powdering was deemed necessary to present a more homogenous sample than introduction of a whole bone piece; which is likely to be prone to high levels of variation in thickness and surface composition. Preparation times of powdered bone samples were approximately 10 min per sample, including waiting time for the evaporation of ethanol from the DART Quickstrip mesh. Even with the added sample preparation time, DART is a suitable method for rapid result generation from lion bone. In addition, further attention is being given to the development of detection and analysis equipment that can be used for ‘in-the-field’ (i.e. decentralised) wildlife trade investigations (Morrison et al. 2018; Masters et al. 2019). The development of robust, portable DART technology (e.g. Wells et al. 2008) further increases the potential utility of such an approach to support lion bone trade investigations, especially in key locations of concern for the lion bone trade which may lie in regions of weaker laboratory capacity for the investigation of wildlife crime (Williams et al. 2017b; Ogden and Mailley 2016).

**Conclusion**

In response to a lack of analytical techniques to differentiate between captive-bred and wild sources of lion bone in trade we tested the potential of Direct Analysis in Real Time (DART) mass spectrometry to differentiate between captive-bred and wild lion bone samples. We found that DART analysis was able to differentiate between two batches of visually indistinguishable lion bone powders; one from contemporary South African captive-bred lion bones and the other from historical Sudanese wild lion bones. We suggest that DART analysis shows good potential as an emerging tool for the investigation of the origin of lion bones in trade. At present the future of the legal trade in lion skeletons from South Africa is uncertain. However, regardless of the future legality of captive-bred lion bone trade, we anticipate a continued requirement for effective methods of captive-bred and wild differentiation; either in the regulation of legal trade or investigation of illegal trade.
**Appendix 1**

See Table 3.

Table 3  Accession numbers of 29 captive-bred lion bone samples at the National Zoological Gardens of South Africa used for DART analysis

| Accession numbers for captive-bred lion bones |
|-----------------------------------------------|
| BN1052                                        |
| BN1046                                        |
| BN1034                                        |
| BN1042                                        |
| BN1496                                        |
| BN1035                                        |
| BN1045                                        |
| BN1485                                        |
| BN1051                                        |
| BN1047                                        |
| BN1037                                        |
| BN1036                                        |
| BN1044                                        |
| BN1038                                        |
| BN1033                                        |
| BN1032                                        |
| BN1054                                        |
| BN1041                                        |
| BN1039                                        |
| BN1040                                        |
| BN1048                                        |
| BN1490                                        |
| BN1492                                        |
| BN1050                                        |
| BN1043                                        |
| BN1494                                        |
| BN1488                                        |
| BN1049                                        |
| BN1053                                        |
## Appendix 2

See Table 4.

### Table 4  Oxford University Museum of Natural History historic wild lion bone samples used for DART analysis

| Object number | Taxon      | Object Name | Locality       | Precise location       | Date collected | Sex     | Physical description                        | DART sample number |
|---------------|------------|-------------|----------------|------------------------|----------------|---------|--------------------------------------------|--------------------|
| ZC-14182      | Panthera leo | Skull       | Sudan          | Anglo-Egyptian Sudan    | 1902–1912      | Unknown | Skull with right ramus of jaw              | 2                  |
| ZC-14185      | Panthera leo | Skull       | Sudan          | Anglo-Egyptian Sudan    | 1902–1912      | Unknown | Skull with jaw                              | 5                  |
| ZC-14186      | Panthera leo | Skull       | Sudan          | Anglo-Egyptian Sudan    | 1902–1912      | Unknown | Skull with jaw                              | 1                  |
| ZC-14188      | Panthera leo | Skull       | Sudan          | Anglo-Egyptian Sudan    | 1902–1912      | Unknown | Skull with jaw                              | 3                  |
| ZC-14189      | Panthera leo | Skull       | Sudan          | Anglo-Egyptian Sudan    | 07/05/1905     | Female  | Skull with jaw                              | 6                  |
| ZC-14190      | Panthera leo | Skull       | Sudan          | Anglo-Egyptian Sudan    | 05/05/1905     | Female  | Skull with jaw                              | 4                  |
Appendix 3

See Table 5.

Table 5  Fisher’s ratios for difference between captive–bred and wild lion bone samples calculated at 10% abundance threshold

| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 0           | WILD       | 494.667523     | 15,523    | 5,740,816 |
| 1           | CAPTIVE    | 935.818658     | 0         | 0        |
| 224.0738068 | –          | –              |           |          |
| 0           | WILD       | 100.364687     | 0         | 0        |
| 1           | CAPTIVE    | 83.481721      | 13,741.1765 | 9,511,833.91 |
| 245.2312012 | –          | –              |           |          |
| 0           | WILD       | 248.70471      | 12,771.4286 | 7,239,183.673 |
| 1           | CAPTIVE    | 172.528972     | 12,771.4286 | 7,239,183.673 |
| 256.2683105 | –          | –              |           |          |
| 0           | WILD       | 7.428018       | 51,539    | 86,436,053.5 |
| 1           | CAPTIVE    | 6.036091       | 39,427.3793 | 200,285,829.9 |
| 257.26651   | –          | –              |           |          |
| 0           | WILD       | 7.018523       | 11,085.7143 | 629,795.9184 |
| 1           | CAPTIVE    | 19.976187      | 11,085.7143 | 629,795.9184 |
| 263.2414856 | –          | –              |           |          |
| 0           | WILD       | 39.045456      | 16,450    | 722,500  |
| 1           | CAPTIVE    | 15.098694      | 14,071.4286 | 6,467,755.102 |
| 265.2565002 | –          | –              |           |          |
| 0           | WILD       | 4.080715       | 18,295.4545 | 57,774,979.34 |
| 1           | CAPTIVE    | 2.70345        | 18,295.4545 | 57,774,979.34 |
| 267.1901855 | –          | –              |           |          |
| 0           | WILD       | 0              | 13,127    | 0        |
| 1           | CAPTIVE    | 0              | 13,127    | 0        |
| 271.2286072 | –          | –              |           |          |
| 0           | WILD       | 1024.084797    | 17,839.5  | 6320.25  |
| 1           | CAPTIVE    | 1024.918582    | 0         | 0        |
| 277.2210083 | –          | –              |           |          |
| 0           | WILD       | 0              | 16,200    | 0        |
| 1           | CAPTIVE    | 0              | 16,200    | 0        |
| 279.1705017 | –          | –              |           |          |
| 0           | WILD       | 0              | 15,444    | 0        |
| 1           | CAPTIVE    | 0              | 15,444    | 0        |
Table 5 (continued)

| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 279.2367859 | –          | –              | –         | –        |
| 280.2583923 | WILD       | 6.385084       | 21,779.5  | 58,993,132.75 |
| 0           | CAPTIVE    | 13.73945       | 17,316.6667 | 17,251,388.89 |
| 281.2542114 | WILD       | 1.451757       | 11,133.3333 | 815,555,5556 |
| 1           | CAPTIVE    | 11.156153      | 10,900    | 0        |
| 0           | WILD       | 280.2583923    | –         | –        |
| 282.2840881 | WILD       | 2.879713       | 66,704.0833 | 579,072,355.9 |
| 1           | CAPTIVE    | 2.886329       | 81,250.8621 | 574,861,538 |
| 283.2862854 | WILD       | 0.027961       | 19,932.4545 | 53,685,652.07 |
| 1           | CAPTIVE    | 0.04513        | 20,270.3704 | 14,844,307.27 |
| 0           | WILD       | 281.2542114    | –         | –        |
| 284.2997131 | WILD       | 309.377798     | 24,700    | 31,360,000 |
| 0           | CAPTIVE    | 850.192718     | 0         | 0        |
| 1           | WILD       | 285.0177917    | –         | –        |
| 285.302887  | WILD       | 3.423868       | 12,741    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 297.2461853 | WILD       | 477.188082     | 19,157    | 17,317,462.83 |
| 0           | CAPTIVE    | 4.096744       | 16,754.1667 | 10,393,315.97 |
| 1           | WILD       | 299.0657959    | –         | –        |
| 299.260498  | WILD       | 128.726333     | 28,054    | 95,770,394 |
| 0           | CAPTIVE    | 344.712732     | 0         | 0        |
| 1           | WILD       | 301.0643921    | –         | –        |
| 0           | CAPTIVE    | 0              | 16,988    | 0        |
| 1           | WILD       | 309.2788086    | –         | –        |
| 0           | CAPTIVE    | 0              | 0         | 0        |
| 1           | WILD       | 311.2598877    | –         | –        |
| 0           | CAPTIVE    | 112.169325     | 0         | 0        |
| 1           | WILD       | 96.734658      | 13,200    | 6,196,250 |
| 313.2727051 | WILD       | 3.809944       | 26,598    | 92,385,346.29 |
| 0           | CAPTIVE    | 3.25468        | 33,848    | 151,227,451.7 |
Table 5 (continued)

| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 314.2771912 | –          | –              | –         | –        |
| 0           | WILD       | 245.482551     | 0         | 0        |
| 1           | CAPTIVE    | 221.619756     | 12,350    | 1,672,500|
| 317.2698975 | –          | –              | –         | –        |
| 0           | WILD       | 963.21163      | 0         | 0        |
| 1           | CAPTIVE    | 595.352823     | 11,450    | 2,102,500|
| 321.3160095 | –          | –              | –         | –        |
| 0           | WILD       | 1003.913804    | 0         | 0        |
| 1           | CAPTIVE    | 829.090214     | 13,800    | 1,000,000|
| 323.262085  | –          | –              | –         | –        |
| 0           | WILD       | 891.125072     | 0         | 0        |
| 1           | CAPTIVE    | 381.026337     | 12,900    | 6,250,000|
| 325.2687988 | –          | –              | –         | –        |
| 0           | WILD       | 114.983589     | 0         | 0        |
| 1           | CAPTIVE    | 94.494314      | 13,626.6667 | 8,753,955.556 |
| 335.2907104 | –          | –              | –         | –        |
| 0           | WILD       | 1.53228        | 30.063.25 | 257,171,939.9 |
| 1           | CAPTIVE    | 1.199443       | 38,099.72 | 549,576,200.2 |
| 338.3445129 | –          | –              | –         | –        |
| 0           | WILD       | 640.810913     | 13,725    | 0        |
| 1           | CAPTIVE    | 391.254555     | 22,600    | 1,960,000 |
| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 340.2948914 | –          | –              | 92.250171 | 0        | 0        |
| 1           | CAPTIVE    | 79.770433      | 17,473.6842 | 12,945,096.95 |
| 341.0227051 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 79.770433      | 17,473.6842 | 12,945,096.95 |
| 1           | CAPTIVE    | 0              | 0         | 0        | 0        |
| 341.3035889 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 17.153188      | 28,070.7143 | 135,611,587.6 |
| 1           | CAPTIVE    | 35.443278      | 15,241.1765 | 11,817,716.26 |
| 342.310791  | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 0              | 18,147    | 0        | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        | 0        |
| 352.3568115 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 1024.968886    | 0         | 0        | 0        |
| 1           | CAPTIVE    | 1024.650075    | 18,150    | 2500     |         |
| 353.2622986 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 128.642658     | 0         | 0        | 0        |
| 1           | CAPTIVE    | 90.471937      | 16,666.6667 | 22,775,555.56 |
| 355.2528992 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 255.6921       | 0         | 0        | 0        |
| 1           | CAPTIVE    | 137.487671     | 15,100    | 19,166,666.67 |
| 355.2802124 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 127.788735     | 24,324    | 0        | 0        |
| 1           | CAPTIVE    | 77.715061      | 15,180    | 10,539,600    |         |
| 356.071106  | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 0              | 13,295    | 0        | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        | 0        |
| 357.3005981 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 427.058485     | 0         | 0        | 0        |
| 1           | CAPTIVE    | 230.292815     | 15,775    | 12,446,875 |
| 360.3265076 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 0              | 0         | 0        | 0        |
| 1           | CAPTIVE    | 0              | 13,000    | 0        | 0        |
| 367.3268127 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 1.438044       | 17,417.5  | 47,052,740.25 |
| 1           | CAPTIVE    | 2.667532       | 15,475    | 16,818,125    |         |
| 369.2341919 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 177.088731     | 0         | 0        | 0        |
| 1           | CAPTIVE    | 117.519312     | 14,755,555.56 | 15,580,246.91 |
| 369.3454895 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 1.822486       | 17,931    | 23,174,926.5 |
| 1           | CAPTIVE    | 1.07154        | 23,771.92 | 351,148,147.6 |
| 370.3526917 | –          | –              | 0         | 0        | 0        |
| 0           | WILD       | 742.999133     | 0         | 0        | 0        |
| 1           | CAPTIVE    | 194.50195      | 22,400    | 47,610,000    |         |
Table 5 (continued)

| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 371.3164063 | –          | –              |           |          |
| 0           | WILD       | 0              | 14,286    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 373.0892944 | –          | –              |           |          |
| 0           | WILD       | 0              | 10,116    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 374.0856018 | –          | –              |           |          |
| 0           | WILD       | 0              | 13,513    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 383.2466125 | –          | –              |           |          |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 383.308905  | –          | –              |           |          |
| 0           | WILD       | 0              | 14,286    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 383.329986  | –          | –              |           |          |
| 0           | WILD       | 0              | 10,116    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 383.3351135 | –          | –              |           |          |
| 0           | WILD       | 0              | 13,513    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 385.3417053 | –          | –              |           |          |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 386.3457031 | –          | –              |           |          |
| 0           | WILD       | 0              | 14,286    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 391.2893982 | –          | –              |           |          |
| 0           | WILD       | 0              | 10,116    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 397.3198853 | –          | –              |           |          |
| 0           | WILD       | 0              | 13,513    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 399.3351135 | –          | –              |           |          |
| 0           | WILD       | 0              | 14,286    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 401.3435974 | –          | –              |           |          |
| 0           | WILD       | 0              | 10,116    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 402.3396912 | –          | –              |           |          |
| 0           | WILD       | 0              | 13,513    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 411.4000854 | –          | –              |           |          |
| 0           | WILD       | 0              | 14,286    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 415.0372925 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 15,896    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 425.3422852 | –          | –              | –         | –        |
| 0           | WILD       | 678.232399     | 0         | 0        |
| 1           | CAPTIVE    | 645.315288     | 11,033.3333 | 228,888.8889 |
| 429.0870056 | –          | –              | –         | –        |
| 0           | WILD       | 678.232399     | 0         | 0        |
| 1           | CAPTIVE    | 645.315288     | 11,033.3333 | 228,888.8889 |
| 429.3714905 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 431.0842896 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 14,300    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 432.0798035 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 10,116    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 437.3510132 | –          | –              | –         | –        |
| 0           | WILD       | 980.865554     | 0         | 0        |
| 1           | CAPTIVE    | 680.521262     | 13,200    | 1,960,000 |
| 439.3474121 | –          | –              | –         | –        |
| 0           | WILD       | 85.021148      | 0         | 0        |
| 1           | CAPTIVE    | 65.975509      | 16,666.6667 | 23,578,888.89 |
| 445.3673096 | –          | –              | –         | –        |
| 0           | WILD       | 483.009379     | 0         | 0        |
| 1           | CAPTIVE    | 373.007232     | 12,500    | 2,385,000 |
| 446.1213074 | –          | –              | –         | –        |
| 0           | WILD       | 61.042662      | 0         | 0        |
| 1           | CAPTIVE    | 44.694887      | 22,814.2857 | 77,968,843.54 |
| 451.3485107 | –          | –              | –         | –        |
| 0           | WILD       | 483.009379     | 0         | 0        |
| 1           | CAPTIVE    | 373.007232     | 12,500    | 2,385,000 |
| 453.353302  | –          | –              | –         | –        |
| 0           | WILD       | 61.042662      | 0         | 0        |
| 1           | CAPTIVE    | 44.694887      | 22,814.2857 | 77,968,843.54 |
| 454.3604126 | –          | –              | –         | –        |
| 0           | WILD       | 400.435913     | 0         | 0        |
| 1           | CAPTIVE    | 365.480993     | 11,060    | 730,400   |
| 455.3648987 | –          | –              | –         | –        |
| 0           | WILD       | 131.389376     | 0         | 0        |
| 1           | CAPTIVE    | 104.027179     | 14,938.4615 | 11,168,520.71 |
| 465.3575134 | –          | –              | –         | –        |
| 0           | WILD       | 72.456875      | 0         | 0        |
| 1           | CAPTIVE    | 57.890394      | 19,247.619 | 32,163,446.71 |
Table 5 (continued)

| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 466.3645935|            |                |           |          |
| 0 WILD      | 0          | 0              | 0         | 0        |
| 1 CAPTIVE   | 0          | 10,500         | 0         | 0        |
| 467.3705139|            |                |           |          |
| 0 WILD      | 46.842267  | 0              | 0         | 0        |
| 1 CAPTIVE   | 35.485521  | 46,653.4231    | 371,767,877.2 |
| 468.3747864|            |                |           |          |
| 0 WILD      | 80.416727  | 0              | 0         | 0        |
| 1 CAPTIVE   | 65.870389  | 17,230         | 20,381,100 |
| 469.3612061|            |                |           |          |
| 0 WILD      | 1017.173183| 0              | 0         | 0        |
| 1 CAPTIVE   | 943.339707 | 11,400         | 250,000   |
| 479.3622131|            |                |           |          |
| 0 WILD      | 88.197345  | 0              | 0         | 0        |
| 1 CAPTIVE   | 66.618892  | 17,464.7059    | 28,004,636.68 |
| 481.3711853|            |                |           |          |
| 0 WILD      | 83.505703  | 0              | 0         | 0        |
| 1 CAPTIVE   | 64.046374  | 20,350         | 37,669,166.67 |
| 482.3829956|            |                |           |          |
| 0 WILD      | 229.467346 | 0              | 0         | 0        |
| 1 CAPTIVE   | 180.992698 | 12,150         | 4,307,500 |
| 483.3699036|            |                |           |          |
| 0 WILD      | 229.467346 | 0              | 0         | 0        |
| 1 CAPTIVE   | 180.992698 | 12,150         | 4,307,500 |
| 489.0563965|            |                |           |          |
| 0 WILD      | 498.31783  | 0              | 0         | 0        |
| 1 CAPTIVE   | 436.423507 | 11,475         | 936,875   |
| 491.3699036|            |                |           |          |
| 0 WILD      | 498.31783  | 0              | 0         | 0        |
| 1 CAPTIVE   | 436.423507 | 11,475         | 936,875   |
| 493.3811951|            |                |           |          |
| 0 WILD      | 49.195914  | 0              | 0         | 0        |
| 1 CAPTIVE   | 37.945101  | 32,538.3462    | 159,525,506.6 |
| 494.3898926|            |                |           |          |
| 0 WILD      | 146.439447 | 0              | 0         | 0        |
| 1 CAPTIVE   | 117.722382 | 14,783.3333    | 9,101,388.889 |
| 495.3893127|            |                |           |          |
| 0 WILD      | 94.336279  | 0              | 0         | 0        |
| 1 CAPTIVE   | 74.720647  | 18,382.3529    | 23,508,512.11 |
| 495.4046021|            |                |           |          |
| 0 WILD      | 93.605749  | 0              | 0         | 0        |
| 1 CAPTIVE   | 73.71548   | 18,176.4706    | 23,808,858.13 |
| 503.1065979|            |                |           |          |
| 0 WILD      | 0          | 18,497         | 0         | 0        |
| 1 CAPTIVE   | 0          | 0              | 0         | 0        |
| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 504.1076965 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 12,741    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 505.104187  | –          | –              | –         | –        |
| 0           | WILD       | 0              | 13,006    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 507.3703003 | –          | –              | –         | –        |
| 0           | WILD       | 324.911024     | 0         | 0        |
| 1           | CAPTIVE    | 278.28293      | 11,283.3333 | 1,641,388.889 |
| 509.3848877 | –          | –              | –         | –        |
| 0           | WILD       | 197.168098     | 0         | 0        |
| 1           | CAPTIVE    | 150.959789     | 12,277.7778 | 5,850,617.284 |
| 519.1381836 | –          | –              | –         | –        |
| 0           | WILD       | 319.783443     | 17,501    | 1,651,225|
| 1           | CAPTIVE    | 620.617193     | 10,900    | 0        |
| 520.5014038 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 10,300    | 0        |
| 521.4511719 | –          | –              | –         | –        |
| 0           | WILD       | 221.147073     | 0         | 0        |
| 1           | CAPTIVE    | 163.687847     | 13,662.5  | 7,407,343.75 |
| 522.1367798 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 18,533    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 523.4718018 | –          | –              | –         | –        |
| 0           | WILD       | 8.298666       | 21,185.75 | 63,512,816.19 |
| 1           | CAPTIVE    | 18.372601      | 15,625    | 12,435,625 |
| 533.1945801 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 11,702    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 536.1657715 | –          | –              | –         | –        |
| 0           | WILD       | 4.920255       | 18,181.6667 | 24,900,656.89 |
| 1           | CAPTIVE    | 36.169174      | 15,800    | 0        |
| 537.1638184 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 10,694    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 537.4810181 | –          | –              | –         | –        |
| 0           | WILD       | 854.695896     | 13,983.5  | 865,830.25 |
| 1           | CAPTIVE    | 1007.161429    | 0         | 0        |
| 538.1621094 | –          | –              | –         | –        |
| 0           | WILD       | 865.58892      | 11,137.5  | 507,656.25 |
| 1           | CAPTIVE    | 1008.490792    | 0         | 0        |
| 547.468689  | –          | –              | –         | –        |
| 0           | WILD       | 96.287302      | 0         | 0        |
| 1           | CAPTIVE    | 77.463056      | 15,341.1765 | 14,849,480.97 |
Table 5 (continued)

| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 548.4746094 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 10,300    | 0        |
| 549.4857178 | –          | –              | –         | –        |
| 0           | WILD       | 52.351399      | 0         | 0        |
| 1           | CAPTIVE    | 39.342647      | 33,545.7083 | 177,687,835.9 |
| 550.4893188 | –          | –              | –         | –        |
| 0           | WILD       | 98.960271      | 0         | 0        |
| 1           | CAPTIVE    | 81.36309       | 16,270.5882 | 14,464,429.07 |
| 551.5015259 | –          | –              | –         | –        |
| 0           | WILD       | 4.677523       | 53,415.3333 | 1,199,762,890 |
| 1           | CAPTIVE    | 9.175364       | 34,249.7917 | 237,381,687.7 |
| 552.5032959 | –          | –              | –         | –        |
| 0           | WILD       | 10.952528      | 28,142.8333 | 139,108,302.1 |
| 1           | CAPTIVE    | 20.483431      | 17,890    | 27,461,900 |
| 553.5032959 | –          | –              | –         | –        |
| 0           | WILD       | 851.949692     | 11,059    | 552,049  |
| 1           | CAPTIVE    | 1006.821486    | 0         | 0        |
| 563.4750977 | –          | –              | –         | –        |
| 0           | WILD       | 246.292214     | 0         | 0        |
| 1           | CAPTIVE    | 223.987971     | 11,862.5  | 1,422,343.75 |
| 563.4927979 | –          | –              | –         | –        |
| 0           | WILD       | 221.233865     | 0         | 0        |
| 1           | CAPTIVE    | 207.645712     | 11,377.7778 | 957,283,9506 |
| 565.4874878 | –          | –              | –         | –        |
| 0           | WILD       | 370.48652      | 0         | 0        |
| 1           | CAPTIVE    | 265.556074     | 12,960    | 4,478,400 |
| 565.5145264 | –          | –              | –         | –        |
| 0           | WILD       | 71.318095      | 20,696.5  | 17,905,214.25 |
| 1           | CAPTIVE    | 230.943078     | 12,100    | 0        |
| 566.5073242 | –          | –              | –         | –        |
| 0           | WILD       | 884.016391     | 10,633    | 400,689  |
| 1           | CAPTIVE    | 1010.672636    | 0         | 0        |
| 573.4857788 | –          | –              | –         | –        |
| 0           | WILD       | 394.919334     | 0         | 0        |
| 1           | CAPTIVE    | 343.167404     | 11,260    | 1,210,400 |
| 575.5028076 | –          | –              | –         | –        |
| 0           | WILD       | 44.855013      | 0         | 0        |
| 1           | CAPTIVE    | 33.476104      | 36,665.1538 | 254,684,070.6 |
| 576.5050049 | –          | –              | –         | –        |
| 0           | WILD       | 85.866945      | 0         | 0        |
| 1           | CAPTIVE    | 70.366712      | 17,805.2632 | 20,332,077.56 |
| 577.1240234 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 16,602    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| Class index | Class name | Fisher's ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 577.5181274 | –          | –              | –         | –        |
| 578.5205078 | –          | –              | –         | –        |
| 579.5328979 | –          | –              | –         | –        |
| 580.5358276 | –          | –              | –         | –        |
| 581.5288086 | –          | –              | –         | –        |
| 589.4833984 | –          | –              | –         | –        |
| 591.4968872 | –          | –              | –         | –        |
| 592.5021973 | –          | –              | –         | –        |
| 593.5101929 | –          | –              | –         | –        |
| 593.5333252 | –          | –              | –         | –        |
| 601.5162964 | –          | –              | –         | –        |
| 603.5314941 | –          | –              | –         | –        |
| 604.5349731 | –          | –              | –         | –        |
| 605.5426025 | –          | –              | –         | –        |
| 608.5656128 | –          | –              | –         | –        |
Table 5 (continued)

| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 609.4887695 | –          | –              | –         | –        |
| 0 WILD      | 0          | 0              | 0         | 0        |
| 1 CAPTIVE   | 0          | 12,200         | 0         | 0        |
| 612.5499268 | –          | –              | –         | –        |
| 0 WILD      | 681.351434 | 0              | 0         | 0        |
| 1 CAPTIVE   | 668.110166 | 10,633.3333    | 82,222.2222 |
| 617.510376  | –          | –              | –         | –        |
| 0 WILD      | 288.790034 | 0              | 0         | 0        |
| 1 CAPTIVE   | 277.503659 | 10,571.4286    | 393,469.3878 |
| 619.5269775 | –          | –              | –         | –        |
| 0 WILD      | 247.653897 | 0              | 0         | 0        |
| 1 CAPTIVE   | 228.050446 | 12,050         | 1,260,000 |
| 626.2260132 | –          | –              | –         | –        |
| 0 WILD      | 0          | 15,830         | 0         | 0        |
| 1 CAPTIVE   | 0          | 0              | 0         | 0        |
| 642.4993286 | –          | –              | –         | –        |
| 0 WILD      | 0          | 0              | 0         | 0        |
| 1 CAPTIVE   | 0          | 11,600         | 0         | 0        |
| 649.6848755 | –          | –              | –         | –        |
| 0 WILD      | 0          | 18,533         | 0         | 0        |
| 1 CAPTIVE   | 0          | 0              | 0         | 0        |
| 656.5111084 | –          | –              | –         | –        |
| 0 WILD      | 981.32276  | 0              | 0         | 0        |
| 1 CAPTIVE   | 683.005018 | 11,850         | 1,562,500 |
| 663.4520264 | –          | –              | –         | –        |
| 0 WILD      | 1.77635    | 59,474.5       | 1,032,452,323 |
| 1 CAPTIVE   | 2.187012   | 46,699.6296    | 601,173,474.3 |
| 664.4577026 | –          | –              | –         | –        |
| 0 WILD      | 0.925652   | 29,827         | 286,433,890 |
| 1 CAPTIVE   | 1.433254   | 25,454.5455    | 103,563,388.4 |
| 665.4603271 | –          | –              | –         | –        |
| 0 WILD      | 203.172127 | 10,105         | 0         | 0        |
| 1 CAPTIVE   | 56.667332  | 11,900         | 1,025,000 |
| 675.6990967 | –          | –              | –         | –        |
| 0 WILD      | 0          | 13,127         | 0         | 0        |
| 1 CAPTIVE   | 0          | 0              | 0         | 0        |
| 677.7145996 | –          | –              | –         | –        |
| 0 WILD      | 0          | 25,482         | 0         | 0        |
| 1 CAPTIVE   | 0          | 0              | 0         | 0        |
| 684.2030029 | –          | –              | –         | –        |
| 0 WILD      | 58.018505  | 20,854         | 149,590,608.7 |
| 1 CAPTIVE   | 287.610803 | 0              | 0         | 0        |
| 688.1956787 | –          | –              | –         | –        |
| 0 WILD      | 0          | 13,513         | 0         | 0        |
| 1 CAPTIVE   | 0          | 0              | 0         | 0        |
| Class index | Class name | Fisher’s ratio | Abundance | Variance |
|-------------|------------|----------------|-----------|----------|
| 691.7000122 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 13,127    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
| 698.255188  | –          | –              | –         | –        |
| 0           | WILD       | 494.667523     | 15,523    | 5,740,816|
| 1           | CAPTIVE    | 935.818658     | 0         | 0        |
| 738.630188  | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 10,000    | 0        |
| 740.633728  | –          | –              | –         | –        |
| 0           | WILD       | 1019.796954    | 0         | 0        |
| 1           | CAPTIVE    | 969.360676     | 11,200    | 160,000  |
| 766.6524048 | –          | –              | –         | –        |
| 0           | WILD       | 329.593048     | 0         | 0        |
| 1           | CAPTIVE    | 294.086201     | 10,916.6667 | 1,091,388.889 |
| 824.7703247 | –          | –              | –         | –        |
| 0           | WILD       | 501.842599     | 23,094    | 12,355,225|
| 1           | CAPTIVE    | 938.074049     | 0         | 0        |
| 825.7698975 | –          | –              | –         | –        |
| 0           | WILD       | 825.934481     | 14,197    | 1,079,521|
| 1           | CAPTIVE    | 1003.501123    | 0         | 0        |
| 848.7681885 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 15,300    | 0        |
| 850.7814941 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 14,100    | 0        |
| 852.8024902 | –          | –              | –         | –        |
| 0           | WILD       | 937.491034     | 19,190    | 763,876  |
| 1           | CAPTIVE    | 1016.565328    | 0         | 0        |
| 853.802002  | –          | –              | –         | –        |
| 0           | WILD       | 1016.982579    | 11,635    | 23,716   |
| 1           | CAPTIVE    | 1024.282225    | 0         | 0        |
| 874.7836304 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 10,700    | 0        |
| 876.7979126 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 17,300    | 0        |
| 877.8029175 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 0         | 0        |
| 1           | CAPTIVE    | 0              | 10,300    | 0        |
| 880.8275146 | –          | –              | –         | –        |
| 0           | WILD       | 0              | 10,086    | 0        |
| 1           | CAPTIVE    | 0              | 0         | 0        |
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Data availability Summary data and sample identification numbers are included as appendices and access to full spectra data may be requested directly from the authors.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication All authors consent to publication.

Ethical approval Bone samples were transferred from South Africa to the UK under CITES scientific institution licence numbers: South Africa, ZA034 (National Zoological Gardens of South Africa); UK, GB031 (SASA).

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