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THE ANALYSIS OF SEA TURTLE AND BOVID KERATIN ARTEFACTS USING DRIFT SPECTROSCOPY AND DISCRIMINANT ANALYSIS*

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We investigated the utility of diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) for the analysis and identification of sea turtle (Family Cheloniidae) and bovid (Family Bovidae) keratins, commonly used to manufacture historic artefacts. Spectral libraries are helpful in determining the class of the material (i.e., keratin versus plastics), but do not allow for inferences about the species source of keratin. Mathematical post-processing of the spectra employing discriminant analysis provided a useful statistical tool to differentiate tortoiseshell from bovid horn keratin. All keratin standards used in this study (n = 35 Bovidae; n = 24 Cheloniidae) were correctly classified with the discriminant analysis. A resulting performance index of 95.7% shows that DRIFTS, combined with discriminant analysis, is a powerful quantitative technique for distinguishing sea turtle and bovid keratins commonly encountered in museum collections and the modern wildlife trade.

KEYWORDS: KERATIN, DRIFT SPECTROSCOPY, DISCRIMINANT ANALYSIS, X-RAY FLUORESCENCE, SEA TURTLE, BOVID, TORTOISESHELL, HORN, WILDLIFE FORENSICS

INTRODUCTION

The keratinous scutes of sea turtles and horn sheaths of bovids have been used for centuries in artefact manufacture (Aikin 1840; Ritchie 1975). Sea turtles have been exploited extensively for their hard cornified scutes, also termed plates (Solomon *et al*. 1986), which cover the carapace and plastron. Known as tortoiseshell, bekko or carey in the antiquities and wildlife trade (Ritchie 1970; Limpus and Miller 1990; Márquez 1990; Edwards *et al*. 1998; van Dijk and Shepherd 2004; Paris *et al*. 2005), sea turtle scutes were commonly used to manufacture jewellery, combs, hand-held fans, snuff boxes, buttons, furniture veneers and numerous other historical artefacts.

The speckled amber and brown appearance of these artefacts is highly distinctive and visually appealing. Due to the scarcity and expense of genuine tortoiseshell, more widely available raw materials, including bovid horn sheaths (Family Bovidae), were modified and used to manufacture items of similar appearance (Wenham 1964; Musser 1978; O'Connor 1987). In more recent times, celluloids and plastics have been used as substitute materials (Reilly 1991; Paris and Coupry 2005; Paris *et al*. 2005). Given that sea turtle and bovid keratin may closely resemble each other in appearance, proper identification of the raw material origin

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of such artefacts is of interest to archaeologists and museum curators, and to law enforcement officials who monitor the illegal trade in wildlife parts and products.

All sea turtle species (Families Dermochelyidae and Cheloniidae) are listed in the Convention on International Trade in Endangered Species of Fauna and Flora (CITES). Those occurring in US waters are also listed in the Endangered Species Act (ESA), and are therefore subject to strict regulations regarding take and trade in their parts. Identification of products manufactured from sea turtle parts is therefore critical to wildlife law enforcement efforts.

Seven sea turtle species are currently recognized by CITES: (1) *Dermochelys coriacea* (leatherback), (2) *Chelonia mydas* (green), (3) *Natator depressus* (flatback), (4) *Eretmochelys imbricata* (hawksbill), (5) *Caretta caretta* (loggerhead), (6) *Lepidochelys kempii* (Kemp's Ridley) and (7) *Lepidochelys olivacea* (Olive Ridley). The leatherback (Family Dermochelyidae) lacks carapacial scutes and is not exploited for the tortoiseshell trade. The most extensively used species in the trade is the hawksbill, though the green and loggerhead sea turtles may also be exploited for their scutes (O'Connor 1987). The flatback and Kemp's Ridley sea turtles are very rare, and to our knowledge are not used in the tortoiseshell trade.

In general, the scutes of the hawksbill are more distinctly patterned than those of the other sea turtle species, though pigmentation in sea turtle scutes can be highly variable (Frazier 1971; Gonzalez and Alvarez 1984; Kobayashi 2001). Hawksbill scutes are typically thicker than those of other sea turtle species and are more conducive to use as a raw material source. Recently, however, green sea turtles raised in captivity on high-protein diets have produced relatively thick scutes that can be used in the same manner as hawksbill scutes (Frazier 2005). Small artefacts and those constructed from scutes that are completely melanistic can be difficult to identify and distinguish from bovid horn sheaths. Morphological methods for distinguishing sea turtle and bovid keratin are reviewed by O'Connor (1987), though forensic identification of such items remains challenging (Colbert *et al*. 1999).

Paris *et al*. (2005) used attenuated total reflection infrared spectroscopy (ATR–FTIR) to differentiate hard keratins (tortoiseshell and horn) from natural synthetic imitations (galalith or bakelite), though they analysed only artefacts and did not compare their results with vouchered specimens of known species origin. Edwards *et al*. (1998) differentiated horn and turtle keratin with Raman spectroscopy, though their sample sizes were relatively small. Here, we present results from a broad range of sea turtle and bovid species, showing that diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), combined with discriminant analysis, is a useful quantitative tool for distinguishing their keratins.

KERATIN BIOCHEMISTRY

Keratins are a broad class of fibrous proteins (Lehninger 1982). The two major classes of keratins have been termed alpha-keratins (α) and beta-keratins (β). Although these keratins can be characterized based on genomic, chemico-physical, ultrastructural and morphological characters (Alibardi 2003a,b), an intrinsic difference in vertebrate keratins is that reptiles and birds produce both α- and β-keratins, whereas mammals produce only α-keratins (Alexander and Parakkal 1969; Marshall *et al*. 1991; Fraser and Parry 1996; Alibardi 2003a,b, 2005). While keratin nomenclature can be confusing (Astbury and Street 1932; Astbury and Woods 1934; Bamford *et al*. 1956; Bendit 1966), α-keratins are characterized by a helical structure, whereas β-keratins have a pleated structure. Either form of keratinization produces extremely hard keratin (turtle shell scutes, horn sheaths, hoof ungues, claw ungues, etc.), which provides external protection to mammals and reptiles (Solomon *et al*. 1986; Alibardi 2005).

The morphological structures and biochemistry of keratin have been systematically described (Lehninger 1982; Marshall *et al*. 1991), whereas Alibardi (2003a,b, 2005) has reviewed the selective advantage of inheriting α- versus β-keratin genes. Most recently, Alibardi (2006) and Alibardi and Toni (2006) characterized β-keratin synthesis in growing turtle scutes.

In this study, we use the terms α-keratin and β-keratin to refer to the entire protein, whereas the terms α-helix and/or β-pleated sheet refer specifically to the structural conformation of the objects analysed and have no taxa implications. For example, Astbury and Street (1932), Astbury and Woods (1934), Bendit (1966) and Lyman *et al*. (2001) have shown that a mammal hair, by definition an α-keratin (Alibardi 2003a,b, 2005), can be modified from its original α-helix configuration to a β-pleated structure.

INFRARED SPECTROSCOPY

Keratins have been studied with various forms of infrared spectroscopy for decades (Ambrose and Elliott 1951). Spectroscopy is now widely used in cultural heritage conservation to characterize a broad range of artefact classes (Derrick *et al*. 2000; Bitossi *et al*. 2005). Fourier transform infrared spectroscopy (FT–IR) is an analytical tool that, when used in examining hard biological tissues, stands out for its robustness, ease of sample preparation, simplicity of operation and the ability to make structural elucidations (Rintoul *et al*. 1998; Lyman *et al*. 2001). The resolving power of FT–IR has been applied in such diverse fields as forensic fibre identification (Kirkbride and Tungol 1999) and bacterial species identification (Timmins *et al*. 1998).

Discriminant analysis (also known as linear discriminant analysis or canonical variates analysis) of vibrational spectra (Raman or infrared) has been successfully used to extend the limitation inherent in vibrational data. Examples include confirmation of edible oils and fats (Baeten and Aparicio 2000), bacterial taxonomy (Amiel *et al*. 2001), sub-typing of nylon polymers (Enlow *et al*. 2005), the forensic characterization of printer toners (Egan *et al*. 2003), geographical sourcing of medicinal plants (Dharmaraj *et al*. 2006), forensic identification of fingernails versus toenails (Widjaja and Seah 2006) and the forensic identification of fibre blends (Espinoza *et al*. 2006).

Prado *et al*. (2005) compared results from two FT–IR methods, attenuated total reflectance (ATR) and DRIFTS, and found that DRIFTS provided better discrimination and quantitative results than the ATR method. In this study, we present our results on differentiating the hard keratin of bovids from those of sea turtles by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), followed by discriminant analysis. Additionally, we describe how we distinguish protein-based plastics (i.e., casein—also known as galalith) from keratin. The use of a DRIFTS accessory permits rapid non-destructive sampling, with no harm to the object in question. The method proved useful in distinguishing bovid and sea turtle keratins commonly encountered in museum collections and the wildlife trade.

METHODOLOGY

FT–IR spectroscopy

A Nicolet, Nexus 470 FT–IR (Omnic v.6 software) with a Nexus Smart Collector™ accessory was used for studying the spectral properties of keratins and for developing a wildlife materials database library at the US National Fish & Wildlife Forensics Laboratory (NFWFL). The DRIFTS analysis was performed using keratin collection parameters that consisted of 50 scans

at a resolution of 4 cm^{-1}, resulting in data spacing of 1.928 cm^{-1}. Every sample was collected by sanding with 320 grid silica carbide collection discs and placing them on the Nexus Smart Collector™ sample holder. The silica carbide collection and the diffuse reflectance device avoid typical FT–IR sample preparation problems (e.g., liquid nitrogen pulverization) and can be accomplished in less than 15 min. The spectrometer instrumentation contained a KBr beam splitter along with a DTGS KBr detector.

Collection parameters for the spectral library were optimized to ensure high spectral accuracy (Kirkbride and Tungol 1999). Keratin samples were scanned 50 times under autogain control, and each spectrum was evaluated for spectral accuracy. The final format of the spectra was recorded as absorbance versus wavenumber (cm^{-1}), with a spectral range of 3800–800 cm^{-1}. There was no correction performed on the resulting spectrum. A background spectrum was taken before each keratin was sampled. Discriminant analysis was performed using the TQ Analyst™ (v.6.0) software package (Nicolet).

Spectral library standards of keratins were obtained from the NFWFL morphology reference collection. Known reference standards used in this study are listed in Table 1. Morphological voucher specimens are curated at NFWFL. Sea turtle and bovid taxonomy follows Pritchard and Mortimer (1999) and Wilson and Cole (2000), respectively. The spectral library includes 35 individual bovids representing 24 different species. We chose to include a broad range of bovid species because of the global nature of the wildlife trade. Three of the bovid standards are represented by hoof ungues, while the remaining are horn sheaths (Table 1). Sea turtle standards are represented by 24 individuals and four species. The two loggerhead standards were taken from keratin beaks of the mandible. All other sea turtle standards were scutes. Of the remaining three sea turtle species recognized by biologists, the leatherback lacks scutes, while the flatback and Kemp's Ridley are so rare that their scutes are not known to be exploited for the tortoiseshell trade.

Casein-based plastic synthesis

Galalith™ standards, a milk-based plastic originally synthesized in France and commonly used as a substitute for tortoiseshell (Paris *et al.* 2005), were difficult to obtain. Two casein plastics were obtained from a commercial source (http://www.redbeartrading.com/picks.html) and validated (see below). Eight batches of casein-based plastics were synthesized in-house. Two hundred millilitres of non-fat milk was heated to 45°C. The milk was removed from the hotplate and 20 ml of 10% acetic acid was added drop-wise while stirring with a glass rod. The solution was stirred continuously for about 4 min after all the acid was added. The casein precipitate was allowed to settle, and the supernatant was decanted. The casein precipitate was filtered through a No. 4 Whatman filter and rinsed with 250 ml of deionized water. After the filtrate had completely drained, the rinsed casein was gently compressed to remove additional water. The casein was next placed in a 200 ml glass beaker and then cooked at maximum power in a 500 W microwave oven for 10 s, which resulted in an agglomerated mass. The warm plastic was hand formed into a small disc and allowed to air dry for one day. Final drying of the plastic was accomplished by a 48 h soak in 35% formalin (Elfick 1996).

X-ray fluorescence spectroscopy (XRF)

Elemental analysis of keratin standards and casein-based plastics was conducted by X-ray fluorescence spectroscopy (XRF). An XRF Eagle II™ by Edax was operated at 40 kVa and at

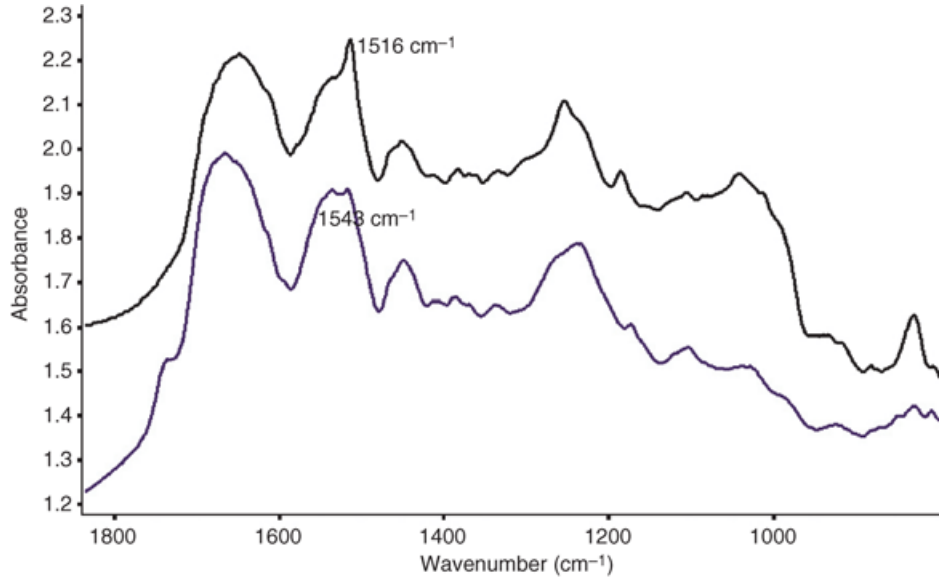

Figure 1 *DRIFTS spectra of two individual hawksbill sea turtle scutes (*Eretmochelys imbricata*). The top spectrum is typical for this class and displays a prominent amide II stretch at 1516 cm^{-1}. The bottom spectrum is atypical, because the amide II stretch of this β-keratin is present at 1543 cm^{-1}, which has been associated with α-keratins (see text).*

1000 μA. Each item was counted for 100 s, and the semi-quantitative results were obtained by using the instrument's standardless fundamental parameters software.

RESULTS AND DISCUSSION

Class character identification

The analysis of materials by DRIFTS is straightforward. The results of the spectra are searched against the spectral library of known reference samples. The best match value infers the class identity of the component. Examples of class identity may include that the sample is of keratin origin, or that the item in question is simply not of biological origin (e.g., celluloid or plastic substitute).

The DRIFTS spectrum in Fig. 1 demonstrates the analytical process. In this case, the top spectrum represents the typical sea turtle keratin spectrum. A search of the spectral library concludes the object is indeed of keratinous origin (91.7 match score), and an examination of the spectrum allows us to corroborate the presence of frequencies characteristic of keratin as demonstrated in Table 2 (Hopkins *et al*. 1991; Joy and Lewis 1991; Akhtar and Edwards 1997; Edwards *et al*. 1998; Carter and Edwards 2001). The presence of these absorptions plus the presence of the 1516 cm^{-1} vibration implies that the keratin sample has a strong β-pleated confirmation, suggestive of reptiles. However, these observations do not allow us to reach a conclusion on the taxon of origin.

Potential errors when using peak assignment to make secondary structure inferences

When using DRIFTS analysis, the absorption of the amide I, amide II and amide III peaks occurs over a broad range, as demonstrated in Table 3. Therefore, any particular stretch is not

Table 1 *Keratin reference samples analysed by DRIFTS and used in this study. Additionally, the table lists the absorption maximum of the amide moieties. Amide II has been listed as 'Amide II, α-like' and 'Amide II, β-like', depending on where the DRIFTS shift was detected*

Family	*Species*	*Common name and material*	*Specimen no.*	*Amide I*	*Amide II, α-like*	*Amide II β-like*	*Amide III*
Bovidae	*Addax nasomaculatus*	Addax horn sheath	MAM #1210	1680	1545		1254
Bovidae	*Bos frontalis*	Gaur horn sheath	NFWFL#1287	1664	1537		1249
Bovidae	*Bos frontalis*	Gaur horn sheath	NFWFL #1285	1675	1543		1246
Bovidae	*Bos frontalis*	Gaur horn sheath	NFWFL #1216	1668	1537		1255
Bovidae	*Bos frontalis*	Gaur horn sheath	NFWFL #1286	1669	1537		1246
Bovidae	*Bos javanicus*	Banteng horn sheath	MAM #774	1673	1539		1245
Bovidae	*Bos taurus*	Cow hoof unguis	MAM #1293	1673	1545		1254
Bovidae	*Bos taurus*	Cow hoof unguis	MAM #1272	1667	1537		1256
Bovidae	*Bos taurus*	Cow hoof unguis	MAM #1293	1673	1546		1255
Bovidae	*Bos taurus*	Cow horn sheath	NFWFL #1330	1677	1546		1244
Bovidae	*Bubalus mindorensis*	Tamaraw horn sheath	NFWFL #1217	1650		1516	1255
Bovidae	*Capra caucasica*	Tur horn sheath	MAM #668	1658	1545		1255
Bovidae	*Capra caucasica*	Tur horn sheath	NFWFL #1560	1661		1513	1250
Bovidae	*Capra caucasica*	Tur horn sheath	MAM #668	1668	1549		1254
Bovidae	*Capra falconeri*	Markhor horn sheath	NFWFL #187	1656		1517	1258
Bovidae	*Connochaetes gnou*	Black wildebeest horn sheath	MAM #995	1667	1543		1255
Bovidae	*Damaliscus pygargus*	Bontebok horn sheath	NFWFL #1561	1680	1544		1250
Bovidae	*Hippotragus niger*	Sable antelope horn sheath	NFWFL #1280	1651		1517	1259
Bovidae	*Oryx dammah*	Scimitar-horned oryx horn sheath	MAM #957	1657		1517	1258
Bovidae	*Oryx gazella*	Gemsbok horn sheath	MAM #961	1666	1538		1256
Bovidae	*Ovibos moschatus*	Muskox horn sheath	MAM #1398	1669	1535		1250
Bovidae	*Ovis aries*	Sheep horn sheath	NFWFL #290	1674	1541		1244
Bovidae	*Ovis aries*	Sheep horn sheath	Wildlife Safari	1661	1545		1250
Bovidae	*Ovis canadensis*	Bighorn sheep horn sheath	NFWFL #208	1679	1555		1252
Bovidae	*Ovis dalli*	Dall's sheep horn sheath	MAM #740	1679	1544		1247
Bovidae	*Ovis dalli*	Dall's sheep horn sheath	NFWFL #677	1680	1550		1249

Bovidae	*Ovis nivicola*	Snow sheep horn sheath	NFWFL #230	1673		1517	1257
Bovidae	*Ovis vignei*	Urial horn sheath	NFWFL #83	1673	1547		1256
Bovidae	*Pantholops hodgsonii*	Tibetan antelope horn sheath	MAM #1100	1677	1546		1247
Bovidae	*Pantholops hodgsonii*	Tibetan antelope horn sheath	MAM #418	1666	1537		1244
Bovidae	*Saiga tatarica*	Saiga horn sheath	NFWFL #1329	1680	1548		1253
Bovidae	*Syncerus caffer*	African buffalo horn sheath	NFWFL#1364	1663	1539		1256
Bovidae	*Taurotragus oryx*	Eland horn sheath	MAM #882	1672	1543		1248
Bovidae	*Tragelaphus eurycerus*	Bongo horn sheath	MAM #872	1670	1543		1255
Bovidae	*Tragelaphus strepsiceros*	Greater kudu horn sheath	MAM #884	1660	1543		1251
Cheloniidae	*Caretta caretta*	Loggerhead sea turtle beak	NFWFL #224	1666		1518	1255
Cheloniidae	*Caretta caretta*	Loggerhead sea turtle beak	NFWFL #225	1664		1516	1254
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AB826	1663		1516	1256
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AB809	1684	1540	1516	1240
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AB810	1670	1537	1517	1240
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AB856	1666	1540	1519	1241
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AE285	1666	1536	1517	1241
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AG638	1664	1539	1516	1241
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AG642	1679	1542	1519	1239
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AJ261	1667	1539	1517	1239
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	AX727	1659	1539	1517	1239
Cheloniidae	*Chelonia mydas*	Green sea turtle scute	LE1062	1666	1544	1516	1256
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	357174	1671	1541	1518	1240
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	1070	1649		1515	1255
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	AA321	1649		1515	1263
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	NFWFL #317	1666		1517	1239
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	BA101	1658		1518	1259
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	BA605	1673	1539	1517	1237
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	NFWFL #187	1650		1516	1253
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	NFWFL #308	1647		1515	1255
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	ST653497 #36	1656		1516	1266
Cheloniidae	*Eretmochelys imbricata*	Hawksbill sea turtle scute	AT560	1663		1517	1236
Cheloniidae	*Lepidochelys olivacea*	Olive Ridley sea turtle scute	NFWFL #408	1660	1544	1517	1241
Cheloniidae	*Lepidochelys olivacea*	Olive Ridley sea turtle scute	NFWFL #411	1654	1542	1519	1239

Table 2 *Assignment of band stretches of DRIFTS keratin spectra*

FT–IR vibration (cm^{-1})	*Approximate assignment*	*Functional group*
3310	υ(NH) symmetric stretch	Amide A
3073	υ(CH) olifinic	
2965	$\upsilon(CH_3)$ asymmetric	
2930	$\upsilon(CH_3)$ symmetric	
2875	$\upsilon(CH_2)$ symmetric	
1672	υ(CO) symmetric β-pleated sheet	Amide I
1665	υ(CO) random coil	Amide I
1655	υ(CO) symmetric α-helix	Amide I
1624	υ(CO) symmetric β-pleated sheet	Amide II
1543	δ(HN) υ(CN) α-helix	Amide II
1516	δ(HN) υ(CN) β-pleated sheet	Amide II
1453	$\delta(CH_2)$ (CH_3) deformation	
1414	$\delta(CH_3)$ deformation	
1336	$\delta(CH_2)$ deformation	
1256	υ(CN)δ(HN) random coil	Amide III
1188	υa(SO) cysteic acid	Cystine oxides
1106	υs(SO) cystine dioxide	Cystine oxides
1075	υs(SO) cystine monoxide	Cystine oxides
1041	υs(SO) cysteic acid	Cystine oxides
1024	υs(SO) cysteine-s-sulphonate	Cystine oxides
831	δ(CCH) aliphatic	

Table 3 *A summary of DRIFTS peak locations in Bovidae and Cheloniidae keratin*

	Amide I	*Amide II, α-like*	*Amide II, β-like*	*Amide III*
Bovid (n = 35)				
Average	1668.8	1543.0	1516.2	1251.8
Range	1650–1680	1535–1555	1513–1547	1244–1259
Standard deviation	8.36	4.71	1.60	4.50
N	35	29	6	35
Chelonid (n = 24)				
Average	1663.3	1539.9	1516.8	1247.0
Range	1647–1684	1536–1544	1515–1519	1236–1266
Standard deviation	9.51	2.41	1.20	9.31
N	24	13	24	24

diagnostic. Figure 1 shows a typical tortoiseshell spectrum (top) and an atypical spectrum (bottom). The amide II moiety of the top spectrum shows an intense absorption at 1516 cm^{-1}, which is characteristic of β-keratins, and thus reptiles. In the Cheloniidae family, the 1516 cm^{-1} absorption was present in all individuals tested but, surprisingly, this peak was also present in 17% ($n = 6$) of the bovids sampled (Fig. 2, bottom spectrum). Conversely, the amide II absorption at 1543 cm^{-1} has been described as diagnostic for α-keratins, and thus mammals. However, in this study it was present in only 83% ($n = 29$) of the bovids tested (Fig. 2, top spectrum) and

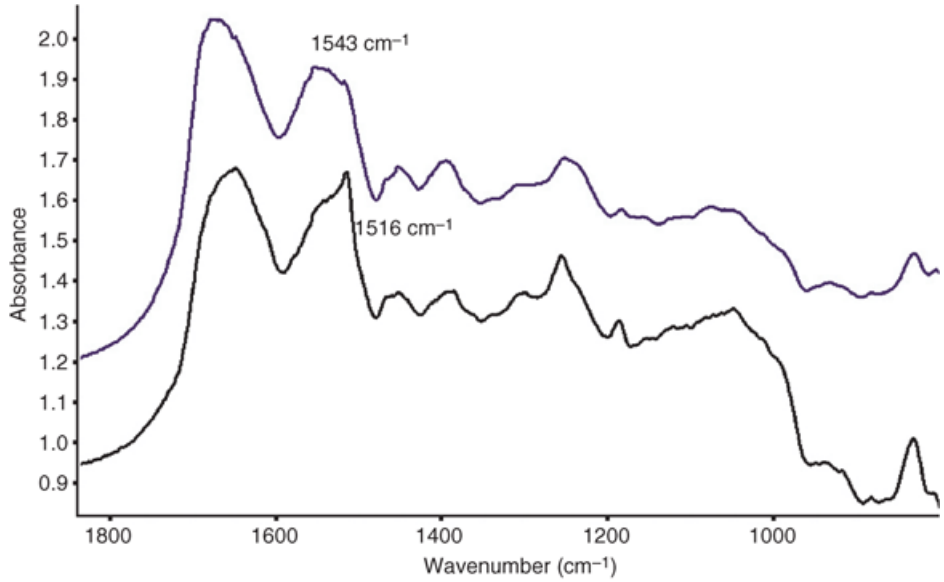

Figure 2 *DRIFTS spectra of two bovids. The top spectrum is of a Gaur horn sheath (*Bos frontalis*) and is typical for this class because it displays a prominent amide II stretch at 1543 cm^{-1}. The bottom spectrum is of a Tamaraw horn sheath (*Bubalus mindorensis*) and is atypical because the amide II stretch of this α-keratin is present at 1516 cm^{-1}, which has been associated with β-keratins.*

in 54% (n = 13) of the Cheloniidae (Fig. 1, bottom spectrum). The amide II stretch of each sample is listed in Table 1 and a summary of the data is displayed in Table 3.

Clearly these data indicate that, when using DRIFTS, making a taxonomic family assignment on the raw spectra alone may cause spurious conclusions. Therefore, we resorted to the statistical power of discriminant analysis to resolve this anomaly. Family assignment of keratin (i.e., Cheloniidae versus Bovidae) was accomplished using discriminant analysis software (TQ Analyst™).

When analysing keratin, the higher resolution of Raman spectroscopy (not used in our analysis) allows one to differentiate between the 1543 cm^{-1} and 1516 cm^{-1} absorptions. Based on the spectrum alone, one can make inferences of α- or β-keratin when using Raman spectroscopy (Akhtar and Edwards 1997; Edwards *et al*. 1998). However, our results show that prudence should be used when attempting to make the same distinctions based on DRIFTS spectra.

Taxonomic family assignment

Discriminant analysis is a multivariate statistical method that assists in the classification of data into distinct groups. Discriminant analysis of spectral data has been comprehensively reviewed by Enlow *et al*. (2005). The rationale of discriminant analysis in the present situation was to establish discriminant functions from known keratin standards (i.e., Bovidae versus Cheloniidae) and then use the discriminant function to classify keratin materials of uncertain origin. The software (TQ Analyst™) compiles an average spectrum from the known standards, and then each sample is assigned a numerical score based on the deviation from the calculated spectrum. These numerical scores are then plotted to provide a graphical representation.

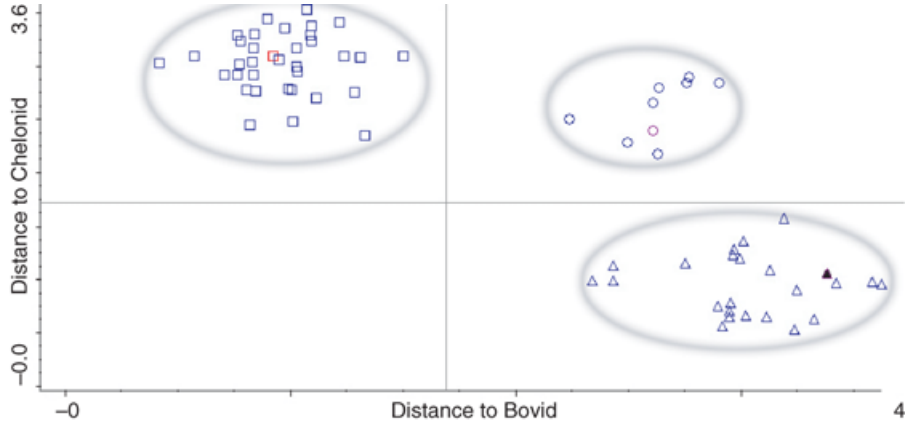

Figure 3 *A graphical representation of the discriminant analysis (DA). Squares (□) represent the Bovidae population, triangles (Δ) represent the Cheloniidae population, and the circles (O) represent the casein-based plastic population. The solid triangle is the DA result for the top spectrum of Fig. 1.*

Each keratin standard is validated by determining the Mahalanobis distance of the sample from the average spectrum. Therefore, each keratin is assigned to the nearest group centroid, based on its calculated Mahalanobis distance. The closer a sample is to a particular centroid class, the higher is the likelihood that it will be classified with that particular sample set (TQ Analyst 1992). In this study, each keratin standard (i.e., $n = 35$ Bovidae; $n = 24$ Cheloniidae) was correctly classified (see Fig. 3).

For the example depicted in Fig. 1 (top spectrum), our hypothesis (based on a spectral library search) is that the keratin was of sea turtle origin. We conducted a discriminant analysis experiment using 35 bovid and 24 sea turtle samples (Table 1) as our reference populations to calculate the discriminant function of each keratin type and to establish a performance index. The performance index is a measure of how well a discriminant analysis method can categorize spectra from calibration standards. The performance index of the discriminant analysis (Fig. 3) was 95.7%, which is an indication of how well the algorithm can differentiate between bovid and sea turtle keratins (Thermo Nicolet 2003). Reliable categorizations occur when the performance index exceeds 90% (TQ Analyst 1992). As demonstrated in Fig. 3, bovid and chelonid standards are segregated within their corresponding groups, and the Fig. 1 spectrum is nested within the chelonid population samples. The best explanation for this is that the questioned material is most similar to the keratins of sea turtle (Cheloniidae). Therefore, it can be inferred that its keratin structure, as characterized by DRIFTS, belongs to a member of the Cheloniidae family.

Identification of casein-based plastics

The identification of casein-based plastics follows the same strategy as described above. Inspection of a given spectrum reveals that it is dominated by the amide I, amide II and amide III moieties, which are ubiquitous of protein-based samples (Paris *et al*. 2005). Spectral library searches produce ambiguous results and occasionally indicate erroneously that a synthetic galalith sample is of keratin origin. Discriminant analysis of casein-based plastics reveals that

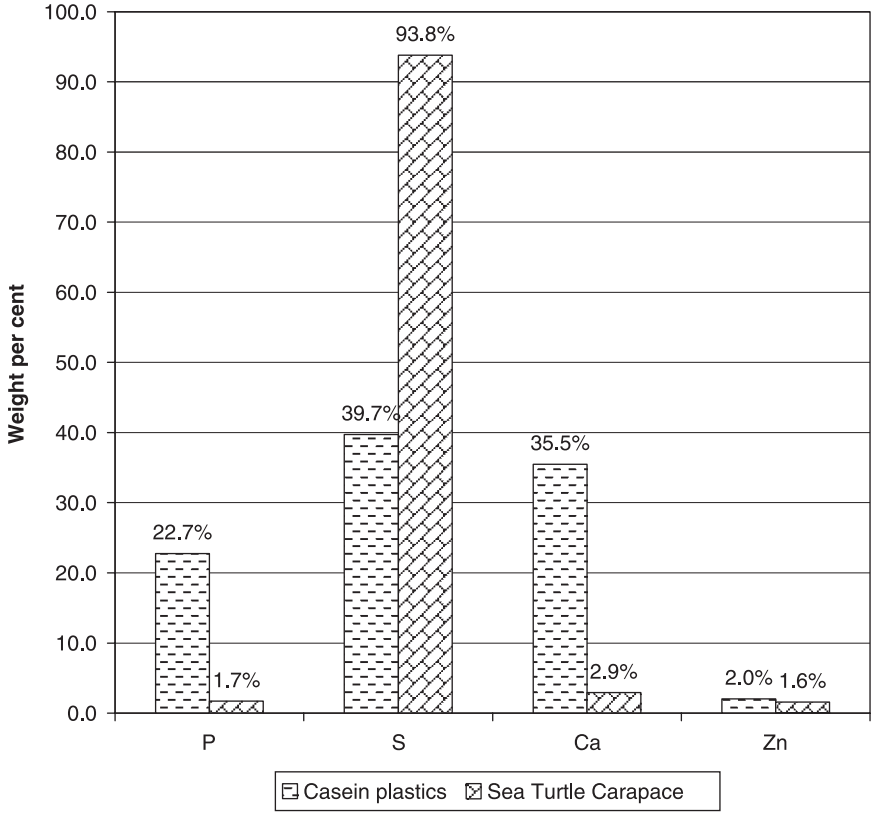

Figure 4 *A bar graph comparing the elemental analysis of sea turtle scutes (n = 21) and casein-based plastics (n = 10). The bars represent the average weight per cent of each population group.*

Table 4 *The per cent weight of selected elements of casein plastics and sea turtle keratin*

	P	*S*	*Ca*	*Zn*
Casein plastics (n = 10)				
Average	22.7	39.7	35.5	2.0
Range	10.1–27.4	14.4–61.2	9.9–65.7	1.0–6.3
Sea turtle (n = 21)				
Average	1.7	93.8	2.9	1.6
Range	0.8–3.2	85.2–97.5	0.2–7.7	0.7–3.9

this class of compound segregates separately from either Bovidae or Cheloniidae keratin; therefore its correct identification can be easily achieved (Fig. 3).

Confirmation of casein plastics relies on elemental analysis by XRF. Table 4 lists the results of the XRF analysis of casein plastics and of known sea turtle keratins. As demonstrated in Table 4, and Fig. 4, casein plastics have a higher average calcium content (>35%) compared

to sea turtle keratin (<3%). These findings are in agreement with the higher levels of calcium found in milk (the source material of casein plastic). Additionally, sea turtle keratin contains a higher sulphur content (>85%) compared to casein plastic (<62%). This is consistent with an abundance of cysteine amino acids in β-pleated reptile keratin.

CONCLUSIONS

DRIFT spectroscopy is a useful tool to assist in the analysis and identification of keratins and casein-based plastics. XRF analysis can be use as a confirmatory method for distinguishing keratins from casein-based plastics. Examination of a single sample is a simple matter when using the diffuse reflectance accessory in conjunction with FT–IR analysis. Spectral library matches operate at a fundamentally different level than discriminant analysis conclusions, but both classification systems are complementary. Spectral library searches are designed to provide the best and closest match to a spectrum, and the similarity is rated by a match score. A high match score implies a higher certainty of accuracy than a lower match score, but the significance of these scores is difficult to evaluate. Discriminant analysis of FT–IR spectra of keratin allows for comparing an unknown to a species population. When examining the graphical display of a discriminant analysis, the unknown is compared to a sample set and not to a single spectrum. Therefore, discriminant analysis allows the analyst to assess the unknown against a normal distribution of samples, rather than rely on the single best answer of a spectral library search.

Discriminant analysis of these spectral data provides strong inference of family provenance (with a resulting performance index of 95.7%). All keratin standards used in this study ($n = 35$ Bovidae; $n = 24$ Cheloniidae) were correctly classified with the discriminant analysis. We have demonstrated that DRIFTS, combined with discriminant analysis, provides a robust method for differentiating bovid and sea turtle keratins. The results provide a quantitative method for identifying keratins used in constructing historical artefacts, and for identifying keratin-based artefacts commonly seen in the wildlife trade.

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