Received: 28 January 2021 | Revised: 12 May 2021 | Accepted: 18 June 2021

DOI: 10.1111/csp2.486

CONTRIBUTED PAPER

Conservation Science and Practice
A journal of the Society for Conservation Biology
Open Access

WILEY

Shining NIR light on ivory: A practical enforcement tool for elephant ivory identification

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Funding information
Royal Thai Government, PhD Scholarship; James Cook University

Abstract

The elephant ivory trade remains controversial because of concerns about the extinction risk of elephants and the different needs of CITES member states. Thailand's situation is particularly contentious because of the different legal status among types of elephant ivory. Thai law allows the local sale of ivory from domesticated Asian elephants, which creates challenges for Thai enforcement officers in identification of ivory provenance. We investigated the capacity of non-destructive Near Infrared (NIR) spectroscopy (600–1700 nm), combined with Partial Least Squares Discriminant Analysis (PLS-DA), to discriminate between ivory from African, wild Asian and domesticated Asian elephants. Ivory spectra of 64 elephants were divided randomly into calibration and validation datasets. We were able to determine elephant ivory provenance at both the interspecies (African and Asian elephant ivory), and within species (wild and domesticated Asian elephant ivory) classifications with 100% accuracy. These results showed the potential use of handheld NIR spectrometers for rapid assessments of ivory provenance, as well as a forensic tool for wider enforcement.

KEYWORDS

African elephant, Asian elephant, CITES, elephant ivory, enforcement, ivory identification, ivory trade control, near infrared (NIR), PLS-DA

1 | INTRODUCTION

Trade in ivory remains an issue of international concern because of its links with the illegal killing of elephants, and the cross-border smuggling of ivory. Currently the international trade in ivory, from both African and Asian elephants, is largely prohibited for commercial purposes, as these species are listed under Appendix I of the Convention for International Trade in Endangered Species of Wild Fauna and Flora (CITES). Exceptions have been made for African elephants from countries if their elephant populations are listed in Appendix II (CITES, 2020). CITES member countries, with a domestic ivory market, are recommended to have comprehensive controls in place to ensure that their markets only operate domestically, and that there are no detrimental effects on elephants in other countries (CITES, 2019). Recommendations include closing the legal domestic ivory markets for commercial purposes if that trade involves illegal activities in other countries.

Thailand permits a domestic trade in ivory from domesticated Asian elephants. The ivory trade of domesticated Asian elephants must comply with the Elephant Ivory Act, while ivory from both wild Asian elephants and African elephants is prohibited from commercial use (Chaitae, Gordon, Addison, & Marsh, in press). Consequently, given the different legal provision of ivory from these three different sources in Thailand, there is a need for a reliable, non-destructive method to identify the provenance (African, wild Asian, or domesticated Asian elephants) of ivory objects to enable the comprehensive control of the domestic ivory trade in Thailand (Chaitae et al., in press). Having such a methodology would ease international pressure for closure of the trade, an action that would jeopardize the livelihoods of local merchants along the supply chain.

An elephant tusk is mainly composed of dentine, surrounded by a thin outer layer of cementum (Baker, Jacobs, Mann, Espinoza, & Grein, 2020). The surface of the tip is coated with a layer of enamel. Dentine largely consists of inorganic components embedded in an organic matrix of collagenous proteins (Locke, 2008; Raubenheimer et al., 1998). Hydroxyapatite is the main inorganic component, while major amino acids in the collagen include glycine, proline, and hydroxyproline (Godfrey, Ghisalberti, Beng, Byrne, & Richardson, 2002). The chemical components in ivory are determined by the elephant's physiology and diet, and the environment in which it lives (Prozesky et al., 1995; Raubenheimer et al., 1998; van der Merwe et al., 1990). The vast ranges of Asian and African elephants (Blanc, 2008; Choudhury et al., 2008), result in location-specific chemical signatures in ivory reflecting environmental and geographical conditions (Prozesky et al., 1995; Raubenheimer et al., 1998; Singh, Goyal, Khanna, Mukherjee, & Sukumar, 2006). The isotope ratios of carbon, nitrogen, hydrogen, sculpture, and oxygen have proved useful for pinpointing the origins of African ivory (Ziegler, Merker, Streit, Boner, & Jacob, 2016). Different elemental and amino-acid signatures have also been documented for ivory obtained from different parts of the range of African elephants (Prozesky et al., 1995; Raubenheimer et al., 1998). Asian and African sourced ivory has also been successfully distinguished based on elemental variation (Singh et al., 2006). Whilst these approaches have potential for forensics to support law enforcement, they are destructive and require specialized laboratories and expertise, making them unsuitable for routine inspection or screening (Baker et al., 2020).

Near-Infrared spectroscopy (NIRS) is an approach suitable for examining the composition of intact samples, using the light absorption of chemical bonds within a range of the NIR spectrum (700–2,500 nm) (Osborne, 2006). Prominent NIR absorption in ivory reflects protein (C–H from collagenous protein, N–H from protein residues), and water (O–H) interacting with proteins (Power, Ingleby, Chapman, & Cozzolino, 2019; Shimoyama, Morimoto, & Ozaki, 2004). The NIRS technique has successfully distinguished elephant ivory from other ivories, bones and horns (Power et al., 2019; Shimoyama et al., 2004), and documented differences in the NIR absorption of the soft and hard ivories of African elephants (Shimoyama et al., 2004). None of these NIRS studies assessed Asian ivory derived from both domesticated and wild populations.

Here, we investigate the potential for the NIRS technique to discriminate between ivory derived from African, wild Asian, and domesticated Asian elephants. As this is an exploratory study, we used three devices developed for the agricultural industry to enable comprehensive spectral coverage from Visible to Long-wave NIR. Our study was in response to enquiries from Thai enforcement officers involved in the routine inspection of ivory about the availability of non-destructive, rapid techniques to screen tusks during routine trade monitoring to identify the provenance of the ivory.

2 | METHODS

2.1 | Samples

Data were collected from 86 samples of raw ivory from 64 elephants as follows:

1. Thirty samples of African elephant ivory (hereafter African ivory), which were the cut pieces of confiscated ivory that had been securely stored at the Thai Department of National Parks, Wildlife, and Plant Conservation. The exact origins of the African ivory samples were not known. We assumed that each specimen was from a different animal.
2. Thirty samples of wild Asian elephant ivory (hereafter wild Asian ivory), mostly whole tusks, which had been removed from 17 free-living elephants from different protected areas in Thailand. These samples were held by the Department of National Parks, Wildlife, and Plant Conservation.
3. Twenty-six samples of domesticated Asian elephant ivory (hereafter domesticated Asian ivory) were tusk tips, and whole tusks with trimmed tips, which had

been removed from 17 government-owned elephants. The elephants were raised in captivity, at Lampang province by the Forest Industry Organization, and regularly provided with a diet of agricultural crops (e.g., banana, sugar cane, corn), cultivated grasses [e.g., Bana (hybrid *Pennisetum* spp), Napier (*Pennisetum purpureum*), Pangola (*Digitaria eriantha*)], and pelleted food. The animals could also access wild forage such as grass and bamboo.

The ivory samples varied in size, shape (e.g., pieces, complete tusks) and tusk position (e.g., tip, base) (Figure S1). Additional information such as sex was available for some of the Asian elephant ivory samples, most of which was from male elephants. Due to the uncontrolled nature of the sources of the ivory, we ignored factors relating to the host animals (e.g., sex, age), and the ivory (e.g., storage condition and duration), reflecting the situation facing enforcement officers.

2.2 | Data collection

NIR spectra were collected using three NIRS portable devices that collectively cover the wavelengths between 600 and 1,700 nm. All three devices were developed by the Near Infrared Technology Laboratory, Department of Food Engineering, Faculty of Engineering at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand (Figure 1a) for use in the agriculture industry. The wavelengths covered by each of the devices follows:

- Device 1 (600–1,100 nm) at 0.46 nm scanning resolution;
- Device 2 (700–1,200 nm) at 0.23 nm scanning resolution, and;
- Device 3 (900–1,700 nm) with scanning resolution of 2.50 nm.

Device 1 and Device 2 collected data in interactance mode; the NIR light penetrates under the scanned surface before reflecting to the device's detector (Pasquini, 2003). For longitudinal positions (see below), therefore, scans of these two devices included data from both cementum and dentine. Whereas the reflectance device, Device 3, sampled the cementum content only. The scans of the cross-sectional cuts for all three devices measured dentine constituents. See Table S1 for the device specifications.

The spectra measurements were taken at positions along and around the sample (longitudinal plane), as well as in the cross-section plane, if possible. Scanning positions avoided areas with notable dirt, cracks and markings. The scanning positions were cleaned with a dry cloth and temporarily marked with tapes to guide positioning. The scans were conducted on a clean area, ensuring that the device's reading probe did not overlap

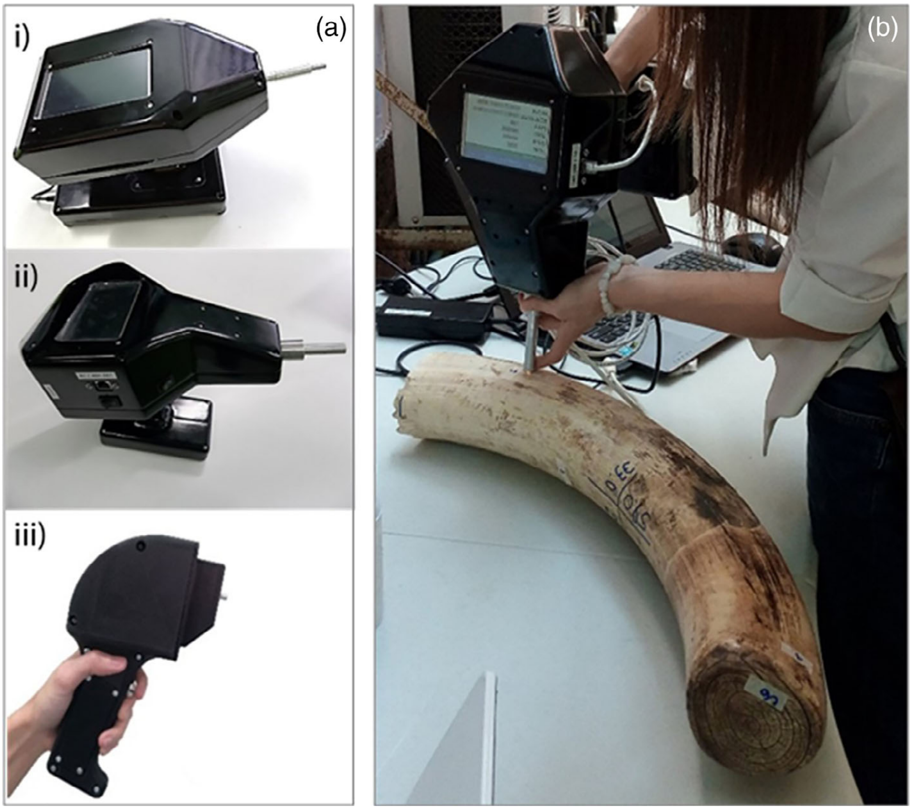

FIGURE 1 Handheld NIR spectrometers (a): Device 1 (i), Device 2 (ii), and Device 3 (iii) used for making a spectral measurement of an ivory sample (b)

with the markings. The reading probe was placed against a smooth surface in a perpendicular position to prevent external light interference (Figure 1b). Each reading area covered 50 mm. diameter. Teflon material was used as a reference before measuring absolute absorbance of ivory samples. The number of scans per tusk sample varied from 2 to 9.

2.3 | Analysis

The spectral data were processed and analyzed using Unscrambler 11 software (CAMO, Oslo, Norway). Exploration of the raw spectra was conducted using line plotting and resulted in the removal from the analyses of spectra that were incomplete due to over-absorption. The few scans at tusk tips, which included enamel, were excluded from analysis due to their small sample size. The numbers of spectra from Device 1, Device 2, and Device 3 used in the analyses were 391, 410, and 406, respectively (Table S2). Analyses were conducted for longitudinal, and cross-sectional planes (if available) for each device. To prevent pseudo-replication, multiple ivory spectra from each elephant individual were averaged to obtain a single mean spectrum for further analyses, resulting in the following samples sizes for testing:

1. *Interspecies classification*: The data from the two types of Asian elephant ivory, wild and domesticated, were combined into one group for comparison with the African ivory samples. Across both species, the sample size was 64 in the longitudinal plane, and 43 in the cross-sectional plane.
2. *Within species classification*: Because few samples of wild Asian ivory were cut in cross-section, only longitudinal scans sourced from Asian ivory, were used for distinguishing between wild and domesticated populations. Across Asian elephant types, the total sample size was 34.

To eliminate noise, mean spectra were processed using a combination of Savitzky–Golay smoothing and derivatives, and standard normal variate (SNV) before further analysis (Table S3). IBM SPSS Statistics 26 software was used to divide the data into calibration (Training) and validation (Test) sets in a 2:1 ratio. In recognition of the limitations of our small sample size, we undertook 10 repeated samplings to generate 10 random calibration and validation sets to examine the robustness of the results.

Discrimination models were developed using the Partial Least Squares Discriminant Analysis (PLS-DA). Each spectrum was assigned a dummy variable (ivory type) as a reference value. The model was then developed from the calibration data sets by regressing the spectral data against the assigned reference value. Samples were classified according to ivory type based on non-overlapping cut-off value(s). The correct classification rate (CC) was based on the percentage of non-overlapping samples to the total sample. The PLS-DA results of the 10 samplings were similar in both interspecies (longitudinal and cross-sectional planes), and within species classifications. We present CC medians and ranges for the 10 samplings (Table 1) and illustrate the first sampling for the classification results. Details for each sampling are in Tables S4 to S6.

3 | RESULTS

In each case, only spectra collected from the best performing NIRS device is presented and discussed here. Additional information on differences between the results obtained using three devices are in - Supporting Information (Result S1).

3.1 | Partial least squares discriminant analysis: Interspecies classification

The correct classification rates ranged from 83 to 100% for the 10 samplings of the 42 spectra obtained in the longitudinal plane with an overall median value of 96% using Device 3 (Table 1, section a-i). All 21 of the cross-sectional samples were correctly classified as African or Asian ivory using Device 1 (Table 1, section a-ii). See Tables S4 and S5 for the results of each sampling.

3.1.1 | Longitudinal plane samples

Ivory spectra clustered within species with some overlap (Figure 2a). NIR light absorbance varied between the two species as noticeable layers in the same wavelength region of the processed spectra (Figure 2b). Negative peaks of the regression coefficients of the PLS-DA model showed the important variables for classification of the ivory types, including the regions at 1,150, 1,215, 1,385, 1,430 and 1,585 nm (Figure 2c).

3.1.2 | Cross-sectional plane samples

The classification results correspond with the grouping of samples illustrated in the PCA score plot (Figure 3a). Absorption of NIR light clearly diverges around the 650–830 nm region (Figure 3b). The discrimination of the ivory species in cross-section scans was influenced by peaks within this region and at 960 nm (Figure 3c).

TABLE 1 PLS-DA results for (a) interspecies classification: African versus Asian ivory, and (b) within species classification: wild versus domesticated Asian ivory

	Calibration			**Validation**			**Total**		
		CC			**CC**			**CC**	
Ivory source	***N***	**Median**	**Range**	***N***	**Median**	**Range**	***N***	**Median**	**Range**
(a) Interspecies classification									
(i) Longitudinal plane (Device 3)									
Af	20	100%	90.00–100%	10	90.00%	60.00–100%	30	96.67%	83.33–100%
As	22	100%	90.91–100%	12	91.67%	58.33–100%	34	95.59%	85.29–100%
Overall	42	100%	92.86–100%	22	90.91%	59.09–100%	64	96.09%	84.38–100%
(ii) Cross-sectional plane (Device 1)									
Af	12	100%	100%	7	100%	100%	19	100%	100%
As	9	100%	100%	5	100%	100%	14	100%	100%
Overall	21	100%	100%	12	100%	100%	33	100%	100%
(b) Within species classification									
Longitudinal plane (Device 3)									
WAs	11	100%	100%	6	100%	100%	17	100%	100%
DAs	11	100%	100%	6	100%	100%	17	100%	100%
Overall	22	100%	100%	12	100%	100%	34	100%	100%

Note: Medians and ranges of correct classification rates (CC) obtained from the ten repeated samplings of the results. Ivory types: Af: African, As: Asian, WAs: wild Asian, DAs: domesticated Asian ivory.

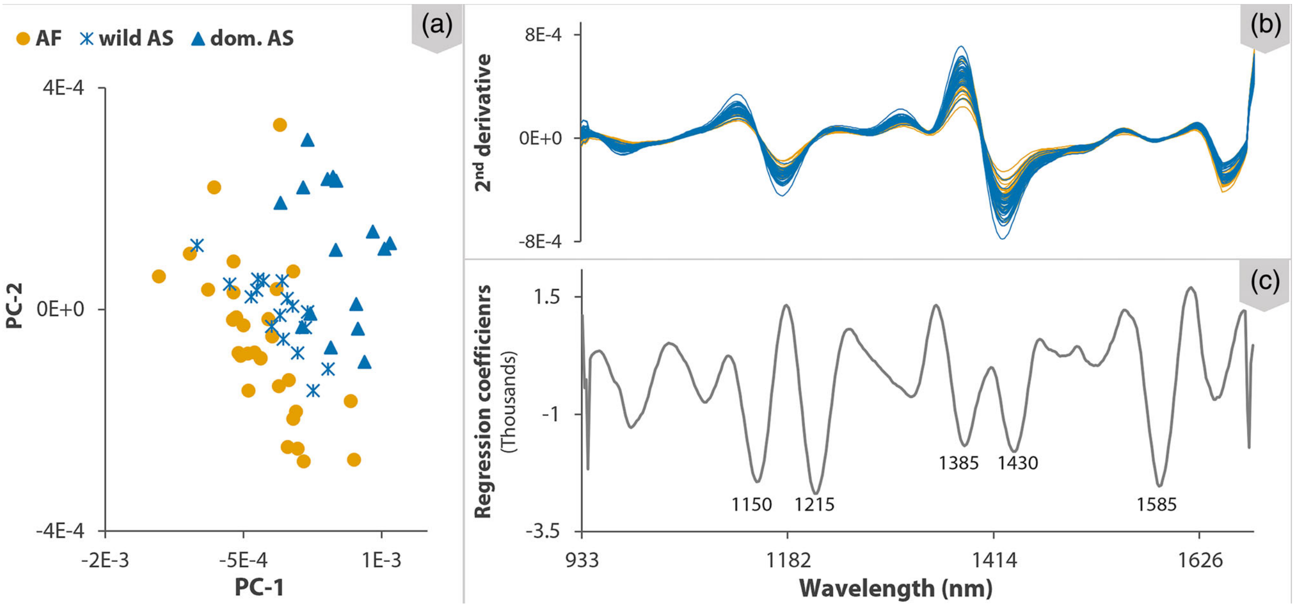

FIGURE 2 PCA score plots for PC-1 and PC-2 for differentiating between African and Asian ivory in the longitudinal plane (a), processed spectra (b), and regression coefficients (c), all using Device 3. Orange markers/lines: African ivory, blue markers/lines: Asian ivory. There was no clear separation of data between two ivory species in PCA score plots (a). However, difference in light absorption intensity was apparent in the longitudinal plane spectra (b). The classification of ivory types was largely based on wavelengths 1,150, 1,215, and 1,585 nm (c)

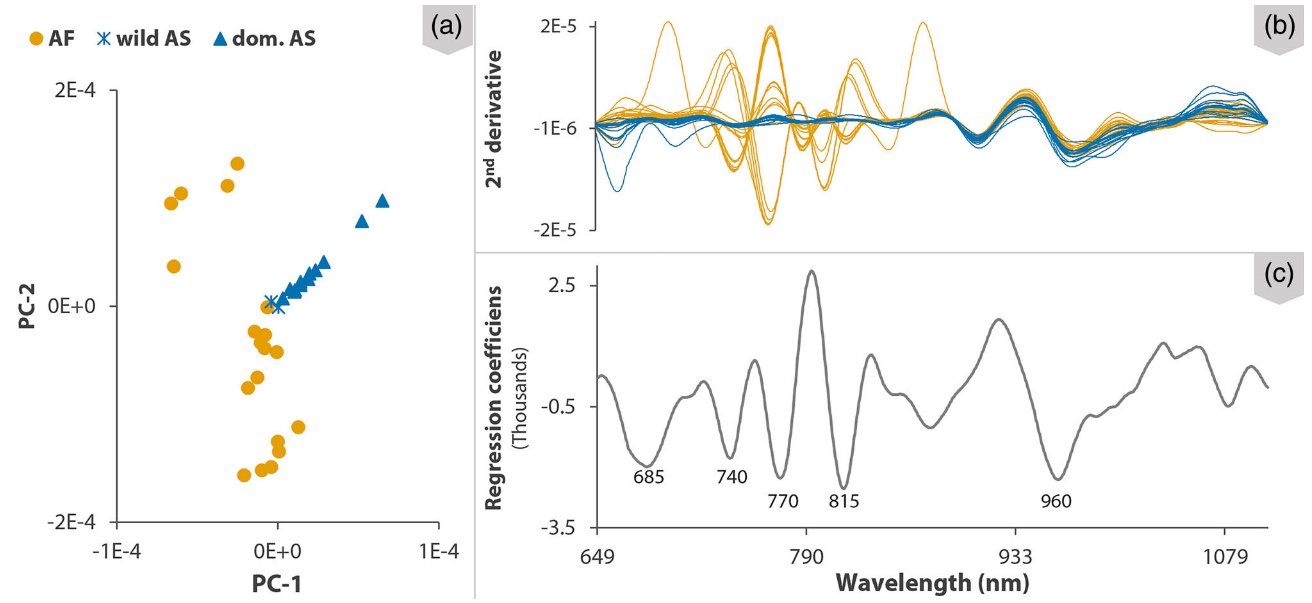

FIGURE 3 PCA score plots for PC-1 and PC-2 for differentiating between African and Asian ivory in cross-sectional plane (a), processed spectra (b), and regression coefficients (c) obtained from Device 1. Orange markers/lines: African ivory, blue markers/lines: Asian ivory. Dentine data was different between two ivory species as presence of the data cluster of Asian ivory in PCA score plots (a). Region 650–830 nm included the majority of distinctive spectra with different light absorption (b) and corresponded to the interspecies classifications (c)

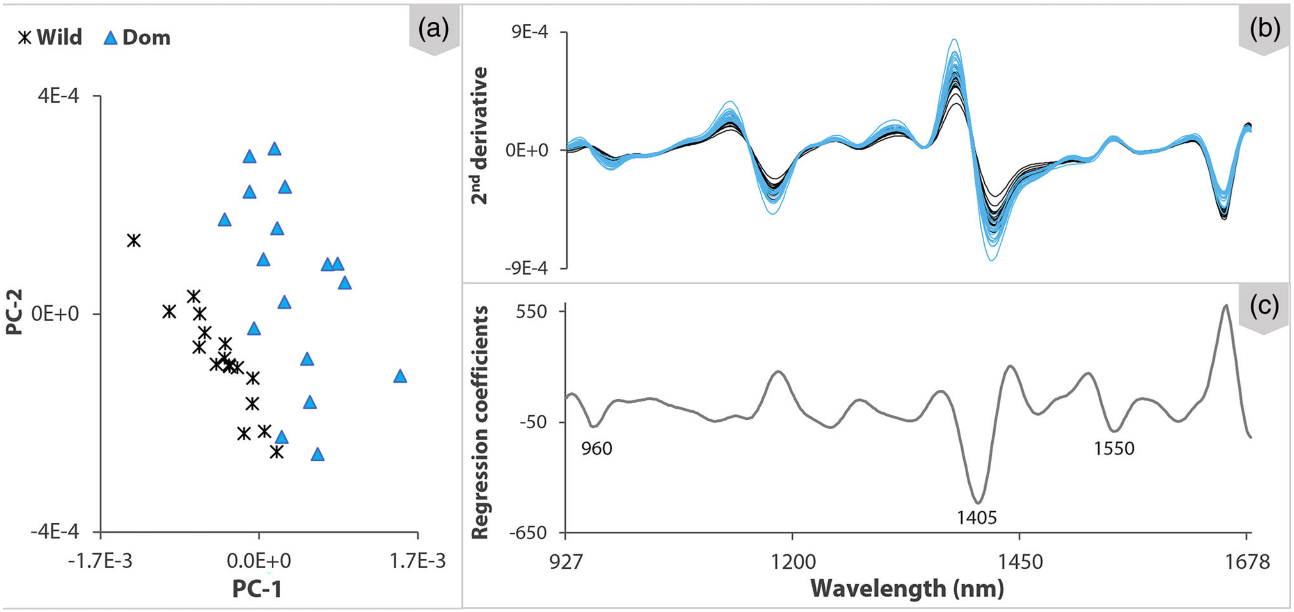

FIGURE 4 PCA score plots for PC-1 and PC-2 for differentiating between wild and domesticated sources of Asian ivory in longitudinal scans (a), processed spectra (b) and regression coefficients (c) obtained from Device 3. Black markers/lines: wild Asian ivory, blue markers/lines: domesticated Asian ivory. Separation of the data was noticeable both in the PCA score plot (a) and ivory spectra (b). Within species classification was enabled by differences between the spectra in the absorption regions at 960, 1,405, and 1,550 nm (c)

3.2 | Partial least squares discriminant analysis: Within species classification

In distinguishing between domestic and wild Asian elephant ivory, Device 3 was 100% correct for both the calibration and the validation sets for all 10 samplings (Table 1, section b). The specific results of the 10 repeated samplings are presented in Table S6.

Separation of ivory sample types were clearly depicted by the PCA score plot (Figure 4a). The spectra from the ivory from the wild and domesticated Asian elephants were generally similar in the pattern of light absorption,

TABLE 2 NIR wavelength regions for ivory discrimination

Region (nm)	Classification (LP: longitudinal plane, XP: cross-sectional plane)	Absorbing functional group	References
685	Interspecies: XP	C–H	Workman Jr. and Weyer (2008)
740	Interspecies: XP	O–H and C–H	Shimoyama et al. (2004)
		C–H	Osborne, Fearn, and Hindle (1993)
770	Interspecies: XP	O–H	Osborne et al. (1993)
815	Interspecies: XP	N–H	Osborne et al. (1993); Stuart (2004)
960	Interspecies: LP	N–H and O–H	Shimoyama et al. (2004)
	Within species: LP	O–H	Kolmas, Marek, and Kolodziejski (2015)
1,150	Interspecies: LP	C–H	Burns and Ciurczak (1992); Workman Jr. and Weyer (2008); Power et al. (2019)
1,215	Interspecies: LP	C–H	Burns and Ciurczak (1992); Power et al. (2019)
1,385	Interspecies: LP	O–H	Kolmas et al. (2015)
1,405	Within species: LP	O–H	Kolmas et al. (2015)
1,430	Interspecies: LP	N–H	Vincke et al. (2014)
		O–H	Kolmas et al. (2015)
1,550	Within species: LP	N–H	Workman Jr. and Weyer (2008)
1,585	Interspecies: LP	O–H	Workman Jr. and Weyer (2008)

with the appearance of peaks in the same region, but with differences in absorbance intensities (Figure 4b). The separation between the spectra of the two Asian elephant ivory types was apparent where spectra of each ivory types were close together and formed separate layers (Figure 4b). The within species classification was significantly influenced by absorption regions at 1,405 nm, coupled with variables at 960 and 1,550 nm (Figure 4c).

3.3 | NIR bands of ivory

The PLS-DA classification models were largely determined by wavelengths that diverged in absorbance as negative peaks in the regression coefficients (Figures 2c, 3c, and 4c). Table 2 presents the important bands for the discrimination of ivory in our samples.

4 | DISCUSSION

4.1 | Ivory classification using NIR spectroscopy

Our findings concurred with the previous studies that indicated great potential of the NIR spectroscopy (NIRS) technique as a non-destructive method of compositional analysis for identify the source of the ivory (Power et al., 2019; Shimoyama et al., 2004). We were able to use NIRS to discriminate ivory from African and Asian elephants, and demonstrate, for the first time, that the technique can also be used to differentiate between wild and domesticated sources of Asian ivory.

4.1.1 | Interspecies classification

NIRS discriminated between African and Asian ivory in both planes (Table 1, section a). Similar to other studies of ivory using NIRS devices, our scans also showed differences in the NIR spectra in regions influenced by protein and water (Power et al., 2019; Shimoyama et al., 2004). Protein contents in ivory are associated with peaks in the N–H and C–H functional groups. Collagenous protein, the main organic component in ivory, largely comprises glycine, proline, and hydroxyproline amino acids (Godfrey et al., 2002). N–H absorption likely represents the presence of these protein residues as they are abundant in ivory (Raubenheimer et al., 1998). In previous studies using NIRS, C–H bonds have been attributed to the chemical constituents of collagen such as CH, CH_2, and CH_3 overtones and stretching of proteins (Power et al., 2019; Shimoyama et al., 2004). Mineralized tissues, including teeth, contain around 10% water per dry weight (Godfrey et al., 2002). In this study, O–H bonds were important for classifying ivory in different wavelength regions, including 740, 960, and 1,385 nm. In a study of hydroxyapatite in human teeth, O–H bands involving hydroxyapatite were attributed to water, structural hydroxyl O–H groups and surface P–OH groups (Kolmas et al., 2015).

Like other teeth, tusks mainly comprise inorganic and organic components, but the proportion of these components varies among species and teeth structures. The ratio of inorganic to organic matter in human dentine is about 70:20, excluding water (Dorozhkin & Epple, 2002; Godfrey et al., 2002). The organic fraction is higher in cementum than dentine (D'souza et al., 2020). Both dentine and cementum are richer in collagen, and less mineralized than enamel (Hillson, 1986). Fresh African elephant ivory contained about 65% inorganic matter (Godfrey et al., 2002). An ivory classification study, using Raman spectroscopy, indicated that the dentine of African ivory has higher mineral content and lower organic component in comparison with Asian ivory (sourced from Thailand) (Edwards, Hassan, & Arya, 2006). Other authors, using X-ray fluorescence to distinguish ivory, reported differences of about 20 elements between the two elephant species, for example, African ivory has higher calcium content, while phosphorus is greater in both Indian and Thai ivory (Buddhachat et al., 2016; Singh et al., 2006). Indian ivory also had higher concentration of hafnium and strontium than African ivory (Singh et al., 2006).

Geochemical factors and the availability of food and water contribute to the composition and properties of ivory (Raubenheimer et al., 1998). The brittle tusks of African elephants living in arid areas, where vitamin C is in short supply show low concentrations of amino acids and under-hydroxylation of protein (Raubenheimer et al., 1998). As collagen synthesis requires vitamin C for the hydroxylation of proline and lysine (D'souza et al., 2020), insufficient vitamin C results in under-hydroxylation and likely further decreased strength in the collagen structure (Raubenheimer et al., 1998). Water content also affects the mechanical properties of ivory, for example, elasticity, strength, and toughness (Vollrath, Mi, & Shah, 2018). It is common knowledge among Asian craftsmen that African ivory, from dry regions, (soft ivory) cracks easily, whilst hard ivory from Asian elephants and African forest elephants is denser and tougher and more suitable for carving (Martin & Stiles, 2003; Walker, 2009).

The origin of our African ivory samples was unknown but assumed to represent the large range of elephants on the African continent, while our samples of Asian ivory were locally sourced in Thailand. This difference is a plausible explanation for the spectra of African ivory being more dispersed in the PCA score plots (Figures 2a and 3a). The PCA score plots (Figure 2a) indicate that the differences between the spectra of African ivory and domesticated Asian ivory are greater than those between African ivory and ivory sourced from wild Asian elephants. This similarity of spectral data might be associated with forest African elephants and Asian elephants inhabiting forests in the two continents, which are the sources of the hard ivory. Moreover, free access to food diversity of free-living African and Asian elephants might also minimizes difference of ivory composition. The spectral differences between domesticated Asian ivory and African ivory were more obvious in the cross-sectional scans in which the Asian ivory was mostly from domesticated sources, (Figure 3a), resulting in 100% correct classifications for all 10 samplings. Thus, the variation across the range of ivory from African elephants lies outside the signature provided by domesticated Asian elephant ivory, indicating that the NIRS technique is potentially useful for distinguishing the legally tradable ivory from domestic elephants in Thailand from illegal ivory from African sources.

4.1.2 | Within species classification

The NIRS device correctly identified the source of each sampled of Asian elephant ivory with 100% accuracy (Table 1, section b). The variation in the cementum spectra contained useful information for the classification of the two types of Asian elephant ivory (Figure 4a). The important contents responsible for classifying two Asian ivory were water (O–H) and protein residues (N–H) (Table 2).

Domesticated elephants were transferred into the tourism industry after the 1989 logging ban in Thailand, and now have less opportunity to forage on their natural diet (Godfrey & Kongmuang, 2009; Phuangkum, Lair, & Angkawanith, 2005). The domesticated elephants regularly feed on agricultural crops, non-native cultivated grasses, and pelleted food plus pineapple plants common in elephant camps in different parts of Thailand. These crops also increase exposure to agricultural chemicals (Phuangkum et al., 2005). In contrast, free-ranging Asian elephants forage on hundreds of different plant species across forest types and seasons; for example, at least 260 plant species of browse and grass plants are consumed by elephants in Thailand's Huai Kha Khaeng Wildlife Sanctuary (Sukmasuang, 2003). Thus, there is diet overlap between the wild and domesticated elephants. Wild elephants have been observed feeding on crops bordering their natural habitats in Thailand (Pla-Ard, Sukmasuang, & Srinopawan, 2020; van de Water & Matteson, 2018; Vinitpornsawan, Bunchornratana, Pukhrua, & Panyawiwatanakul, 2016), while domesticated elephants may occasionally access to natural forage. This overlap in forage is likely to be reflected in the NIR spectra from the two ivory types.

Studies of carbon isotopes in elephant tooth enamel indicate that browse plants dominate the diet of wild elephants today (Cerling, Harris, & Leakey, 1999). Like Indian elephants (Joshi & Singh, 2008), wild elephants in Thailand spend larger amounts of time browsing than grazing (Sukmasuang, 2003). The protein content of browse plants is higher than grasses, and can contribute about 70% of organic carbon for bone collagen synthesis in Asian elephants (Sukumar, 2003; Sukumar & Ramesh, 1995). The food provided to two domesticated populations of Thai elephants was low in protein and calcium (Romain et al., 2014). These elephants mainly obtained protein from Bana grass, corn cobs, and pellets. Calcium is the most abundance mineral in ivory (Buddhachat et al., 2016; Raubenheimer et al., 1998) and is part of hydroxyapatite crystals that account for the rigidity of the tusk (Vollrath et al., 2018). Tusk material is largely composed of dentine, the main component of ivory products (Baker et al., 2020). Further NIRS work on dentine will be beneficial for identifying products of Asian ivory.

4.2 | Application of NIRS technique for ivory trade control

Reliable identification of the source of ivory has been an important challenge facing enforcement officers, particularly for in-field tasks (Baker et al., 2020). In circumstances such as those found in Thailand, where the legal domestic trade is only allowed for ivory derived from domesticated Asian elephants, ivory identification is challenging and important. Ivory investigations include screening and routine inspections, which need to be conducted in a non-destructive manner. A portable identification tool, like that used in the analysis presented here, has the potential to facilitate in-field investigations by enforcement officers. Moreover, separate analyses between longitudinal and cross-sectional spectra from tusks would improve discrimination between major ivory forms; that is, use of longitudinal discrimination for whole tusk, applying cross-sectional analysis for identifying ivory products.

The NIRS technique is already widely used by the agro-food industry and pharmaceutical industries (Osborne, 2006; Workman Jr. & Weyer, 2008). A portable NIR spectrometer costs less than the equipment and chemicals used in conventional analyses. Commercial handheld NIR spectrometers can be manufactured at low cost, and are easy for non-specialists to use (Beć, Grabska, Siesler, & Huck, 2020; Vance, Tolleson, Kinoshita, Rodriguez, & Foley, 2016). The locally built NIRS devices we used cost around USD 5,000 for each of these prototype models. We applied available NIRS devices varying in specifications (e.g., measuring mode, resolution, and wavelength) to expand coverage of our exploratory study. Our results can inform the customization of an NIR spectrometer for ivory discrimination. The rapid and reliable results we obtained fulfill the needs of enforcement officers, particularly for routine inspection or initial screening. The modest cost of this approach should facilitate their accessibility for existing regional wildlife enforcement units in Thailand. Availability of commercial devices could further enable use in wider enforcement communities.

It is important to note that our findings are based on local samples of Asian elephant ivory in Thailand. The application for other uses and in other countries needs to be done with caution as different geological and environmental conditions influence ivory composition. Complete characterization for spectra peaks needs comprehensive attention in relation to the chemical aspect because of the broad overlapping appearance of spectra in the NIR region (Stuart, 2004).

5 | CONCLUDING REMARKS

This study clearly established the potential of NIRS to discriminate between both: (a) Asian and African ivory, and (b) wild versus domesticated Asian ivory. NIRS technology is very simple to use in the field, and has the potential to enable the timely and cost-efficient identification of ivory by enforcement officers using portable NIR spectrometers. Equipping enforcement officers with such devices should enable efficient and effective screening and routine inspection of ivory in the field, including in remote areas. Implementation of this technique, particularly for confiscation and prosecution, will require further research to capture the variation in ivory among elephants with diverse feeding regimes and the development of a customized device. This advance would enable the effective implementation of the Thai laws designed to enable the sustainable use of ivory derived from the routine management of domesticated Asian elephants.

ACKNOWLEDGMENTS

Department of National Parks, Wildlife and Plant Conservation, and Forest Industry Organization have provided the required approvals to use ivory samples for this study. We thank A. Noochdumrong, W. Jianratanasawat, N. Khanha, and K. Laochote for assists in sample acquiring;

and S. Phoungmalee for help with data collecting. This research was financially supported by James Cook University. The authors would like to thank the Thai government for sponsoring the first author's PhD at James Cook University.

CONFLICT OF INTEREST

The first and last authors are officers in Thai's Department of National Parks, Wildlife and Plant Conservation. The other authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Apinya Chaitae: Conceptualization, study design, data collection and facilitating, analysis, manuscript drafting, editing and reviewing. **Ronnarit Rittiron:** Conceptualization, study design, analysis, editing and reviewing. **Iain J. Gordon:** Conceptualization, study design, editing and reviewing. **Helene Marsh:** Study design, editing and reviewing. **Jane Addison:** Study design, editing and reviewing. **Suttahatai Pochanagone:** Study design, data collection and facilitating, analysis, editing and reviewing. **Nattakan Suttanon:** Data collection and facilitating, editing and reviewing

DATA AND MATERIALS AVAILABILITY

Relevant data used in this study are available as Supporting Information files.

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REFERENCES

Baker, B., Jacobs, R., Mann, M., Espinoza, E., & Grein, G. (2020). *CITES identification guide for ivory and ivory substitutes* (4th ed.). Washington, DC: World Wildlife Fund Inc.

Beć, K. B., Grabska, J., Siesler, H. W., & Huck, C. W. (2020). Handheld near-infrared spectrometers: Where are we heading? *NIR News*, *31*(3–4), 28–35.

Blanc, J. (2008). Loxodonta africana. The IUCN Red List of Threatened Species 2008: e.T12392A3339343. Retrieved from https://www.iucnredlist.org/species/12392/3339343

Buddhachat, K., Thitaram, C., Brown, J. L., Klinhom, S., Bansiddhi, P., Penchart, K., … Nganvongpanit, K. (2016). Use of handheld X-ray fluorescence as a non-invasive method to distinguish between Asian and African elephant tusks. *Scientific Reports*, *6*, (24845), https://doi.org/10.1038/srep24845.

Burns, D. A., & Ciurczak, E. W. (1992). *Handbook of near-infrared analysis*. New York, NY: M. Dekker.

Cerling, T., Harris, J., & Leakey, M. (1999). Browsing and grazing in elephants: The isotope record of modern and fossil proboscideans. *Oecologia*, *120*, 364–374.

Chaitae, A., Gordon, I. J., Addison, J., & Marsh, H. (In press). From protection of elephants to sustainable use of ivory in Thailand. *Oryx*. https://doi.org/10.1017/S0030605321000077

Choudhury, A., Lahiri Choudhury, D.K., Desai, A., Duckworth, J. W., Easa, P.S., Johnsingh, A.J.T., Fernando, P., Hedges, S., Gunawardena, M., Kurt, F., Karanth, U., Lister, A., Menon, V., Riddle, H., Rübel, A. & Wikramanayake, E. (2008). Elephas maximus. The IUCN Red List of Threatened Species 2008: e. T7140A12828813. Retrieved from https://doi.org/10.2305/IUCN.UK.2008.RLTS.T7140A12828813.en

CITES. (2019). Conf. 10.10 (Rev. CoP18): Trade in elephant specimens. Retrieved from https://www.cites.org/sites/default/files/document/E-Res-10-10-R18.pdf

CITES. (2020). Appendices I, II and III. Retrieved from https://cites.org/sites/default/files/eng/app/2020/E-Appendices-2020-08-28.pdf

D'souza, Z., Chettiankandy, T. J., Ahire (Sardar), M. S., Thakur, A., Sonawane, S. G., & Sinha, A. (2020). Collagen – structure, function and distribution in orodental tissues. *Journal of Global Oral Health*, *2*, 134–139. http://dx.doi.org/10.25259/jgoh_4_2020.

Dorozhkin, S. V., & Epple, M. (2002). Biological and medical significance of calcium phosphates. *Angewandte Chemie, International Edition*, *41*(17), 3130–3146.

Edwards, H. G. M., Hassan, N. F. N., & Arya, N. (2006). Evaluation of Raman spectroscopy and application of chemometric methods for the differentiation of contemporary ivory specimens I: Elephant and mammalian species. *Journal of Raman Specroscopy*, *37*, 353–360.

Godfrey, A., & Kongmuang, C. (2009). Distribution, demography and basic husbandry of the Asian elephant in the tourism industry in northern Thailand. *Gajah*, *30*, 13–18.

Godfrey, I. M., Ghisalberti, E. L., Beng, E. W., Byrne, L. T., & Richardson, G. W. (2002). The analysis of ivory from a marine environment. *Studies in Conservation*, *47*(1), 29–45.

Hillson, S. (1986). Archaeology and the study of teeth. *Endeavour*, *10*(3), 145–149.

Joshi, R., & Singh, R. (2008). Feeding behaviour of wild Asian elephants (*Elephas maximus*) in the Rajaji National Park. *Journal of American Science*, *4*(2), 34–48.

Kolmas, J., Marek, D., & Kolodziejski, W. (2015). Near-infrared (NIR) spectroscopy of synthetic hydroxyapatites and human dental tissues. *Applied Spectroscopy*, *69*(8), 902–912.

Locke, M. (2008). Structure of ivory. *Journal of Morphology*, *269*(4), 423–450.

Martin, E., & Stiles, D. (2003). *The ivory markets of East Asia*. Nairobi and London: Save the Elephants.

Osborne, B. G. (2006). Near-infrared spectroscopy in food analysis. In R. A. Meyers & R. J. McGorrin (Eds.), *Encyclopedia of analytical chemistry*. New York, US: John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470027318.a1018.

Osborne, B. G., Fearn, T., & Hindle, P. H. (1993). *Practical NIR spectroscopy with applications in food and beverage analysis*. Singapore: Longman Singapore Publisher (Pte) Ltd.

Pasquini, C. (2003). Near infrared spectroscopy: Fundamentals, practical aspects and analytical applications. *Journal of the Brazilian Chemical Society*, *14*, 198–219.

Phuangkum, P., Lair, R. C., & Angkawanith, T. (2005). *Elephant care manual for mahouts and camp managers*. Thailand: Bannakij Printing.

Pla-Ard, M., Sukmasuang, R., & Srinopawan, K. (2020). Population characteristics and habitat suitability of Asian elephants

(*Elephas maximus* Linnaeus, 1758) in the Khao Yai National Park, Thailand. *European Journal of Ecology*, *5*(2), 62–71.

Power, A., Ingleby, S., Chapman, J., & Cozzolino, D. (2019). Lighting the ivory track: Are near-infrared and chemometrics up to the job? A proof of concept. *Applied Spectroscopy*, *73*(7), 816–822.

Prozesky, V. M., Raubenheimer, E. J., Van Heerden, W. F. P., Grotepass, W. P., Przybylowicz, W. J., Pineda, C. A., & Swart, R. (1995). Trace element concentration and distribution in ivory. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, *104*(1), 638–644.

Raubenheimer, E. J., Brown, J. M. M., Rama, D. B. K., Dreyer, M. J., Smith, P. D., & Dauth, J. (1998). Geographic variations in the composition of ivory of: The African elephant (*Loxodonta africana*). *Archives of Oral Biology*, *43*(8), 641–647.

Romain, S., Angkawanish, T., Bampenpol, P., Pongsopawijit, P., Sombatphuthorn, P., Nomsiri, R., & Silva-Fletcher, A. (2014). Diet composition, food intake, apparent digestibility, and body condition score of the captive Asian elephant (*Elephas maximus*): A pilot study in two collections in Thailand. *Journal of Zoo and Wildlife Medicine*, *45*(1), 1–14.

Shimoyama, M., Morimoto, S., & Ozaki, Y. (2004). Non-destructive analysis of the two subspecies of African elephants, mammoth, hippopotamus, and sperm whale ivories by visible and short-wave near infrared spectroscopy and chemometrics. *Analyst*, *129*(6), 559–563.

Singh, R. R., Goyal, S. P., Khanna, P. P., Mukherjee, P. K., & Sukumar, R. (2006). Using morphometric and analytical techniques to characterize elephant ivory. *Forensic Science International*, *162*(1), 144–151.

Stuart, B. H. (2004). *Infrared spectroscopy: Fundamentals and applications*. New York, NY: John Wiley & Sons Ltd.

Sukmasuang, R. (2003). Ecology and population density of Asian elephants in Huai Kha Khaeng wildlife sanctuary. *Thai Journal of Forestry*, *11*(1), 13–36.

Sukumar, R. (2003). *The living elephants: Evolutionary ecology, behavior, and conservation*. New York, NY: Oxford University Press.

Sukumar, R., & Ramesh, R. (1995). Elephant foraging: Is browse or grass more important? In J. C. Daniel & H. Datye (Eds.), *A week with Elelphants* (pp. 368–374). Oxford: Oxford University Press.

van de Water, A., & Matteson, K. (2018). Human-elephant conflict in western Thailand: Socio-economic drivers and potential mitigation strategies. *PLoS One*, *13*(6), e0194736.

van der Merwe, N., Lee-Thorp, J., Thackeray, F., Hall-Martin, A., Kruger, F., Coetzee, H., … Lindeque, M. (1990). Source area determination of elephant ivory by isotopic analysis. *Nature*, *346*, 744–746.

Vance, C. K., Tolleson, D. R., Kinoshita, K., Rodriguez, J., & Foley, W. J. (2016). Near infrared spectroscopy in wildlife and biodiversity. *Journal of near Infrared Spectroscopy*, *24*(1), 1–25.

Vincke, D., Miller, R., Stassart, É., Otte, M., Dardenne, P., Collins, M., … Fernández Pierna, J. A. (2014). Analysis of collagen preservation in bones recovered in archaeological contexts using NIR Hyperspectral imaging. *Talanta*, *125*, 181–188.

Vinitpornsawan, S., Bunchornratana, K., Pukhrua, A., & Panyawiwatanakul, R. (2016). *Population and age structure of wild Asian elephants in eastern forest complex*. Thailand: Wildlife Conservation Office, Department of National Park, Wildlife and Plant Conservation.

Vollrath, F., Mi, R., & Shah, D. U. (2018). Ivory as an important model bio-composite. *Curator*, *61*(1), 95–110.

Walker, J. F. (2009). *Ivory's ghosts: The white gold of history and the fate of elephants*. New York, NY: Atlantic Monthly Press.

Workman, J., Jr., & Weyer, L. (2008). *Practical guide to interpretive near-infrared spectroscopy*. Boca Raton, FL: CRC Press.

Ziegler, S., Merker, S., Streit, B., Boner, M., & Jacob, D. E. (2016). Towards understanding isotope variability in elephant ivory to establish isotopic profiling and source-area determination. *Biological Conservation*, *197*, 154–163.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Chaitae, A., Rittiron, R., Gordon, I. J., Marsh, H., Addison, J., Pochanagone, S., & Suttanon, N. (2021). Shining NIR light on ivory: A practical enforcement tool for elephant ivory identification. *Conservation Science and Practice*, e486. https://doi.org/10.1111/csp2.486