4TH EDITION

IDENTIFICATION GUIDE FOR IVORY AND IVORY SUBSTITUTES



Authored by:
Barry W. Baker
Rachel L. Jacobs
Mary-Jacque Mann
Edgard O. Espinoza
Giavanna Grein

Edited by: Crawford Allan

WWF
CITES
TRAFFIC
the wildlife trade monitoring network

IDENTIFICATION GUIDE FOR IVORY AND IVORY SUBSTITUTES

© 2020 by CITES Secretariat and World Wildlife Fund. All rights reserved. No part of this book may be reproduced without permission of the CITES Secretariat and World Wildlife Fund.

COVER IMAGE
© Martin Harvey–WWF
Photo credits: U.S. Fish & Wildlife Service unless otherwise listed

DISCLAIMER
The geographical designations employed in this document do not imply the expression of any opinion whatsoever on the part of the CITES Secretariat (or the United Nations Environment Programme), World Wildlife Fund Inc. or TRAFFIC International concerning the legal status of any country, territory, or area, or concerning the delimitation of its frontiers or boundaries. The responsibility for the contents of this document rests exclusively with its authors and the editor.

FUNDERS
This publication was made possible with the support from the European Union (EU) (through the CITES CoP17 Decisions implementation project) and World Wildlife Fund.

ABOUT THE AUTHORS
The authors of the morphology section of this guide are current and former forensic and wildlife identification experts working at the United States Fish & Wildlife Service Forensic Laboratory. Mary-Jacque Mann and Edgard O. Espinoza were collaborators on previous versions of this identification guide. Giavanna Grein is the author of the section on online trade in ivories and is an expert on online trade in wildlife, who coordinates the Coalition to End Wildlife Trafficking Online for TRAFFIC and WWF.

ACKNOWLEDGEMENTS
Crawford Allan edited the Guide and was responsible for production oversight. Giavanna Grein authored the section on online trade, conducted additional research and was project manager. Barry W. Baker, Rachel L. Jacobs, Mary-Jacque Mann and Edgard O. Espinoza authored the morphology section.

Thanks to Abigail Hehmeyer of WWF; Robin Sawyer, Hallie Sacks, and Stephanie Pendry of TRAFFIC.

We are grateful for the support and inputs of the CITES Secretariat staff, including Haruko Okusu, Thea Carroll and Sofie Herrmann Flensborg.

Thank you to the International Consortium on Combating Wildlife Crime (ICCWC), the United Nations Office of Drugs and Crime (UNODC) and Jorge Rios of UNODC for the reproduction of the laboratory analysis section of their 2014 report, *Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis*.

Design by Fuszion. French language translation provided by Martin Collette and Spanish language translation provided by Lindsay Walsh.

SUGGESTED CITATION
Baker, B., Jacobs, R., Mann, M., Espinoza, E., Grein, G. (2020). CITES Identification Guide for Ivory and Ivory Substitutes (4th Edition, Allan, C. (ed.)), World Wildlife Fund Inc., Washington DC. Commissioned by CITES Secretariat, Geneva, Switzerland.

IDENTIFICATION GUIDE FOR IVORY AND IVORY SUBSTITUTES

Authored by:
Barry W. Baker
Rachel L. Jacobs
Mary-Jacque Mann
Edgard O. Espinoza
Giavanna Grein

Edited by: Crawford Allan

Foreword by
Ivonne Higuero, CITES Secretary-General

4th Edition

WWF
CITES
TRAFFIC
the wildlife trade monitoring network

This Guide is also available in Chinese, French and Spanish language versions through CITES (www.cites.org) and WWF (www.worldwildlife.org).

TABLE OF CONTENTS

FOREWORD vi

INTRODUCTION viii

ELEPHANT AND MAMMOTH TUSKS 12

WALRUS 28

SPERM WHALE AND ORCA 34

NARWHAL 38

HIPPOPOTAMUS 42

WARTHOG 50

NATURAL IVORY SUBSTITUTES 56

MANUFACTURED IVORY SUBSTITUTES 60

SUGGESTED READING 64

MODERN FORENSIC METHODS FOR IVORY IDENTIFICATION 70

DETECTION AND IDENTIFICATION OF ELEPHANT IVORY SOLD ONLINE 87

FOREWORD

On behalf of the 183 Parties to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the CITES Secretariat, I am honoured to welcome the much-awaited 4th edition of the Identification Guide for Ivory and Ivory Substitutes.

CITES regulates more than 36,000 species of animals and plants. Parties are expected to implement the Convention for all listed species, which means that administrators, scientists, and enforcement officers must be able to differentiate the many species and their products. Establishing the identity of the specimen is one of the first pieces of information that Parties need to be able to regulate international trade in accordance with the Convention.

Identification of different types of ivory, and of objects and products made of materials that imitate or look like ivory, is the main scope of this identification guide. It responds to Decision 17.162 adopted at the Seventeenth meeting of the CITES Conference of the Parties (Johannesburg, 2016), whereby Parties requested the Secretariat to prepare a revised and updated version of the Identification Guide for Ivory and Ivory Substitutes, taking into account modern identification methods. Considering that the third edition of the Guide was published in 1999, we are pleased that significant progress can be found in the present edition – both in the science and in the visual presentation of the publication.

FOREWORD

I would like to express my appreciation to the European Union for its generous financial support that allowed this update, and to the colleagues at TRAFFIC, WWF-US and the U.S. Fish and Wildlife Service Forensic Laboratory for their valuable contributions.

We remain committed to continuing our collaboration with the experts and partners in advancing our collective efforts to support CITES Parties and to ensure the conservation and sustainable use of the world's wildlife.

Ivonne Higuero
Secretary-General
Convention on International Trade in Endangered Species of Wild Fauna and Flora

Our hope is that this handbook continues to prove useful to the international wildlife enforcement community tasked with identifying ivory-bearing species commonly encountered in commercial trade



© TOM STAHL / WWF

INTRODUCTION

The information contained within this book was originally developed for the wildlife law enforcement community in connection with its mandate to enforce international endangered species trade regulations and restrictions. Thousands of copies of previous editions of this guidebook have been distributed in three languages throughout the world. As with previous editions, the goal is to provide wildlife law enforcement officers, scientists and managers with a visual and non-destructive means of tentatively identifying the authenticity and species origin of suspected ivory for enforcement purposes, including a "probable cause" justification for seizure of suspected illegal material, at ports of entry. Emphasis also remains on carved ivory, mostly because whole teeth are easily identified. Importantly, international regulations related to conservation and wildlife trade generally define protections based on species names (or in some cases subspecies names). Since ivory originates from a wide range of species whose protection status varies, species identification is critical to CITES enforcement efforts. Our hope is that this handbook continues to prove useful to the international wildlife enforcement community tasked with identifying ivory-bearing species commonly encountered in commercial trade.

A note on species names and listings: Herein we use the scientific names of animals as followed by agreement of the signatory countries of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). For example, CITES currently recognizes two species of living elephants, the African elephant *(Loxodonta africana)* and the Asian elephant *(Elephas maximus).* Many scientists consider the African forest elephant to be a unique species of its own *(Loxodonta cyclotis),* though here we follow CITES nomenclature for enforcement purposes. Similarly, while most recent taxonomic references recognize the pygmy hippopotamus as *Choeropsis liberiensis,* we use the scientific name recognized by CITES, *Hexaprotodon liberiensis.* Importantly, CITES may adopt taxonomic and nomenclatural changes over time. Readers are encouraged to remain current on changes through the CITES website (www.cites.org and www.speciesplus.net). At the beginning of each identification section, it is noted if the species referenced is listed on CITES Appendix I, II, III or non-listed as of May 2020. Status updates to CITES-listed species can be found through the Checklist of CITES Species (http://checklist.cites.org).



© KRISTA LYONS / WWF

GLOSSARY

Casein: a protein found within mammalian milk

Cementum: a layer surrounding the dentine of tooth and tusk roots

Dentine: a mineralized dental tissue which normally comprises the majority of the tooth mass

FT-IR (Fourier Transform Infrared Spectroscopy): a non-destructive technique for the chemical analysis of materials based upon molecular interaction with infrared radiation. The analytical product of this technique is expressed in an interferogram.

Haversian systems / canals: a series of canals through which fluid flows in compact bone

Lingual surfaces: surfaces towards the tongue

Netsuke: a small carved ornament, especially of ivory or wood, worn as part of Japanese traditional dress as a toggle by which an article may be attached to the sash of a kimono

Pulp cavity: the innermost part of a tooth which contains organic soft tissue called pulp

Proboscidea: the Order in which elephants and their extinct relatives (e.g., mammoths and mastodons) are grouped together by biologists and paleontologists. A member of this Order is referred to as a proboscidean.

Schreger lines: a diagnostic morphological feature seen in elephant and mammoth ivory cross-sections

Scrimshaw: engraved or shallowly carved bone or ivory, traditionally on whale teeth

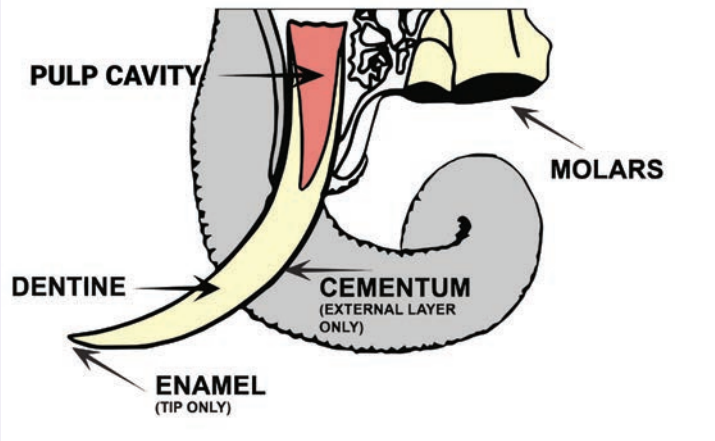
Taphonomic state: state of decay and fossilization

Tusk interstitial zone (TIZ): an area of growth convergence at the center of the tooth/tusk for the developing dentine

Tusk nerve: the nerve and associated micro-canal that runs longitudinally through the center of a tusk

WHAT IS IVORY?

FIGURE 1.1



↑ **Figure 1.1** Diagram of tusk morphology.

The word "ivory" was traditionally applied only to the tusks of elephants. However, the chemical structure of the teeth and tusks of mammals is the same regardless of the species of origin, and trade in certain teeth and tusks other than elephant is well-established and widespread. Therefore, the term "ivory" can correctly be applied to any mammalian tooth or tusk of commercial interest that is large enough to be carved or scrimshawed.

Teeth and tusks (a specific type of tooth) have the same origins. Teeth are specialized structures primarily adapted for processing food. Tusks, which are extremely large teeth projecting beyond the lips, have evolved to perform a variety of specialized functions. The teeth of most mammals consist of a root, a neck, and a crown. A tusk consists of a root and the tusk proper. Teeth and

tusks have the same physical structures: Pulp cavity, dentine, cementum, and enamel (Figure 1.1). The innermost area is the pulp cavity. The pulp cavity is a space within the tooth that in life contains organic soft tissue called pulp.

Odontoblastic cells line the pulp cavity and are responsible for the production of dentine. Dentine, which is the main component of carved ivory objects, forms a thick layer around the pulp cavity and comprises the bulk of most teeth and tusks. Dentine is a mineralized connective tissue with an organic matrix of collagenous proteins. The inorganic component of dentine consists of hydroxyapatite with the general formula $Ca_{10}(PO_4)_6(CO_3)H_2O$. Dentine contains microscopic structures called dentineal tubules, which are micro-canals that radiate outward through the dentine from the pulp cavity to the cementum border. These canals have different configurations in different teeth and tusks, and can be taxonomically informative.

Exterior to the dentine lies the cementum layer. Cementum forms a layer surrounding the dentine of tooth and tusk roots. Its main function is to adhere the tooth and tusk root to the mandible and maxilla. Incremental lines are commonly seen in cementum.

Enamel, the hardest animal tissue, covers the surface of the tooth or tusk that receives the most wear, such as the tip or crown. Ameloblasts are responsible for the formation of enamel and are lost after the enamel process is complete. Enamel exhibits a prismatic structure with prisms that run perpendicular to the crown or tip. Enamel prism patterns can have taxonomic and functional significance.

INTRODUCTION

Tooth and tusk ivory can be carved into an almost infinite variety of shapes and objects. Carved ivory has been observed in the form of netsukes, jewelry, flatware handles, furniture inlays, and piano keys. Additionally, tusks and teeth (e.g., warthog and sperm whale) can be scrimshawed or superficially carved, thus retaining their original shapes as morphologically recognizable objects.

The identification of ivory and ivory substitutes can be accomplished using physical, chemical, or genetic techniques. Since the first publication of this guide, advances in methods in forensic genetics have revolutionized the toolsets available to scientists identifying ivory in a law enforcement context. However, these techniques typically require expensive instrumentation and extensive training in genetics and biochemistry. The approach taken here focuses on the identification of ivory using the visual macroscopic and microscopic physical characteristics of ivory in combination with a simple chemical test using ultraviolet light. With some basic training, many ivory pieces are readily identifiable to species based on visually evident morphological characters. For ivory pieces lacking species diagnostic morphological characters, genetic analyses can be powerful tools in their identification.

PROCEDURE FOR THE IDENTIFICATION OF IVORY AND IVORY SUBSTITUTES

The following is the basic procedure we use to morphologically identify ivory and ivory substitutes. These steps are simple to follow, and the morphological characters we describe and illustrate are easy to learn. However, as biological structures, teeth exhibit variability. Training, experience, and access to a diverse comparative research collection of raw and carved ivory specimens are important factors to consider when identifying ivory. As technology continues to advance, one must also remain current on novel processes and materials used as ivory substitutes. In many cases, the first steps in this identification procedure can exclude these substitute materials:

1. Examine the object using long-wave ultraviolet light (we use 365 nm). The chemical composition of ivory, other teeth, and bones (hydroxyapatite) is such that it fluoresces brightly under long-wave ultraviolet light. In contrast, most plastics and resins appear darkly colored, dull purple or dark blue when examined under long-wave ultraviolet light (Figures 1.2A and 1.2B). This simple step should be conducted with comparison to known references of ivory/bone and known plastic/resin material. It can be used to quickly screen for objects of potential biological origin (in this case, ivory/tooth/bone). Note: long-wave ultraviolet radiation is hazardous to the eyes. Never look directly into a UV light.

2. Examine the object for the presence of significant diagnostic morphological features (see flow chart pages 10–11).

3. If Schreger angles are present (described and illustrated in detail below), see the section of this guide on elephant and mammoth tusks (pages 12–27).

4. If no specific identification is suggested by steps 1-3, consider submitting the object to a laboratory for instrumental analysis.

FIGURE 1.2A



FIGURE 1.2B



↑ Collection of objects suspected of being made from ivory. Figure 1.2B shows the reaction of the objects to long-wave UV light (365 nm). Only the hair comb has UV fluorescence characteristic of hydroxyapatite. Top – plastic letter opener. Clockwise from top left – casein nail buffer case; casein button; resin turtle carving; resin whale tooth; resin lion tooth; ivory hair comb; resin dragon.

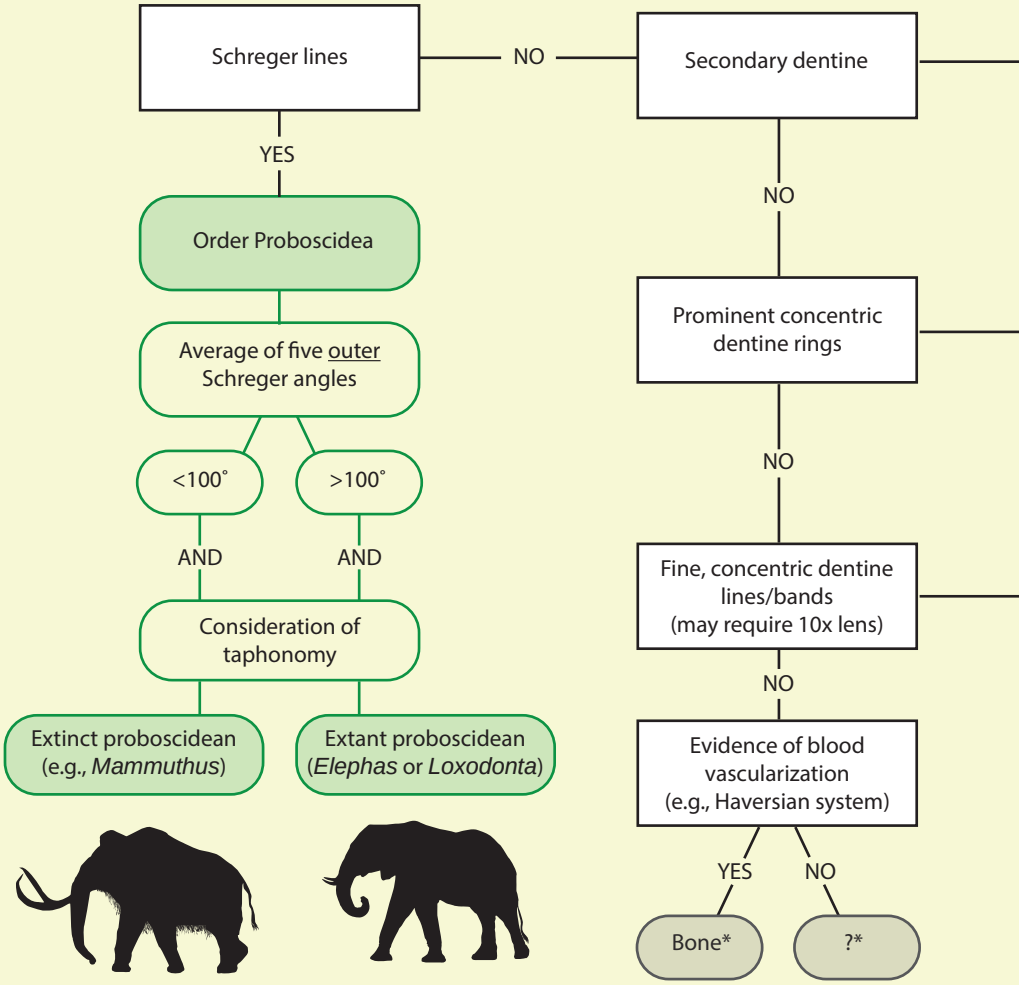
Note: Long-wave ultraviolet radiation is hazardous to the eyes. Never look directly into a UV light.

CLASS CHARACTERISTICS OF SELECTED COMMERCIAL IVORIES

TABLE 1

Source	Modified Tooth	Macroscopic Characteristic	Microscopic Characteristic (10x)	Enamel
Elephant (Asian & African)	Upper incisors	Average Schreger angles > 100° in cross-section		Tip, worn away
Mammoth	Upper incisors	Average Schreger angles < 100° in cross-section		
Walrus tusk	Upper canines	Secondary dentine in cross-section		Tip, worn away
Walrus teeth	All teeth	Cementum rings in cross-section; hypercementosis		Tip, may be worn
Orca/Sperm Whale	All teeth	Dentine rings in cross-section		Tip
Narwhal	Upper canine	Spiral; hollow center in cross-section		Tip, worn away
Hippopotamus	Upper canines	Oval cross-section; angular TIZ	Fine concentric lines in cross-section	Longitudinal band
Hippopotamus	Lower canines	Triangular cross-section; angular TIZ	Fine concentric lines in cross-section	Longitudinal band
Hippopotamus	Lower incisors	Peg-shaped; small TIZ (dot only)	Fine concentric lines in cross-section	None
Warthog	Upper canines	Squared cross-section; linear TIZ	Fine concentric lines in cross-section	Longitudinal band

PROCESS FOR IDENTIFYING COMMONLY OBSERVED IVORY IN TRADE BASED ON CROSS-SECTION MORPHOLOGY



*Instrumental analysis may be required for further identification

The following chart assumes that the item under examination produces fluorescence under UV light that is consistent with hydroxyapatite. If the item does not fluoresce in a manner consistent with hydroxyapatite, it likely represents an ivory substitute and instrumental analyses are recommended.

YES — *Odobenus rosmarus* (walrus)



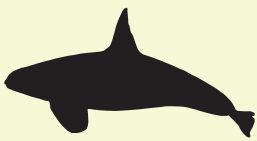
YES — Prominent white transition ring, hollow center — *Monodon monoceros* (narwhal)



YES — Thick cementum, round/oval shape — *Physeter macrocephalus* (sperm whale)



YES — Thin cementum, rectangular shape — *Orcinus orca* (orca)



YES — Hippopotamidae or Suidae

- Linear TIZ and "waisted" shape → *Phacochoerus* (warthog)



- Small or angular TIZ and round or triangular shape → Hippopotamidae → Large size → *Hippopotamus amphibius*** (hippopotamus)



**See text for discussion of the pygmy hippopotamus

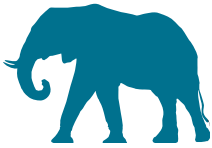
ELEPHANT AND MAMMOTH TUSKS

CITES Listings (as of 2020)

Loxodonta africana	Appendix I, except the populations of Botswana, Namibia, South Africa and Zimbabwe, which are included in Appendix II subject to Annotation 2
Elephas maximus	Appendix I
Mammuthus	Extinct, non-CITES listed



© STEVE MORELLO / WWF



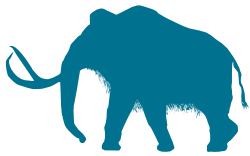
Modern (extant) elephants and their extinct relatives (e.g., mammoths and mastodons, among others) are grouped together by biologists and paleontologists in the Order Proboscidea. The most common proboscidean ivory in the wildlife trade comes from the two upper incisors of extant elephants. The international and domestic commercial trade in African and Asian elephant ivory *(Loxodonta africana, Elephas maximus,* respectively) is highly regulated, and in many instances is illegal due to prohibitions based on domestic legislation or CITES listing status.

Ivory from the extinct mammoth species *Mammuthus primigenius* (one of many species of mammoths) is also commonly observed in trade. Bruemmer (1989) has estimated that in the last 350 years, over 7,000 tons of mammoth ivory have been recovered and placed in trade, and Vereshchagin (1974) estimates that over 550,000 tons of mammoth tusks are still buried in Siberia. Because the mammoth's prehistoric range included Alaska and Siberia, the tusks of mammoths found in permafrost can be well-preserved, and the color and condition can resemble modern elephant ivory. Mammoth tusks

that have been deposited in soil, on the other hand, often exhibit blue to brown staining, depending on the burial conditions, which can facilitate distinguishing them from extant elephant.

Ivory from mastodons has also been found in paleontological environments, but of the thousands of mastodon tusks uncovered in North America, only two tusks were pristine enough to have the appearance of modern elephant ivory (Personal Communication. D. Fisher, July 9, 2018). As such, mastodon ivory can generally be excluded from consideration when identifying proboscidean ivory in the wildlife trade.

In proboscidean tusks, enamel is only present on the tusk tip of young animals, and is soon worn off. The full cross-section of proboscidean tusks is either rounded or oval (Figure 2.1). Dentine composes 95 percent of the tusk and sometimes displays broad concentric bands, called Owen's lines. Cementum covers the exterior of the tusk and can present a layered appearance.



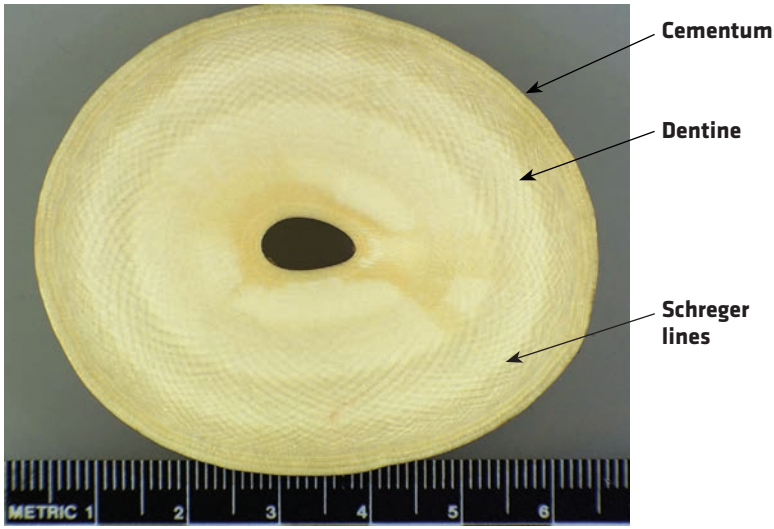
IDENTIFICATION OF ELEPHANT AND MAMMOTH TUSKS

Intact and complete elephant tusks are characterized by their shape and size. Historically, elephant tusks were extremely large. However, in part due to the continued illegal harvesting of ivory, the average tusk size in African elephants is under rapid decline (Chiyo et al. 2015). Whole mammoth tusks are large and have asymmetrical curvature. These rarer whole tusks also generally exhibit more degraded taphonomic states, and are not typically confused with those of modern elephants. Other materials, including hippopotamus teeth, warthog tusks, bone, resin, and plastic, are often used to craft items that resemble elephant tusks. These look-alikes can be easily distinguished by careful examination and analysis as described in this book.

IDENTIFICATION OF CARVED ELEPHANT IVORY

Determining if a carved ivory object (Figure 2.2) is from a proboscidean source is based on the presence of a diagnostic morphological feature seen in elephant and mammoth ivory cross-sections called “Schreger lines”. Sir Richard Owen in 1845 (Owen 1845) first described these lines as “curvilinear”, “decussation” and “lozenge”, but Espinoza and Mann (1993) first used the term “Schreger pattern” to describe these morphological features as a tool to distinguish the ivory of extant elephants from those of mammoths. The histogenesis and development of the Schreger pattern is described by Virag (2012) and Alberic et al. (2017), and is produced by the expression of sinusoidal dentineal tubules.

ELEPHANT AND MAMMOTH TUSKS



↑ **Figure 2.1** Typical image of a cross-section of an elephant tusk. The exterior is composed of cementum layers which surround the exterior of the tusk. The most abundant component is the dentine, which in this photograph shows the angular Schreger lines. The apex of the angles point toward the cementum layers. The oval interior is the space occupied by the pulp in a living elephant, and therefore can be described as the pulp cavity.

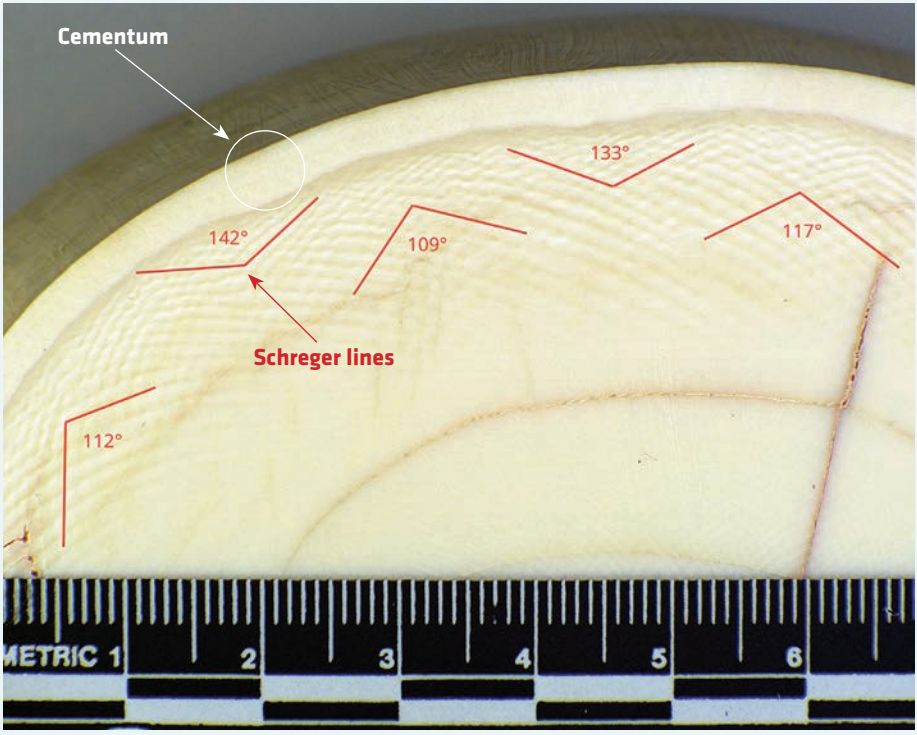


↑ **Figure 2.2** Three examples of typical ivory netsukes showing the small details of these miniature carvings.

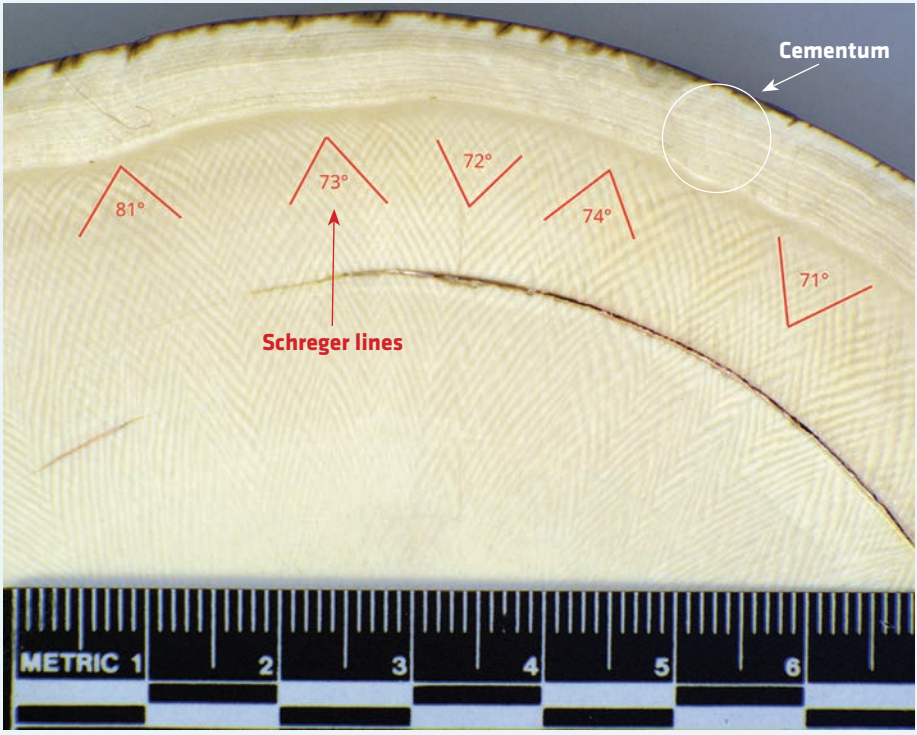
Since a carved ivory object is three dimensional, a careful examination of the item will often reveal a location where the carving exposes a cross-section surface. Schreger lines have been described as cross-hatchings, engine turnings or stacked chevrons in the elephant dentine. Schreger lines can be divided into two groups: 1) conspicuous lines that are adjacent to the cementum, which we refer to as "the outer Schreger lines", and 2) the faintly discernible Schreger lines found surrounding the tusk nerve (Figure 2.1). The angles formed by the intersection of the outer Schreger lines are measured to distinguish between extinct from extant proboscideans, whereas the "the inner Schreger lines" are not helpful in classifying the taxonomic source of ivory (Figure 2.1).

In order to make taxonomic determinations, the orientation of the Schreger angles is critical. When examining a proboscidean ivory cross-section, the cementum layer surrounds the periphery. Adjacent to the cementum, the Schreger lines intersect to form either 1) concave angles (which resemble lancet or gothic doorway arches) with the apex (point of the angle) pointing toward the cementum, or 2) convex angles formed by the outer Schreger lines, where the apex (point of the angle) points toward the tusk center. The database created by Espinoza, et al. (1990) and Espinoza and Mann (1993) measured both outer concave and outer convex Schreger angles when the cementum was observable. These authors obtained reference ivory from 27 elephants and 27 mammoths. For each specimen, five concave Schreger angles were measured, and five convex Schreger angles were measured; the resulting averages were calculated (Figures 2.3 and 2.4). In total, 270 angles were measured in each taxonomic group.

Modern elephants exhibit a Schreger angle average greater than 100° (Figure 2.3), whereas the average for mammoth is less than 100° (Figures 2.4 and 2.5). Averages of both concave and convex angles were >100° for all 27 elephants, and <100° for all 27 mammoths. Accordingly, the average measurement of either concave or convex angles (n= ≥ 5) is useful for separating extant elephant ivory from that of mammoth.



↑ **Figure 2.3** Close up of an extant elephant tusk cross-section showing the measurement results of the Schreger angles. The range of measurement is 109° to 142°. The average Schreger angle measurement is 122.6°. Notice that the apex of the angles measured either faced the cementum or the pulp cavity.



↑ **Figure 2.4** Close up of an extinct mammoth tusk cross-section showing the measurement results of the Schreger angles. The range of measurement is 71° to 81°. The average Schreger angle measurement is 74.2°. Notice that the apex of the angles measured either faced the cementum or the pulp cavity.

The classification of proboscidean taxa based on Schreger angle measurement has been corroborated by Fisher, et al. (1998), Palombo and Villa (2001) and Ábelová (2008).

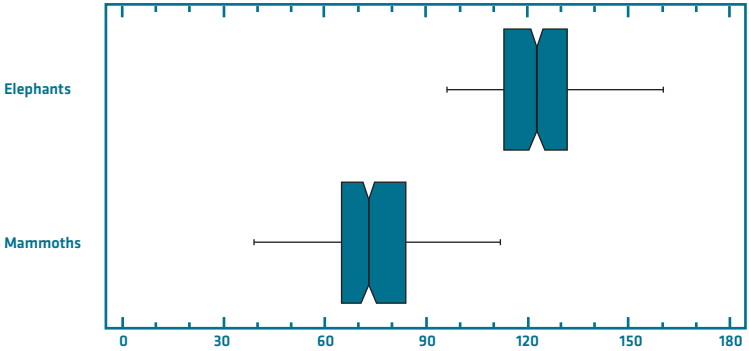
Table 2.1 below shows that the directionality of the Schreger angle does not affect the conclusion. A reasonable strategy when examining an ivory object is to combine concave and convex angles, especially when the size of the object is small.

TABLE 2.1

	Concave angles (apex facing cementum)	Convex angles (apex facing tusk center)	All angles combined (n=540)
Elephants:			
average	131.0°	117.3°	124.2°
range	105.0 – 162.0°	96.0 – 149.0°	96.0 – 162.0°
Mammoths:			
average	74.8°	72.7°	73.7°
range	39.0 – 115.0° [1]	42.0 – 115.0°	39.0 – 115.0°

[1]Ábelová (2008) recorded individual Schreger angle measurements ≥120° in mammoth.

FIGURE 2.5



↑ **Figure 2.5** Box-and-whisker plot of the Schreger angles measured from elephant and mammoth ivory.

HOW TO MEASURE THE SCHREGER ANGLES

1) Schreger angles can be captured using digital photography or by capturing the image with a photocopy machine.

2) Orient the image so that the cementum is noted (See Figures 2.6A, 2.6B, and 2.7).

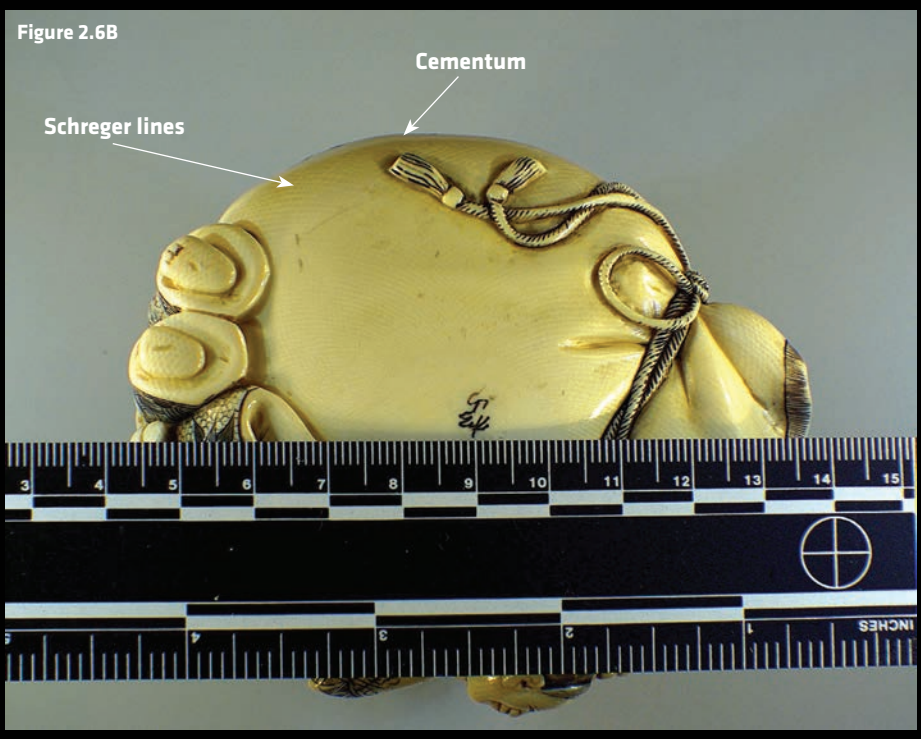
3) Make at least five angle measurements of either the concave or convex angles and calculate the average. If the image is digital, there are many imaging tools that have a built-in angle calculator. If the image was captured on a photocopy machine, then a protractor is needed for angle calculation.

4) If the **average** of the angles measured is greater than 100°, and the taphonomic state of the dentine does not show degradation (see note below), then it is reasonable to infer that the object is from an elephant. Conversely, if the **average** of the angles measured is less than 100°, it is reasonable to infer that the object is from a mammoth.

Figure 2.6A

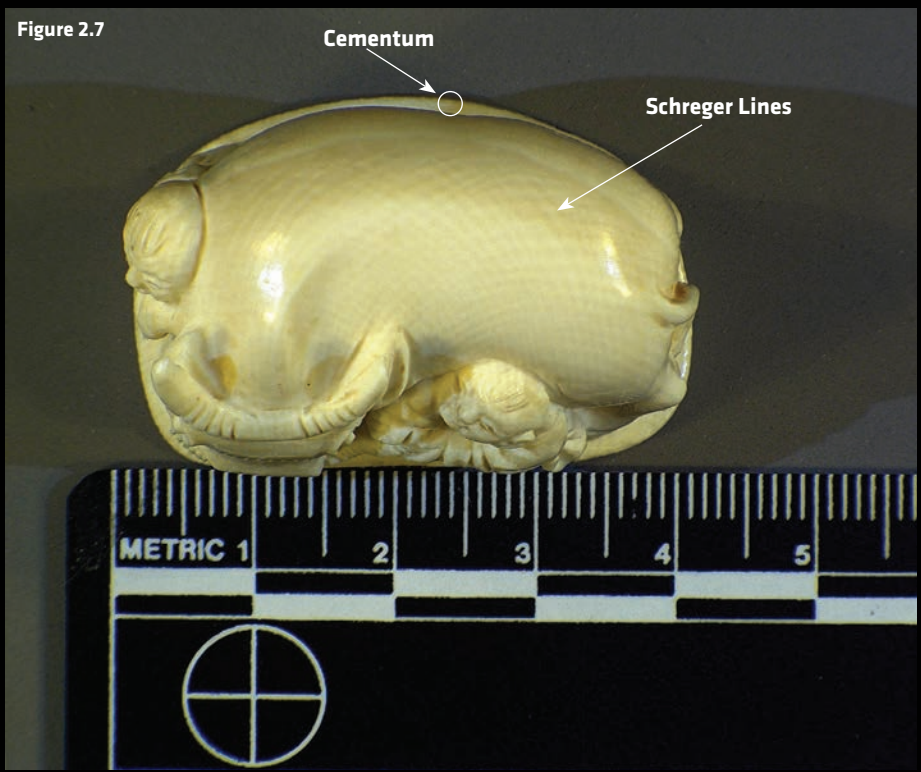


Figure 2.6B



← **Figure 2.6A** Ivory figurine showing the presence of Schreger lines on diverse surfaces of the carving. These lines confirm that the object has a proboscidean origin.

← **Figure 2.6B** Same figurine from Figure 2.6A, but in this image the base is shown. Careful examination shows the cementum layers, and the Schreger angles adjacent to it are measurable. Analysis showed this object is extant elephant ivory.



↑ **Figure 2.7** A small figurine which exhibits the exterior cementum layer as well as obtuse Schreger angles which identify it as extant elephant ivory.

NOTES OF CAUTION

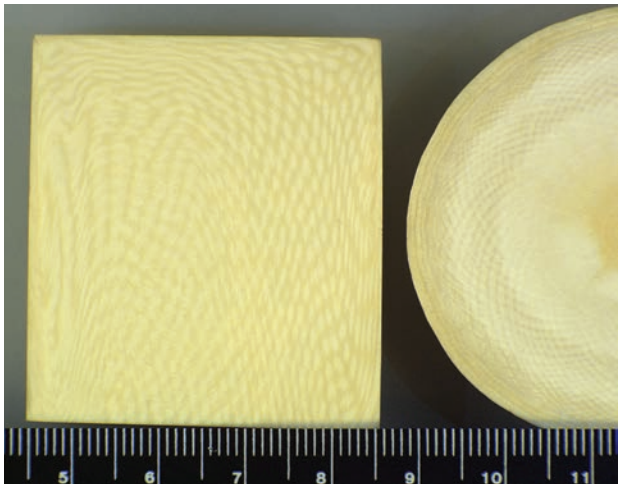
1) If an ivory object cannot be oriented so as to determine the location of the cementum, then there is a high probability that an incorrect conclusion will be reached. This is because:
a. The object could have been carved from the tusk center; in which case, the angles observed correspond to inner Schreger angles. Inner Schreger angles exhibit acute angles and are misleading for measurement.
b. The angles are parallel to the cementum, and these will yield spurious conclusions. The correct measurement is of angles that are **perpendicular** to the cementum.

2) Fisher et al. (1998) reported that extinct mastodon ivory has obtuse Schreger angles >100° (average ~125°). Thus, based on angle measurements alone, mastodon ivory could potentially be confused with modern elephant ivory. However, of the thousands of mastodon tusks uncovered in North America and Europe, only two tusks were in such good condition to have the appearance of modern elephant ivory (Personal Communication. D. Fisher, July 9, 2018). Therefore, the taphonomic state of the ivory must be taken into consideration before inferring the identity of extant proboscidean ivory.

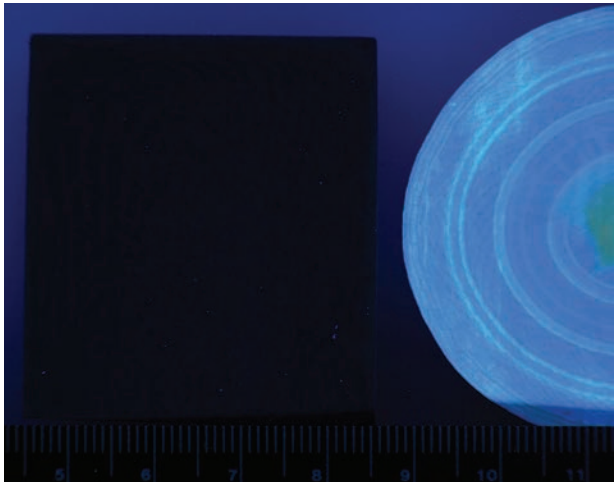


3) In cases where the item cannot be properly oriented or equivocal features are observed, genetic analysis should be conducted to identify species.

4) There are casein and other polymers manufactured by successively depositing layers, which result in Schreger-like lines and angles (Figures 2.8A and 2.8B). Such items can be distinguished from



← **Figure 2.8A** An alkyd resin manufactured to resemble Schreger angles (left) next to a cross-section of an elephant tusk showing the Schreger angles adjacent to the cementum (right).



← **Figure 2.8B** Alkyd resin (left) next to an elephant ivory cross-section under UV light (312 nm). The resin absorbs the UV light whereas the ivory reflects it.

real proboscidean ivory by visually examining the ultraviolet fluorescent properties of the material, and/or using analytical chemical instrumentation, such as Fourier-transform infrared spectroscopy (FT-IR).

Lastly, the analysis of the Schreger angles is only to determine if an object is from an elephant or mammoth. This analysis cannot differentiate African from Asian

See page 70 for other forensic methods for ivory identification



© RICHARD BARRETT / WWF-UK

elephants. This type of query requires DNA analysis. The UNODC book entitled *Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis* (2014), as well as the dedicated CITES Wildlife Forensics webpage (cites.org/eng/prog/imp/Wildlife_forensics), have comprehensive suggestions for further testing.



WALRUS
(ODOBENUS ROSMARUS)

CITES Listings (as of 2020)

Odobenus rosmarus	Appendix III (Canada)



© WILD WONDERS OF EUROPE / OLE JOERGEN LIODDEN / WWF

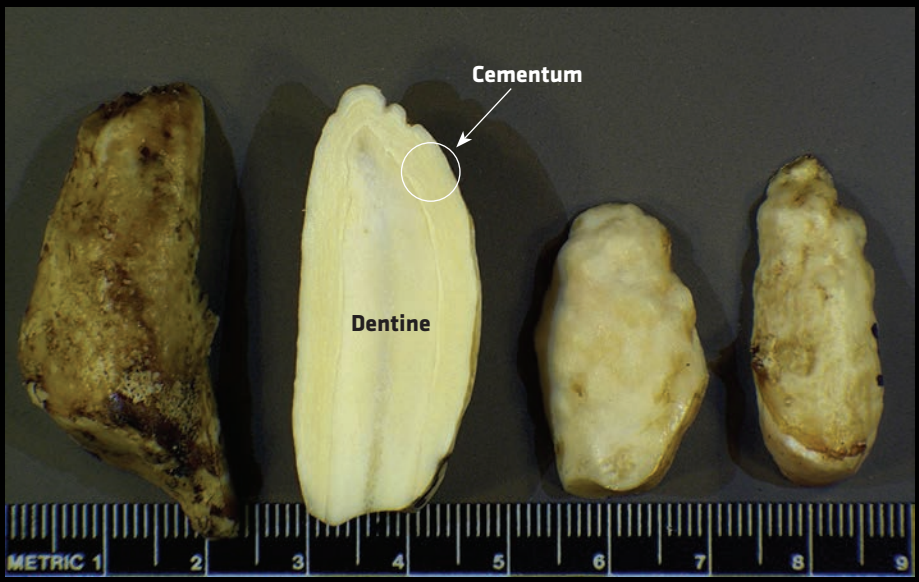


TUSK

The ivory from walrus tusks comes from two altered upper canines. The tusks of the Pacific walrus may attain a length of one meter (Figure 3.1). Walrus cheek teeth are also carved and commercially traded (Figure 3.2). The average walrus cheek tooth is rounded and irregularly shaped and grows to ~5 cm in length. The tip of the tusks of a juvenile walrus contains enamel, but this is worn away as the walrus matures. Walrus tusks often show longitudinal cracks throughout the length of the tusk, which originate in the cementum (outer layer) and penetrate through to the dentine.



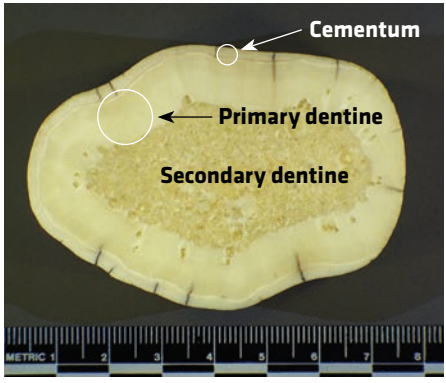
↑ **Figure 3.1** A pair of typical walrus tusks. Notice the presence of repeated longitudinal fissures in the tusks.



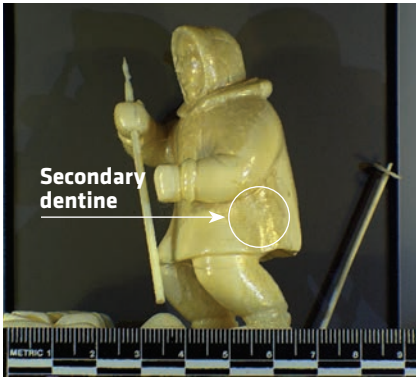
↑ **Figure 3.2** Examples of walrus teeth. The globular appearance of these teeth is due to the overproduction of cementum, called hypercementosis. The second tooth from the left has been cut in half longitudinally in order to show the thick layer of cementum that surrounds the primary dentine. In this instance the amount of cementum is almost equal to the amount of dentine.

CARVED OBJECTS

Walrus tusk cross-sections, typically seen in trade, are generally oval with a widely corrugated cementum exterior. Carvings and/or cross-sections are distinguished by a unique morphological feature termed secondary dentine, which is located in the core (center) of the cross-section and has a marbled appearance (Figure 3.3). Interior to the cementum is a wide layer of dentine which has no outstanding morphological features. Radial fissures can be seen crossing the cementum through the dentine and at times reaching the interior secondary dentine (Figures 3.1 and 3.3). The presence of secondary dentine identifies an object as originating from a walrus tusk (Figures 3.1 and 3.4).



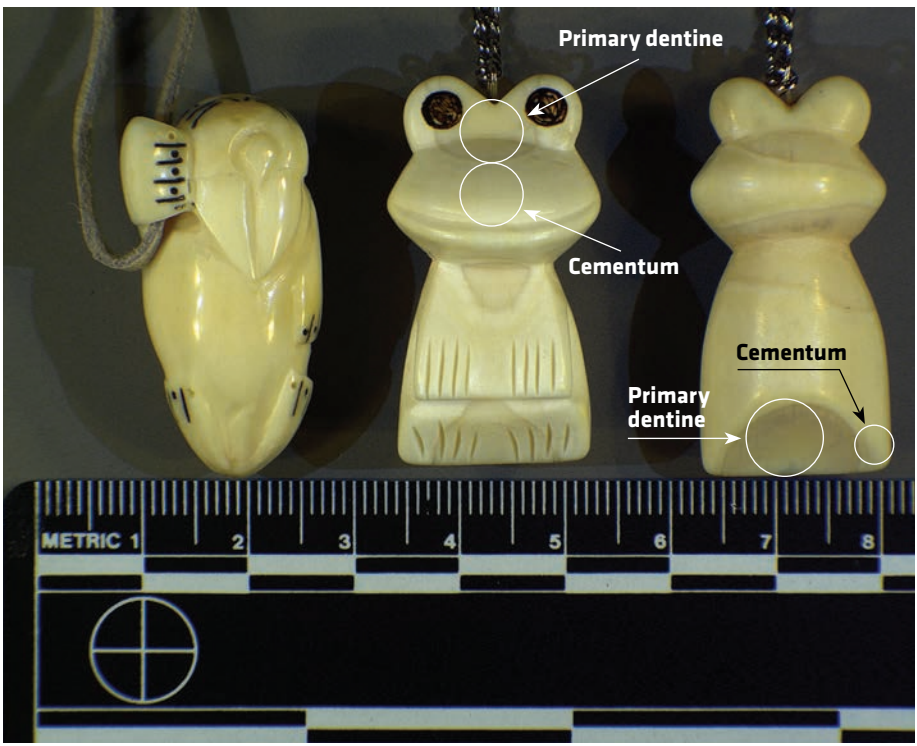
↑ **Figure 3.3** Typical example of a walrus tusk cross-section. The exterior is composed of cementum layers which surround the interior of the tusk. Interior to the cementum are two types of dentine. The marbled looking tissue is called secondary dentine and is found at the center or core of the tusk. The smooth tissue is the traditional primary dentine. Notice that the cementum has cracks that sometimes extend into the primary dentine; these cracks are caused by the longitudinal fissures seen in Figure 3.1.



↑ **Figure 3.4** Ivory carving made from walrus tusks. This figurine shows the secondary dentine (i.e., marbled) and primary dentine. A careful examination of the left hand of the figure also shows traces of cementum.

WALRUS CHEEK TEETH

The cheek teeth in the maxilla and mandible of a walrus have an anomalous appearance due to the excessive amount of cementum that coats the exterior and is termed hypercementosis (Figure 3.2). In cross-section and in carvings, a walrus cheek tooth will show very thick cementum with prominent cementum rings (Figure 3.5). Interior to the cementum is a layer of dentine, which is separated from the cementum by a clearly defined narrow transition ring. The center of the tooth may contain a small core of secondary dentine, depending on the tooth size.



↑ **Figure 3.5** Three miniature carvings using walrus teeth. The center figurine shows a thick cementum layer which surrounds the primary dentine.



© STEVE MORELLO / WWF

SPERM WHALE AND ORCA

(PHYSETER MACROCEPHALUS AND ORCINUS ORCA)

CITES Listings (as of 2020)

Physeter macrocephalus	Appendix I
Orcinus orca	Appendix II



© BRIAN J. SKERRY / NATIONAL GEOGRAPHIC STOCK / WWF

SPERM WHALE AND ORCA



Sperm whale teeth can be quite large (Figure 4.1) and are often carved with nautical themes in a tradition termed scrimshaw (Dyer 2018). Scrimshaw typically involves etching the exterior of a whole tooth (Figure 4.2). The average height of a whole sperm whale tooth is approximately twenty centimeters. Orca teeth are much smaller, though a very small sperm whale tooth may overlap in size with a very large orca tooth. Both species display conical teeth with a small amount of enamel at the tips. The rest of the tooth is covered by cementum. The full cross-section of a sperm whale tooth is rounded or oval, while that of an orca is rectangular (Figures 4.3 and 4.4). The dentine is deposited in a progressive laminar fashion. As a result of this laminar deposition, orca and sperm whale teeth will show prominent concentric dentine rings in cross-section. One orca tooth specimen has been observed to exhibit faint dentine lines that should not be confused with Schreger lines (Figure 4.4). Similar structural features have only been observed in one other non-proboscidean ivory specimen (Sims 2010) in the cumulative 50+ years of ivory identification at the United States National Fish and Wildlife Forensic Laboratory.

The dentine of orca and sperm whale teeth is separated from the cementum by a clearly defined transition ring. Carved sperm whale teeth can typically be easily distinguished from carved orca teeth based on cementum thickness. Sperm whale teeth have very thick cementum, while that of orca is comparatively thin (Figure 4.4) (Yates and Sims 2010).

SPERM WHALE AND ORCA

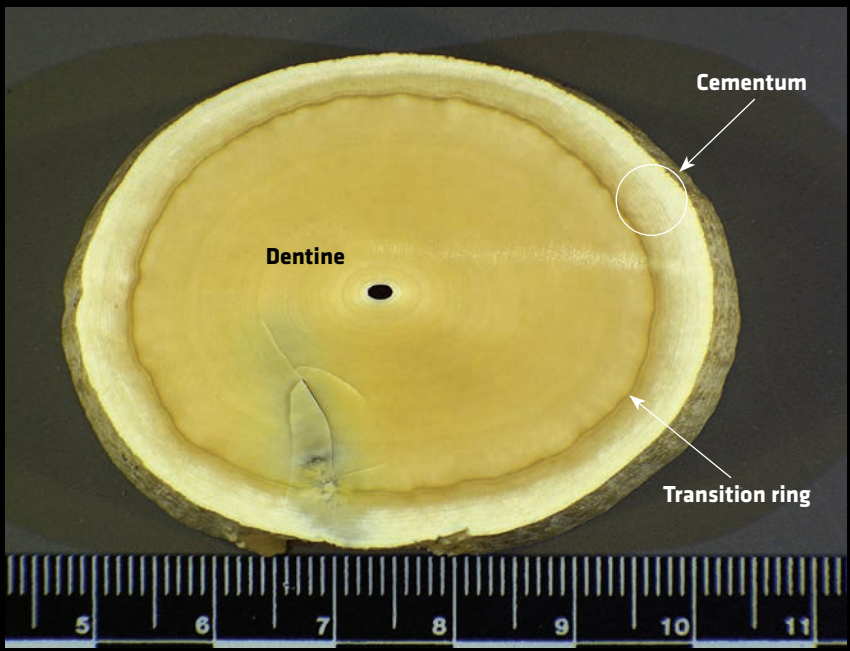


↑ **Figure 4.1** Uncarved sperm whale teeth.

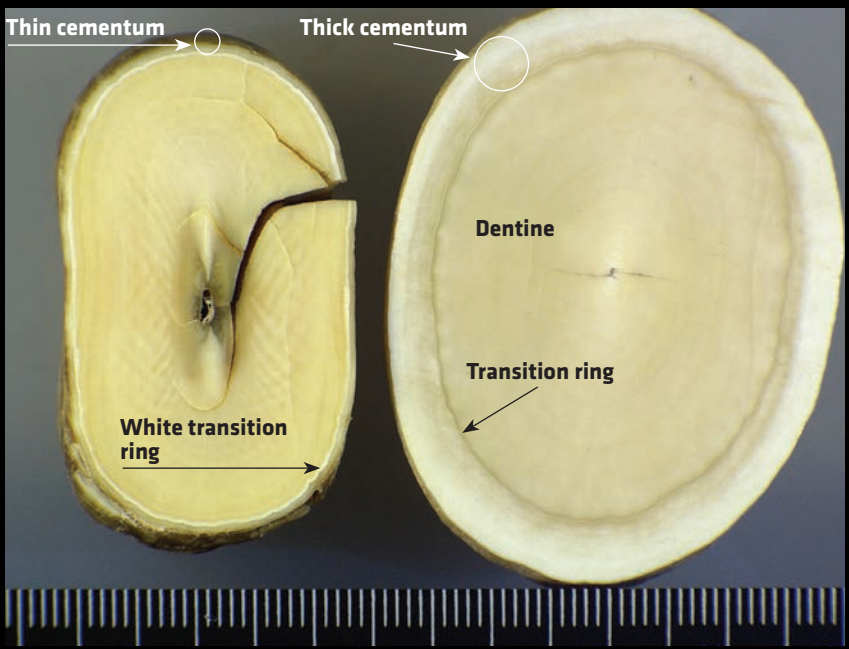


↑ **Figure 4.2** Carved and modified sperm whale teeth.

SPERM WHALE AND ORCA



↑ **Figure 4.3** Cross-section of a sperm whale tooth. Note the thick outer cementum, the dark transition ring separating the cementum from the dentine, and the dentine composed of fine circular rings.



↑ **Figure 4.4** Cross-sections of an orca tooth (left) and a sperm whale tooth (right). Note the rectangular shape of the orca tooth and its characteristically thin cementum.

NARWHAL
(MONODON MONOCEROS)

CITES Listings (as of 2020)

Monodon monoceros	Appendix II



© PAUL NICKLEN / NATIONAL GEOGRAPHIC CREATIVE / WWF-CANADA

NARWHAL

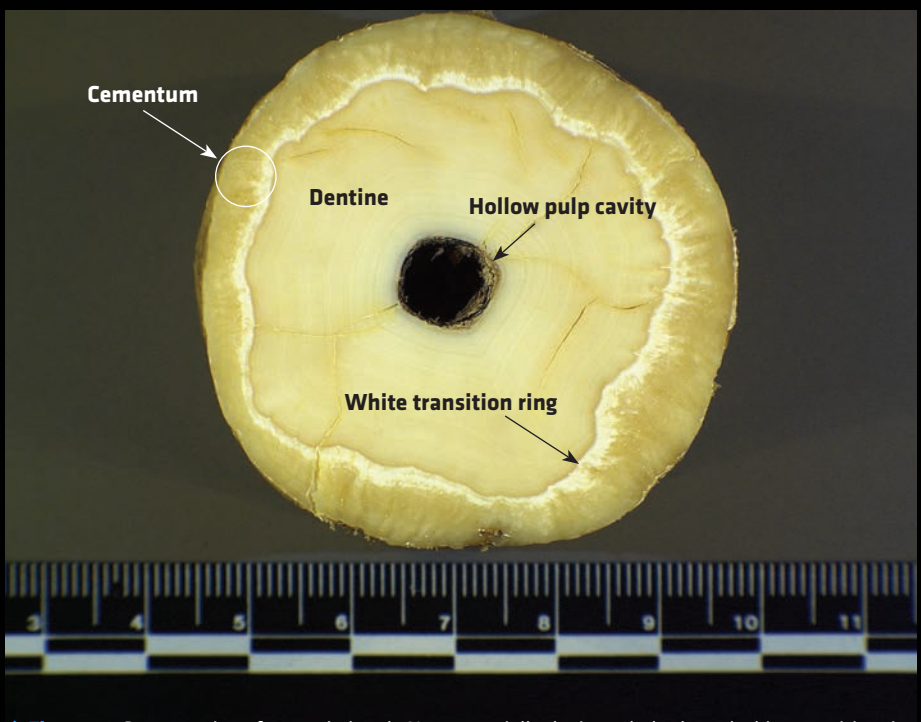


The narwhal is a rarely seen arctic whale. The male of this species has a single tusk, which is a modified canine. The tusk is spirally twisted, usually counter-clockwise (Figure 5.1). In a mature specimen, the tusk can reach a length of two to seven meters. Enamel may be present at the tip of the tusk. The cementum frequently displays longitudinal cracks which follow the depressed areas of the spiral pattern. As a result, narwhal tusk cross-sections are rounded with peripheral indentations. The cementum on a narwhal tusk is separated from the dentine by a clearly defined white transition ring. Like orca and sperm whale, the dentine can display prominent concentric rings, though those of narwhal are irregular in shape. The pulp cavity of a narwhal tusk extends throughout most of its length, giving cross-sections a hollow interior (Figures 5.1 and 5.2).

NARWHAL



↑ **Figure 5.1** Sections of narwhal tusks illustrating their spiral structure and hollow pulp cavity. The pulp cavity of the tusk on the right has been plugged.



↑ **Figure 5.2** Cross-section of a narwhal tusk. Note especially the irregularly shaped white transition ring and the hollow pulp cavity.



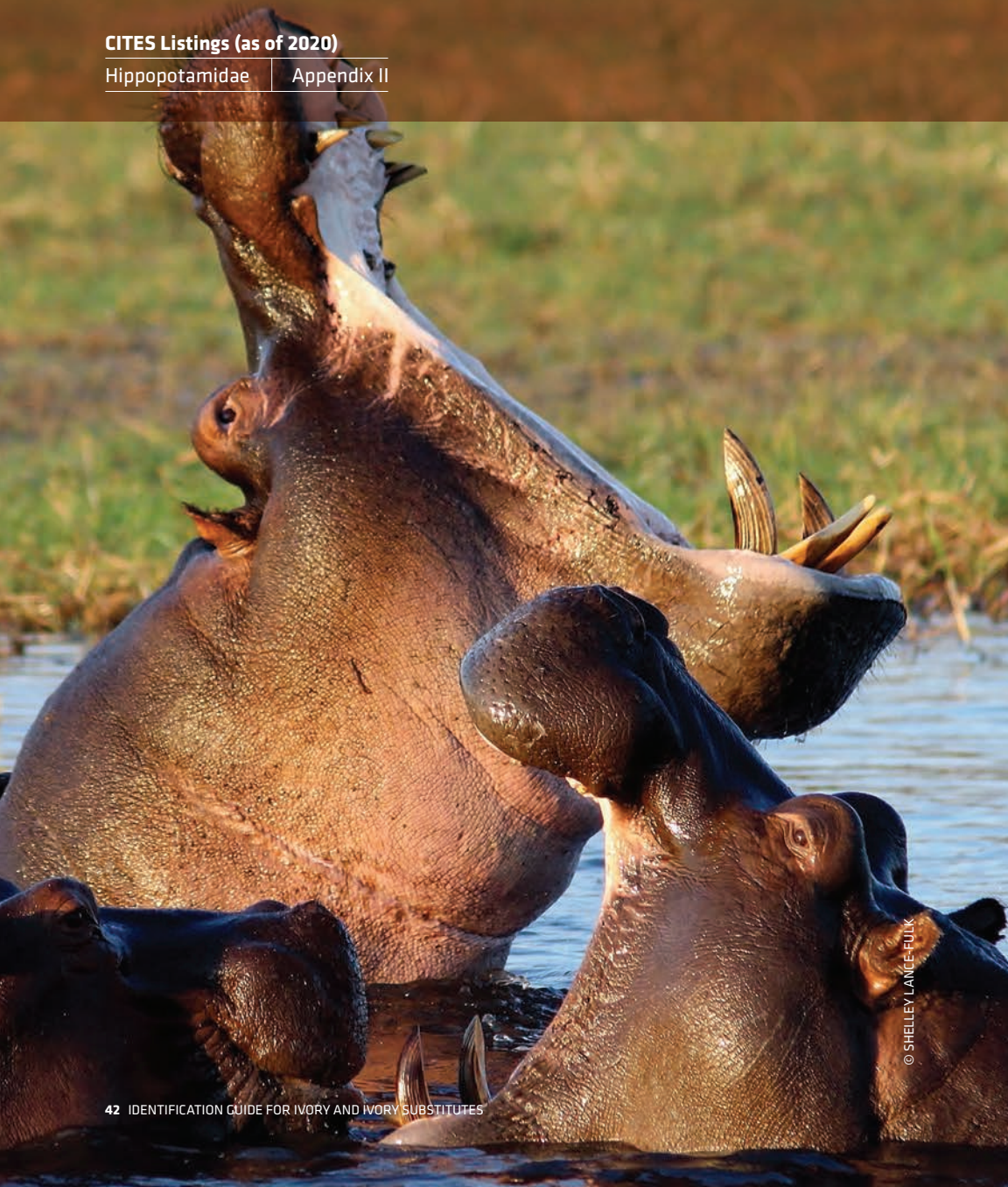
In a mature specimen, the tusk can reach a length of two to seven meters.

© NATUREPL.COM/ BRYAN AND CHERRY ALEXANDER/ WWF

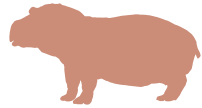
HIPPOPOTAMUS

CITES Listings (as of 2020)

Hippopotamidae	Appendix II



© SHELLEY LANCE-FULK



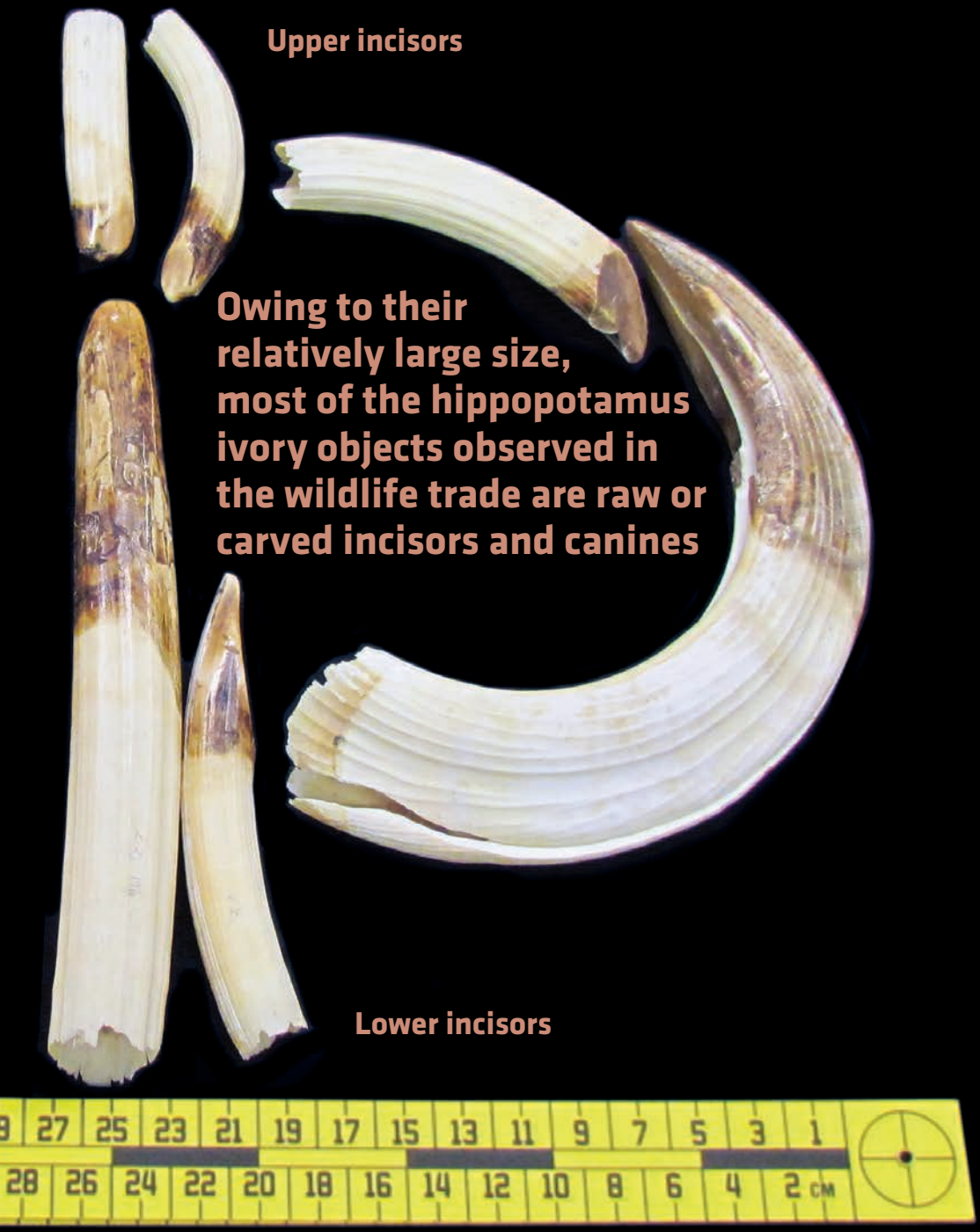
HIPPOPOTAMIDS EXHIBIT LARGE CANINES AND INCISORS THAT ARE COMMONLY OBSERVED IN THE IVORY TRADE. There are two extant species of hippopotamid: common hippopotamus *(Hippopotamus amphibius)* and pygmy hippopotamus *(Hexaprotodon liberiensis)*. These two taxa differ markedly in size. They also differ in their global population numbers, with the latter being relatively rare and having a much more restricted distribution (Wilson and Mittermeier 2011). Given the larger size of *H. amphibius* teeth, as well as its higher population numbers, this taxon is more commonly observed in the ivory trade. *Hex. liberiensis* is presently considered rare in the ivory trade. The features described below are based on observations of *H. amphibius* teeth/tusks, but at least some of these features may be observed in *Hex. liberiensis* for which sufficient comparative data are lacking. Accordingly, while it is unclear whether all features apply to the family level (i.e., Hippopotamidae), we recommend caution in excluding *Hex. liberiensis,* particularly when size is equivocal (e.g., small carved objects).

RAW TEETH/TUSKS

Owing to their relatively large size, most of the hippopotamus ivory objects observed in the wildlife trade are raw or carved incisors and canines, which can be distinguished based on differences in shape (Figure 6.1).



↑ **Figure 6.1** Incisors and canines of *Hippopotamus amphibius*.


Upper incisors
Owing to their relatively large size, most of the hippopotamus ivory objects observed in the wildlife trade are raw or carved incisors and canines
Lower incisors
27 25 23 21 19 17 15 13 11 9 7 5 3 1
28 26 24 22 20 18 16 14 12 10 8 6 4 2 CM

INCISORS: Hippopotamus lower incisors are generally straight and peg-shaped. Upper incisors are similar but may also exhibit slight curvature (Figure 6.1). Incisors may or may not have enamel on the surface. Lower central incisors of *H. amphibius* lack enamel, but exhibit an external cementum layer; other incisors may have longitudinal enamel bands on the surface or cementum where enamel is lacking (Locke 2013).

CANINES: Hippopotamids have a single set of upper and lower canines that are curved and larger than their incisors. The lower canines are generally larger and more strongly curved, almost semi-circular in shape, compared to upper canines (Figure 6.1). Enamel can be found on the outer surfaces of both upper and lower canines, and cementum is present on the lingual surfaces.

CROSS-SECTIONAL MORPHOLOGY

One of the primary distinguishing characters for hippopotamus ivory is related to the morphology of the dentine of both canines and incisors, which exhibits fine concentric lines/bands that may be visible with the naked eye or require additional magnification (a 10x hand lens is generally sufficient; Figures 6.2A and 6.2B). Some areas of the dentine might not exhibit these lines, and this variation is related to the surface structure of the tooth (i.e., whether enamel or cementum is present). Dentine with fine lines/bands begins directly below enamel surfaces; below cementum surfaces, these lines/bands begin nearer to the tusk interstitial zone (hereafter TIZ), which is an area of growth convergence at the center of the tooth/tusk for the developing dentine (Figures 6.3 and 6.4).

FIGURE 6.2A

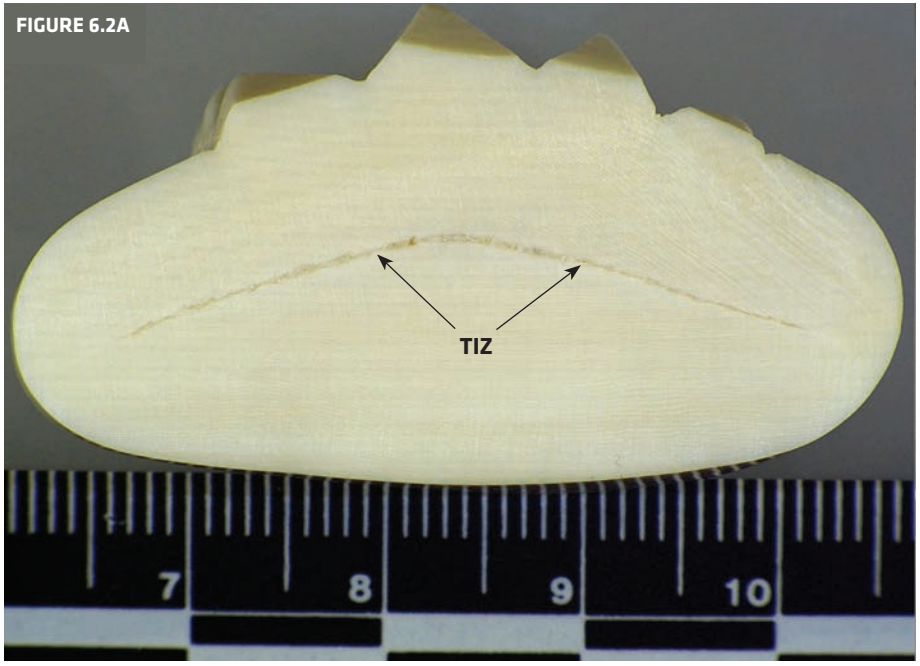
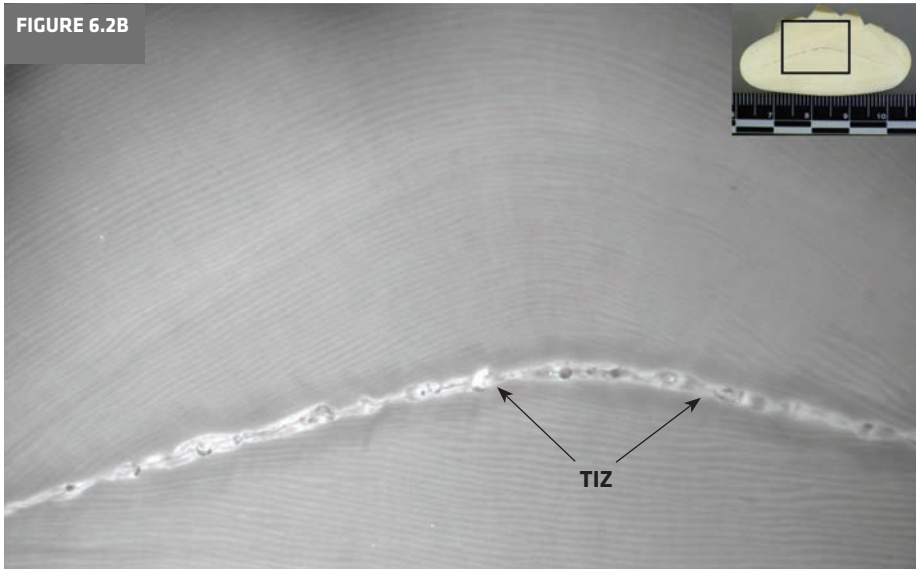


FIGURE 6.2B

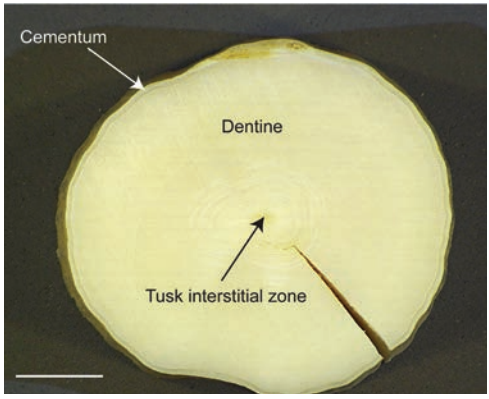


↑ **Figure 6.2A** Cross-section of a lower canine of *Hippopotamus amphibius*.
Figure 6.2B Fine concentric lines in the dentine of the *H. amphibius* lower canine in Figure 6.2A. The image was taken at 30.48 magnification under spot fluorescence with the light source set at 485-590 nm and infrared filter at 645 nm to facilitate visualization. Note the angular TIZ at the center of the tooth in both Figures 6.2A and 6.2B.

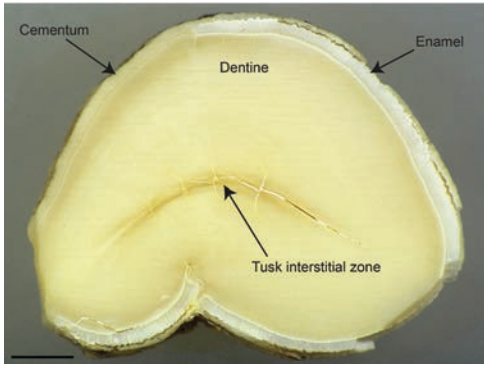
HIPPOPOTAMUS

INCISORS: Hippopotamus incisors are round in cross-section with a small TIZ. Lower central incisors have cementum surfaces and lack enamel (Locke 2013). Accordingly, the dentine directly below the cementum lacks the distinctive fine concentric lines, but they are observable closer to the TIZ (Figure 6.3).

CANINES: Upper canines are oval, rounded, or somewhat heart-shaped in cross-section (Figure 6.4). Lower canines on the other hand are triangular in cross-section (Figure 6.2A). Both upper and lower canines exhibit angular TIZs that follow the shapes of the teeth/tusks. Because canines may exhibit both enamel and cementum surfaces, the fine concentric dentine lines/bands may be visible directly below the surface (enamel) or closer to the TIZ (cementum).



← **Figure 6.3** Cross-section of an incisor of *Hippopotamus amphibius*. Note the small TIZ in the center, and the fine concentric lines visible near the TIZ but absent closer to the cementum. Scale bar is 5 mm.



← **Figure 6.4** Cross-section of an upper canine of *Hippopotamus amphibius*. Scale bar is 5 mm.

CARVED OBJECTS

Many carvings of hippopotamus teeth/tusks maintain the structure of the tooth in the design (Figure 6.5). Accordingly, while many external features (e.g., enamel and cementum) may be removed, hippopotamus ivory may be identified based on the shape and size of the overall piece, as well as the cross-section shape. In smaller carved pieces, hippopotamus ivory may be identifiable based on the presence of the fine concentric lines/bands described above and the shape of the TIZ when present (Figures 6.6A and 6.6B).



Figure 6.5 Raw and carved lower canines of *Hippopotamus amphibius*.



Figure 6.6A Carvings of an upper (left) and a lower (right) canine of *Hippopotamus amphibius*. Scale bar represents 1 cm. **Figure 6.6B** Undersides of the respective carvings in Figure 6.6A. Note the angular TIZ, as well as the fine concentric lines visible on each piece.

WARTHOG

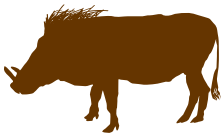
CITES Listings (as of 2020)

Phacochoerus	Non-CITES listed

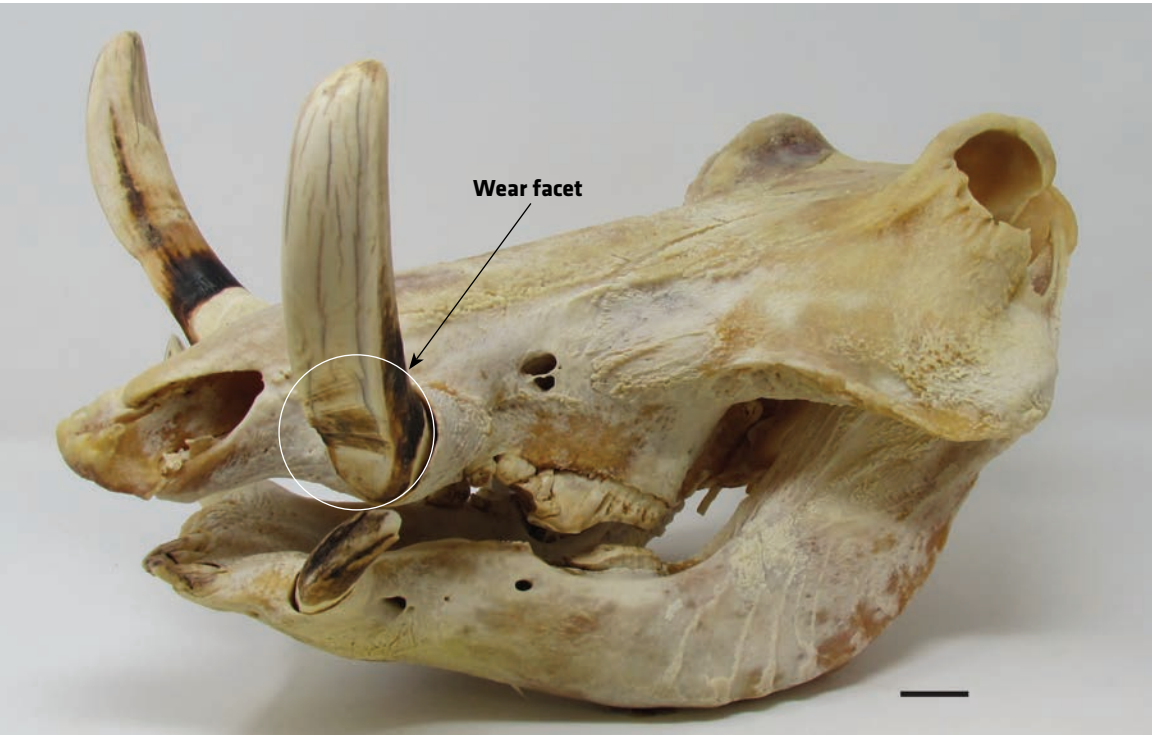


© MARTIN HARVEY/ WWF

WARTHOG



THE LARGE CANINES OF SOME SUIDS ARE ENCOUNTERED IN THE IVORY TRADE, and those commonly observed represent the ever-growing tusks of males. Across the Family Suidae, there is variation in the size of male canines, but those generally observed in the trade are some of the largest and most robust, in particular the upper canines of warthogs (genus *Phacochoerus,* non-CITES listed) (Figure 7.1). The features described below are based on observations of *Phacochoerus* upper canines. This taxon has relatively short lower canines, which can be distinguished from upper canines by cross-section shape and dentine morphology. Specifically, lower canines have a triangular cross-section and lack fine concentric lines/bands occurring in upper canines. Importantly, another species, the giant forest hog *(Hylochoerus meinertzhageni)* exhibits similarly-sized canines as *Phacochoerus,* and they also appear to share similar cross-section shape and dentine morphology (Locke 2013). We recommend caution in any attempt to distinguish these taxa, which also requires comparative reference material.



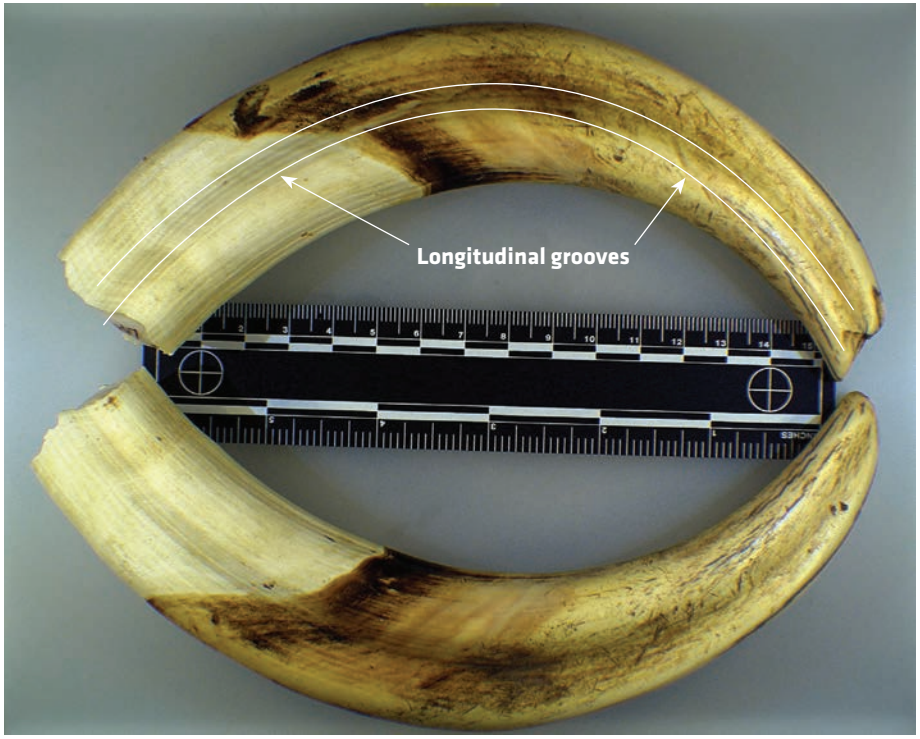
↑ **Figure 7.1** Skull of *Phacochoerus* sp. Note the large upper canines, which are commonly observed in the wildlife trade. Scale bar is 20 mm.

Canines of the common wild boar *(Sus scrofa)* might also be encountered in the wildlife trade and thus have the potential to be confused with *Phacochoerus.* However, the large S. *scrofa* tusks are lower canines and can be distinguished from *Phacochoerus* tusks based on their triangular cross-section morphology and lack of fine concentric lines/bands. For small, modified objects, however, excluding *Sus scrofa* or other suids with smaller tusks (e.g., *Potamochoerus*) may require DNA analysis.

The features described below in conjunction with consideration of size should be considered to apply to *Phacochoerus,* and potentially *Hylochoerus,* although sufficient comparative material for the latter are lacking.

RAW TEETH/TUSKS

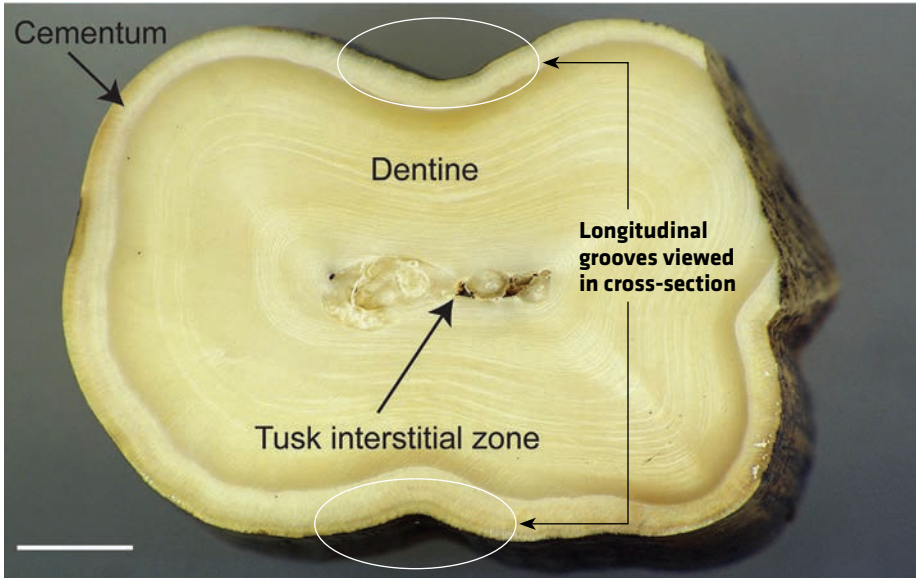
The raw or carved upper canines of *Phacochoerus,* which are commonly observed in the trade, can be distinguished from other ivory objects based on their overall shape. The upper canines are strongly curved. The anterior surface has a wear facet from contact with the lower canine (Figure 7.1), and the medial and lateral sides of the tooth exhibit a longitudinal groove along the length (Figure 7.2).



↑ **Figure 7.2** Upper canines of *Phacochoerus* sp.

CROSS-SECTIONAL MORPHOLOGY

One of the primary distinguishing characters for *Phacochoerus* ivory is related to the shape of the cross-section, which is generally rectangular and "waisted" (pinched centrally) (Figure 7.3). This "waisted" morphology results from the longitudinal grooves along the length of the medial and lateral surfaces. The dentine also exhibits fine but irregular concentric lines/bands that may be visible with the naked eye or require a 10x hand lens. In this feature, *Phacochoerus* ivory appears to resemble hippopotamus ivory, but the fine lines of hippopotamus ivory are generally more regularly spaced. The fine dentine lines/bands are also wavy in *Phacochoerus,* following the overall shape of the cross-section (Figure 7.3). Finally, the upper canines of *Phacochoerus* exhibit a linear TIZ that differs from the TIZ of hippopotamus teeth/tusks, which are angular, or small and round.



↑ **Figure 7.3** Cross-section of an upper canine of *Phacochoerus* sp. Scale bar is 5 mm.

CARVED OBJECTS

Many carvings of *Phacochoerus* upper tusks maintain the structure of the tooth in the design (Figure 7.4). Accordingly, *Phacochoerus* ivory may be identified based on the shape of the overall piece, as well as the cross-section shape and size. *Phacochoerus* identification can be further supported by the presence of the irregular fine concentric lines/bands described above, and the shape of the interstitial zone when present.

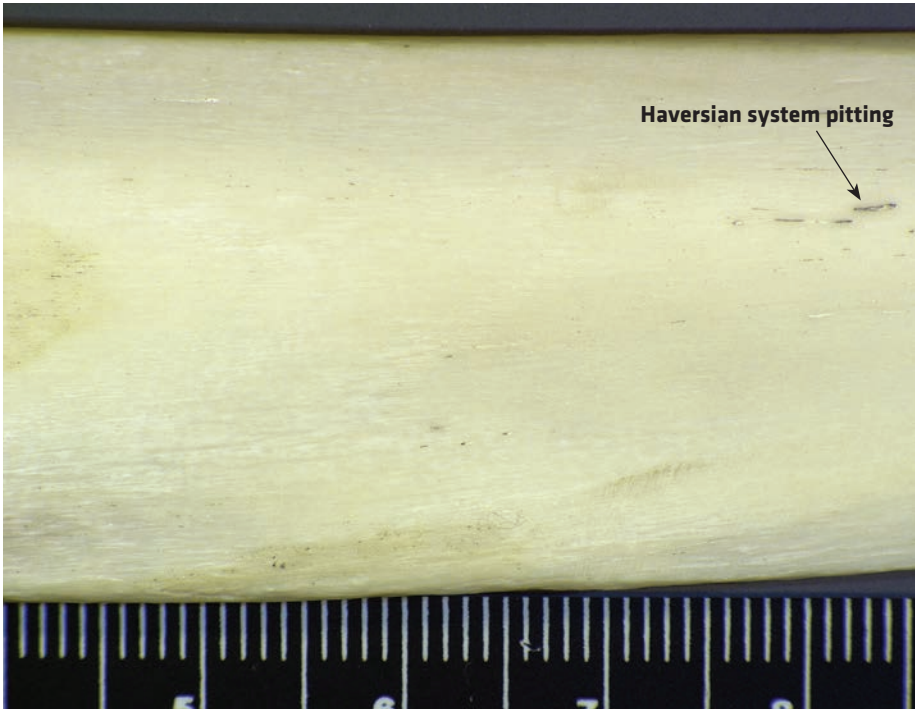


↑ **Figure 7.4** Carved upper canines of *Phacochoerus* sp.

NATURAL IVORY SUBSTITUTES



BONE - Bone is a mineralized connective tissue consisting of hydroxyapatite, proteins and lipids. Compact bone, which is most often used as an ivory substitute (Figure 8.1), is extensively permeated by a series of canals through which fluid flows. These are called Haversian systems. The Haversian canals can be seen on a polished bone surface using a 10x hand lens. These canals appear as pits or scratch-like irregularities (Figure 8.2). Their appearance is often accentuated by the presence of discolored organic material that adheres to the pit walls.



← **Figure 8.1** A large figure made from a collage of polished bone pieces. Each bone chip measures about 1 cm^2. The bone chips were glued onto a wooden base.

↑ **Figure 8.2** A close up of a polished bone. The pitting seen in this image are the Haversian canals, which are diagnostic that an object is made from bone and not from dentine.

CITES Listings (as of 2020)

Rhinoplax vigil	Appendix I

HELMETED HORNBILL *(Rhinoplax vigil)* - The casque of the CITES Appendix I-listed helmeted hornbill (Figure 8.3), a bird which occurs in Southeast Asia, can be carved and polished. The ivory colored casque is distinctive by virtue of its size, up to approximately 8 x 5 x 2.5 cm, and its peripheral color, which is a bright red. Other names for hornbill casque "ivory" are "ho-ting" and "golden jade".



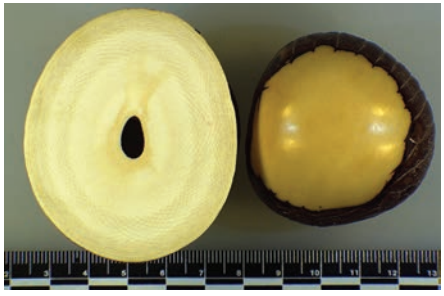
↑ **Figure 8.3** Casques of helmeted hornbill (*Rhinoplax vigil*). Although sometimes called "ivory" casques, these hornbill casques are composed of keratin and not dentine. The right two casques are the complete skull, whereas the left casque has been removed from the skull plate.

VEGETABLE IVORY - Vegetable ivory or ivory nuts are primarily the nuts of the Tagua palm tree *(Phytelephas macrocarpa),* although other palms of the same subfamily also produce ivory nuts. Tagua trees grow mainly in moist locations in northern South America. The mature nut, which can reach the size of an apple, has a very white, exceedingly hard cellulose kernel, which is worked like ivory. The husk of the nut (Figure 8.4) has a dark brown appearance and is frequently incorporated into the carving.

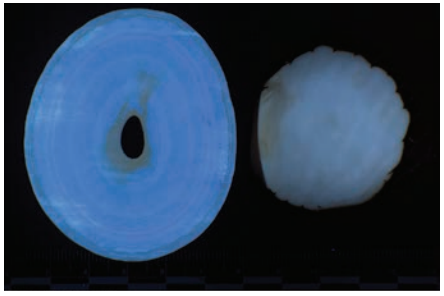
Examination of the cellulose in carved vegetable ivory reveals a series of fine, regularly spaced concentric lines similar to those seen in the hippopotamus. Close examination with a low-powered microscope reveals a grainy or lined appearance. These features may not always be obvious on highly curved surfaces. Vegetable ivory UV fluorescence is very similar to ivory fluorescence (Figures 8.5A and 8.5B). In the absence of obvious morphologically identifying features, identification of vegetable ivory is best done using Fourier transform infrared spectroscopy (FT-IR).



↑ **Figure 8.4** Examples of two tagua nut carvings and an intact tagua nut (*Phytelephas macrocarpa*). These plant products are sold as vegetable "ivory" and are composed of cellulose and not from dentine.

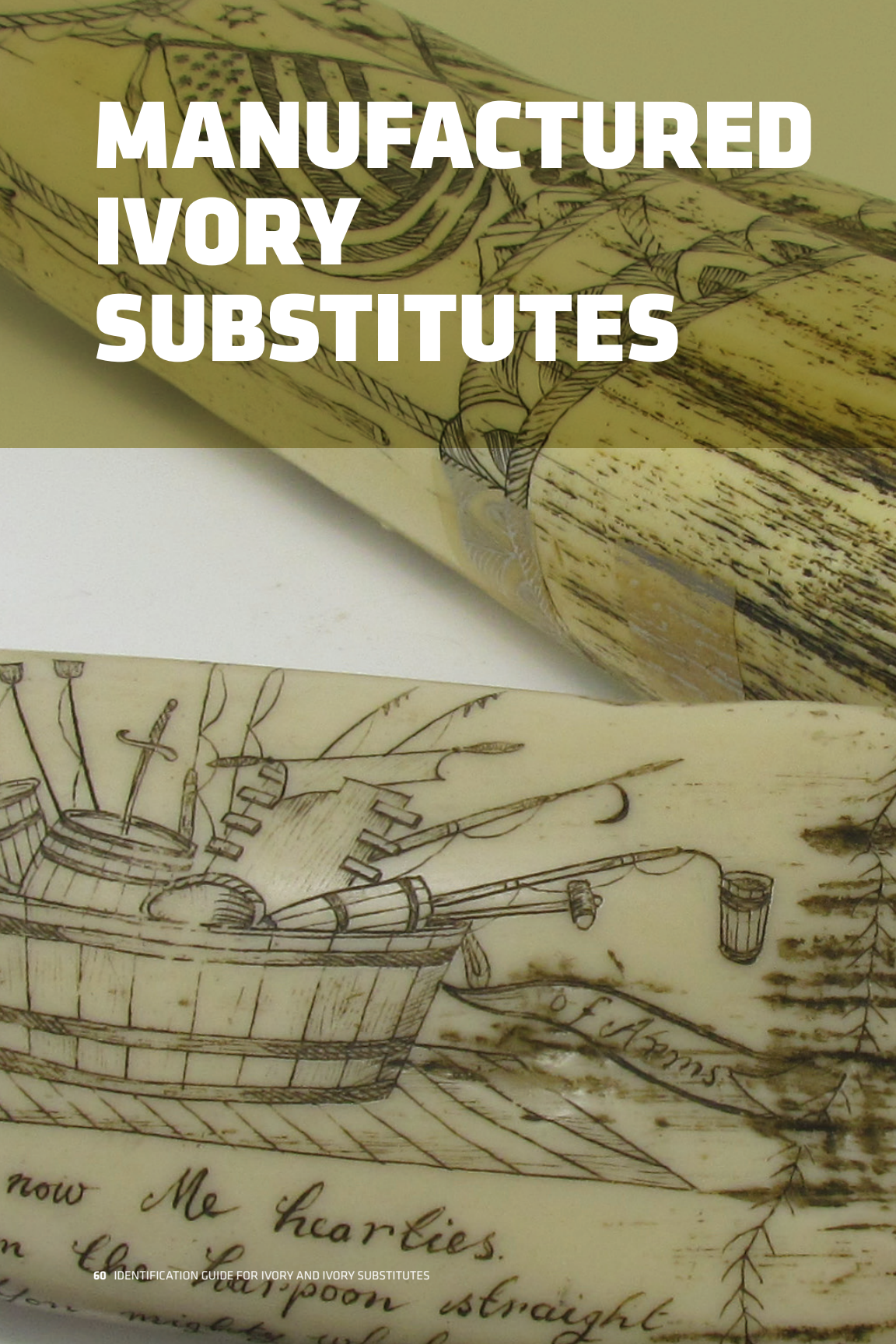


↑ **Figure 8.5A** Comparison of elephant ivory (left) and vegetable "ivory" (right) under normal lighting conditions.



↑ **Figure 8.5B** Comparison of UV fluorescence of elephant ivory (left) and vegetable "ivory" (right) at 365 nm.

MANUFACTURED IVORY SUBSTITUTES



MANUFACTURED IVORY SUBSTITUTES fall into two broad categories:
1) composites made from organic and/or inorganic materials, and
2) composites manufactured with casein, a milk-derived protein. Trade names for some manufactured ivory substitutes vary depending on the manufacturer. Figures 9.1, 9.2A, and 9.2B show examples of manufactured ivory substitutes.

Regardless of the appearance of the manufactured ivory or their chemical composition, they all share a common characteristic that allows for the identification of an ivory substitute: they fluorescence differently when viewed under ultraviolet light. Ivory has a white/blue fluorescence when illuminated with a long-wave UV light source (365 nm), whereas manufactured ivory substitutes exhibit a dull blue or yellowish appearance, depending on the

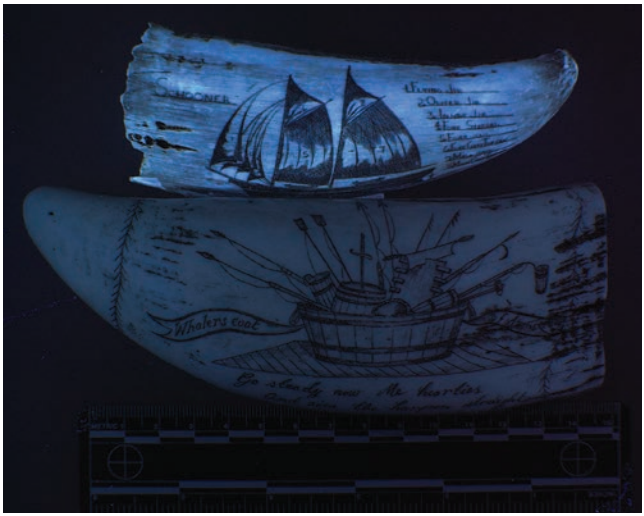


↑ **Figure 9.1** Examples of two items made to resemble ivory. The imitation whale tooth (front) and the imitation walrus tusk (back) were manufactured from a composite resin. Although the exterior has the appearance of a whale tooth and walrus tusk, a careful examination shows that it lacks the natural morphological features.

manufactured source (Figures 9.2B). Identification of manufactured ivory substitutes using a 365 nm UV light should be done in a darkened room, and the analysis should be performed with comparative reference materials using both ivory and manufactured ivory substitutes.



← **Figures 9.2A** Suspected ivory whale teeth.



← **Figure 9.2B** Reaction of the objects to long-wave UV light (365 nm). Only the top tooth has UV fluorescence characteristic of dentine while the bottom object had fluorescence characteristic of a manufactured resin.



© MARTIN HARVEY/ WWF

SUGGESTED READING AND LITERATURE REFERENCED

CITES Wildlife Forensics Page:
https://cites.org/eng/prog/imp/Wildlife_forensics

Ábelová, M. 2008. Schreger pattern analysis of *Mammuthus primigenius* tusk: Analytical approach and utility. Bulletin of Geosciences 83(2):225–232.

Alberic, M., et al. 2017. Relation between the macroscopic pattern of elephant ivory and its three-dimensional micro-tubular network. PLOS ONE https://doi.org/10.1371/journal.pone.0166671.

Barfod, A.S. 1989. Rise and fall of vegetable ivory. Principes 33(4):181-190.

Best, R.C. 1981. The tusk of the narwhal *(Monodon monoceros L.)*: Interpretation of its function (Mammalia: Cetacea). Canadian Journal of Zoology 59:2386-2393.

Brown, G., Moule, A.J. 1977. The structural characteristics of elephant ivory. The Australian Gemmologist 13(1):13-17.

Brown, G., Moule, A.J. 1977. The structural characteristics of various ivories. The Australian Gemmologist 13(2):47-60.

Bruemmer, F. 1989. Arctic treasures. Natural History 98:39-46.

Burack, B. 1984. Ivory and its uses. Charles E. Tuttle Co., Vermont, United States.

Butynski, T.M., de Jong, Y.V. 2018. Common warthog *Phacochoerus africanus* (Gmelin, 1788). In *Ecology, Conservation and Management of Wild Pigs and Peccaries* (Melletti, M. and Meijaard, E., eds.). Cambridge University Press: Cambridge, United Kingdom:85-100.

CITES Wildlife Forensics: https://cites.org/eng/prog/imp/Wildlife_forensics

Chiyo, P.I., Obanda, V., Korir, D.K. 2015. Illegal tusk harvest and the decline of tusk size in the African elephant. Ecology and Evolution 5(22):5216-5229.

Dyer, M.P. 2018. Scrimshaw. In, Encyclopedia of Marine Mammals, 3rd edition, edited by B. Wursig, et al., Academic Press, New York, United States: 841-845.

Espinoza, E.O., et al. 1990. A method for differentiating modern from ancient proboscidean ivory in worked objects. Current Research in the Pleistocene 7:81-83.

Espinoza, E.O., Mann, M.J. 1993. The history and significance of the Schreger pattern in proboscidean ivory characterization. Journal of the American Institute for Conservation 32(3):241-248.

Fisher, D.C., Trapani, J., Shoshani, J., Woodford, M.S. 1998. Schreger angles in mammoth and mastodon tusk dentine. Current Research in the Pleistocene 15:105–107.

Granfield, K., et al. 2014. The narwhal *(Monodon monoceros)* cementum-dentine junction: A functionally graded biointerphase. Journal of Engineering in Medicine 228(8):754-767.

Ishida, Y. , Georgiadis, N. J., Hondo, T., Roca, A. L. 2013. Triangulating the provenance of African elephants using mitochondrial DNA. Evol Appl, 6: 253-265. doi:10.1111/j.1752-4571.2012.00286.x

Kingsley, M.C.S., Ramsay, M.A. 1988. The spiral in the tusk of the narwhal. Arctic 41(3):236-238.

Lambert, W.D. 2005. The microstructure of proboscidean ivory and its application to the subordinal identification of isolated ivory specimens. Bulletin of the Florida Museum of Natural History 45:521-530.

Leslie, Jr., D.M., Huffman, B.A. 2015. *Potamochoerus porcus* (Artiodactyla: Suidae). Mammalian Species 47(919):15-31.

Liang, J., et al. 2014. Identification characteristics of natural and imitation hornbill ivory. The Journal of Gemmology 34(1):42-49.

Locke, M., Dean, R.L. 2003. Vascular spaces in compact bone. The American Biology Teacher 65(9):701-707.

Locke, M. 2004. Structure of long bones in mammals. Journal of Morphology 262(2):546-565.

Locke, M. 2008. Structure of ivory. Journal of Morphology 269-423-450.

Locke, M. 2013. Bone, Ivory, and Horn: Identifying Natural Materials. Schiffer Publishing, Ltd.: Atglen, Pennsylvania, United States.

Loxodonta Localizer. https://www.loxodontalocalizer.org/

Manger Cats-Kuenen, C.S.W. 1961. Casque and bill of *Rhinoplax vigil* (Forst.) in connection with the architecture of the skull. Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen. Afdeling Natuurkunde. Series 2. 53(3):1-51.

Mayer, J.J., Brisbin, I.L. Jr. 1988. Sex identification of *Sus scrofa* based on canine morphology. Journal of Mammalogy 69(2):408-412.

McDonnel, D. 1965. Crystal chemistry of hydroxyapatite: its relation to bone mineral. Archives of Oral Biology 10:421-431.

MacKinnon, J. 1981. The structure and function of the tusks of babirusa. Mammal Review 11(1):37-40.

Maskell, A. 1966. Ivories. Charles E. Tuttle Co., Vermont, United States.

Miles, A.E.W., Boyde, A. 1961. Observations on the structure of elephant ivory. Journal of Anatomy 95(3):450.

Miles, A.E.W., White, J.W. 1960. Ivory. Proceedings of the Royal Society of Medicine 53:775–80.

Miller, W. D. 1890. Studies on the anatomy and pathology of the tusks of the elephant. Dental Cosmos 32:337–48.

Nweeia, M.T., et al. 2014. Sensory ability in the narwhal tooth organ system. The Anatomical Record 297:599-617.

Owen, R. 1845. Odontography. London, United Kingdom.

Owen, R. 1856. Ivory and the teeth of commerce. Journal of the Royal Society of Arts 5:65–71.

Palombo, M.R., Villa, P. 2001. Schreger lines as support in the Elephantinae identification. In, The World of Elephants, edited by G. Cavarretta, et al., Rome, Italy: 656-660. Proceedings of the 1st International Congress. Consiglio Nazionale delle Ricerche, Rome, Italy.

Penniman, T. K. 1952. Pictures of ivory and other animal teeth, bone and antler. Occasional Paper on Technology 5:4–40.

Raubenheimer, E.J., Bosman, M.C., Vorster, R., Noffke, C.E. 1998. Histogenesis of the chequered pattern of ivory of the African elephant *(Loxodonta africana)*. Archives of Oral Biology 43:969–977.

Raubenheimer, E.J. 2000. Early development of the tush and the tusk of the African elephant *(Loxodonta africana)*. Archives of Oral Biology 45:983–986.

Reyna-Hurtado, R., d'Huart, J.P., Turkalo, A.K. 2018. Forest hog *Hylochoerus meinertzhageni* (Thomas 1904). In Ecology, Conservation and Management of Wild Pigs and Peccaries (Melletti, M. and Meijaard, E., eds). Cambridge University Press: Cambridge, United Kingdom:114-121.

Rorimer, J.J. 1931. Ivory and bone. Ultraviolet rays and their use in the examination of works of art. Metropolitan Museum of Art, New York, United States.

Schabilion, S. 1983. All in a nutshell: The story of the vegetable ivory nut. Keystone Comedy, Flora, Mississippi, United States.

Scheffer, V.B., Myrick, A.C. Jr. 1970. A review of studies to 1970 of growth layers in the teeth of marine mammals. Reports of the International Whaling Commission, Special Issue 3:51-63.

Schreger, B.N.G. 1800. Beitrag zur geschichte der zähne. Beitrage für die Zergliederungskunst 1:1–7.

Sims, M.E. 2010. Unusual appearance of Schreger-like pattern in *Hippopotamus amphibius* ivory: Wildlife forensics investigation of a netsuke. Forensic Science International 200(1-3):e19-e20.

Sims, M.E., Baker, B.W., Hoesch, R.M. 2011. Tusk or bone? An example of ivory substitute in the wildlife trade. Ethnobiology Letters 2:40-44.

St. Aubyn, F., editor. 1987. Ivory: An international history and illustrated survey. Harry N. Abrams, Inc., New York, United States.

Trapani, J., Fisher, D.C. 2003. Discriminating proboscidean taxa using features of the Schreger pattern in tusk dentine. Journal of Archaeological Science 30:429-438.

UNODC. 2014. Guidelines on methods and procedures for ivory sampling and laboratory analysis. International Consortium on Combating Wildlife Crime (ICCWC). United Nations, Vienna.

Van der Merwe, N.J., et al. 1990. Identifying ivory. The Rhino and Elephant Journal 4(July):12-15.

Vereshchagin, N.K. 1974. The mammoth "cemeteries" of north-east Siberia. Polar Record 17(106):3-12.

Virag, A. 2012. Histogenesis of the unique morphology of proboscidean ivory. Journal of Morphology 273(12):1406-1423.

Weissengruber, G.E., Egerbacher, M., Forstenpointner, G. 2005. Structure and innervation of the tusk pulp in the African elephant *(Loxodonta africana)*. Journal of Anatomy 206(4):387-393.

Wilson, D.E., Mittermeier, R.A. (eds). 2011. Handbook of the mammals of the world. Vol. 2. Hoofed Mammals. Lynx Edicions: Barcelona, Spain.

Witztum, A., Wayne, R. 2012. Button botany: plasmodesmata in vegetable ivory. Protoplasma 249:721-724.

Yates, B.C., Sims, M.E. 2010. Tupilak transformations: Traditional ivory objects as modern souvenirs. Anthropological Approaches to Zooarchaeology: Colonialism, Complexity, and Animal Transformations, edited by Campana, D., Crabtree, P., deFrance, S.D., Lev-Tov, J., Choyke, A. Oxbow Books/David Brown Book Co., Oakville, Connecticut, United States: 230-234.



© MARTIN HARVEY/ WWF

MODERN FORENSIC METHODS FOR IVORY IDENTIFICATION

The morphological identification methodologies outlined in the previous section may be used to quickly, and without destruction, compare lookalike and natural ivory samples. If it is not feasible to identify a sample through macroscopic and microscopic morphological characters, other forensic methods may be helpful to answer questions regarding species identification, calendar year of death, geographic origin, and individual elephant identification. These forensic methodologies are rapidly evolving, with some processes still under development and others well-supported and applied. They may include both proprietary technologies as well as open-source data and techniques, which are publicly accessible to forensic labs and other users.

The United Nations Office on Drugs and Crime (UNODC) published the *Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis* in 2014 in partnership with the International Consortium on Combating Wildlife Crime (ICCWC). The Laboratory Analysis section (Part II) has been reproduced below with their kind permission, as it provides a comprehensive description of the wide range of tools, methods and tests that can be applied for forensic examination of ivory. The methods include FT-IR (see section 14.1.2), Raman spectroscopy (section 14.1.2), DNA analyses including mitochondrial DNA (mtDNA) (sections 14.1.3, 14.3.1), and isotope analysis (sections 14.2.2, 14.3.2).

While the Guidelines cover relevant laboratory methods and analyses, since its publication in 2014, there is a more recently developed resource that is important to reference. 'Loxodonta Localizer' is a web-based platform that provides the user with the ability to analyze mtDNA data to determine the geographic provenance of African elephant ivory samples. This open-source tool is free to use, the source data have been peer-reviewed by independent experts, and it is accessible at https://www.loxodontalocalizer.org/.
Loxodonta Localizer is a database tool based on mtDNA sequence data from African elephants, both forest and savanna, from 24 countries. The dataset is described in Ishida et al., 2013 and has been updated with additional sequences. The user enters a 316 bp mitochondrial control region sequence from an African elephant sample, and the localities from which elephant samples with similar sequences have been reported are displayed.

There are numerous tests and analyses under development that may be applicable and soon available, but are not included in this CITES Ivory Identification Guide published in 2020. They may be included in future editions once the methods are validated and replicable.

Disclaimer for *Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis* (2014) by UNODC in partnership with ICCWC.

The following text is an extracted section, directly reprinted from the 2014 Guidelines by UNODC in partnership with ICCWC and does not reflect the views or opinions of the authors of this Ivory Identification Guide, CITES, United Nations, USFWS, TRAFFIC or WWF.

Operating and experimental conditions are reproduced from the original reference materials, including unpublished methods, validated and used as per the list of references. A number of alternative conditions and substitution of named commercial products may provide comparable results in many cases, but any modification has to be validated before it is integrated into laboratory routines. Mention of names of firms and commercial products does not imply the endorsement of the CITES, TRAFFIC, United Nations, USFWS or WWF.

Copyright for the extract from *Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis* (2014) by UNODC in partnership with ICCWC.

© United Nations, November 2014. All rights reserved worldwide. The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the United Nations concerning the legal status of any country, territory, city or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries. This publication has not been formally edited. Publishing production: English, Publishing and Library Section, United Nations Office at Vienna, Austria.



© CLAIRE CLARKE

UNODC
United Nations Office on Drugs and Crime



Guidelines on
Methods and Procedures for Ivory Sampling and Laboratory Analysis

CITES INTERPOL WORLD CUSTOMS ORGANIZATION UNODC United Nations Office on Drugs and Crime

INTERNATIONAL CONSORTIUM ON COMBATING WILDLIFE CRIME

Part II. Laboratory analysis

Laboratory analysis may provide a powerful means of confirming the species, age and origin of ivory samples, as well as to link samples to individual elephants. The steps in laboratory analysis may be different depending on the sample type and purpose of the analysis. This section is technical and aimed at laboratory analysts with expertise in specific areas.

14. Considerations for laboratory analysis

Ivory sample types fall into one of two broad categories:

- Raw ivory: unprocessed whole or sectioned tusk
- Worked ivory: sections of tusk such as carved ornaments, hankos (seals), ear-rings, pendants, etc.

The type of the ivory sample needs to be considered in taking decisions on the entire process and procedures to be used for the analysis.

The scientific methodology to be performed for testing the ivory samples depends on the question being addressed and the forensic/scientific capacity available, including appropriate equipment and skills. Typically, investigative questions dealing with ivory fall into the following categories:

- Is it ivory? If so, what is the species source?
- When was the animal killed? How old is the ivory sample?
- Where was the elephant killed?
- How many elephants were killed?

It is essential that all those conducting the procedures below understand the need for good laboratory practice. All processes should be validated and standard operating procedures should be in place. The laboratory performing the analysis needs to be confident that the methods are reliable, robust and reproducible. Method verification is required when a laboratory is using published, validated methods for the first time. This ensures reproducibility of results in another laboratory.

14.1 Methods for the identification of species

Ivory can come from several species. There is well-established and widespread trade in tusks other than elephant, therefore it is important to determine from which animal species the ivory originates. For this purpose, a number of tests can be used. The applicability of these methods to different types of ivory sample is outlined in table 1. The methods vary from the non-destructive and skill-based, to destructive, equipment-based tests.

14.1.1 Morphology

Morphology using a visual inspection may be all that is needed. The whole tusk may not require any further scientific testing. If the samples are sections of tusks presumed to be ivory, then microscopy can be employed as a first test. A guide for the identification of ivory through visual, non-destructive means has been developed. It includes information on ivory identification through examination of Schreger lines, which can be used to distinguish between ivory of different species [10].

14.1.2 Vibrational spectroscopy: Fourier transform infrared spectroscopy and Raman spectroscopy

Certain molecules, when exposed to infrared or laser energy, display diagnostic vibrational patterns. These patterns (bands) give a general indication of the composition of the material being studied. In ivory, these techniques have been used primarily for two purposes:

- To determine if a carved object is made from ivory or plastic
- To determine if a carved object originated from mammoth or modern elephant ivory

Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy are the best tools to distinguish ivory-looking plastics from genuine hydroxyapatite-based ivory. The vibrational spectroscopy techniques are non-destructive and quick to perform. However, these analytical tools cannot determine the exact species of the many animals that produce ivory [25-32].

14.1.3 Mitochondrial DNA

Mitochondrial DNA is used in many areas of taxonomy and forensic science, for both human and wildlife subjects. Mitochondrial DNA typing has the potential to identify a species and requires access to specific equipment. It is a destructive method; however, only a small-sized sample is needed for this analysis.

Table 1. Applicability of methods of species identification

Test	Tusk	Processed tusk	Tissue
Morphology • Non-destructive • Little equipment required	Based on the whole structure Morphology can be used depending on amount of detail available	Based on the whole structure	Not always possible
Raman spectroscopy • Non-destructive **FTIR** • Non-destructive • Requires specific instrument	Can get to family or genus level depending on the quality of sample available		Not ideal
DNA • Destructive • Requires equipment	Mitochondrial DNA loci such as cytochrome b (cyt b) or cytochrome oxidase 1 (COI) are species-informative and can be analysed in all sample types		

14.2 Methods for the determination of age

Age determination can give an indication whether seized tusks are from recent poaching incidents by providing useful intelligence surrounding how recently an animal was alive. Furthermore, determination of age provides crucial information for CITES Management Authorities to decide whether ivory can be classified as pre-convention material, and thus whether the provisions of the Convention apply to that specimen. The applicability of methods to determine the age of ivory based on type of sample is outlined in table 2.

14.2.1 Morphology

In some cases, the physical characteristics of the sample can be used to determine age; for example, the presence of blood, the presence of a strong carcass odour, or carving in a style that is indicative of a specific era. However, care must be applied when providing an opinion of age based on appearance, as it is possible to make recent ivory look like an old sample.

14.2.2 Isotopes

A more definitive scientific method to determine sample age involves isotope analyses. Isotopes are different forms of an element that have dissimilar massing because of different neutron numbers in the nucleus. Most isotopes on Earth are stable, but some are radioactive and decay over a period of time characteristic of their half-life. Ivory is secreted at the margin of the inner pulp cavity of a tusk; therefore, the youngest ivory is found along this margin, becoming progressively older as the distance outwards from the margin increases. The transverse growth rate is approximately 5 mm per year and the longitudinal growth rate is approximately 5 cm per year [33]. Ivory consists of bioapatite (dentine) and collagen, with percentages of circa 70:30. The bioapatite and collagen in ivory do not exchange elements or isotopes once formed and therefore provide a time sequence of the isotope ratios [34]. For stable isotopes, this records a history of diet (C, N, S incorporated in food) or water (H, O in food and water) using natural abundances of these isotopes.

Radioisotopes can be used to determine the age of raw or worked elephant ivory. If whole tusks are available, the year of death can often be determined; if worked ivory is available, the year in which the available sample grew can often be determined. The nuclear weapons testing of the 1950s and early 1960s almost doubled the concentration of carbon 14 (^{14}C) in the atmosphere. Since then, the concentration has gradually decreased due to natural processes, primarily absorption and recycling in the biosphere and oceans. In 2014, it dropped to a level of about 4 % above the natural concentration. Because of the long half-life of radiocarbon (5,730 years), the well-calibrated "bomb curve" over the past 50 years is widely used for dating samples formed since the mid-1950s. Measured ^{14}C concentrations in plants and animal tissues can be assigned an age of formation to within a few years with appropriate sampling protocols; in some particular situations, the age of formation can be determined to within ± 0.5 years. If the samples were taken along the active growing interface, the age of death can be determined [31, 33]. Thus, the "bomb curve" is useful for accurate dating from circa 1955 to the present.

However, the "bomb curve" had a rapid rise, peaking circa 1964, followed by a gradual decline. Thus, most samples from seizures are likely to be on the declining limb of the bomb curve. However, in certain cases, two findings of age may be obtained (one from 1955 to 1965; the other from 1965 to the present). One approach to verify the results is to use a different isotope with a history unlike that of ^{14}C, e.g. ^{90}Sr, as recent research suggests [35]. Another method is based on the relative uptake of radium (bio-available) compared to thorium (not bio-available). It determines the absolute time since the tissue was formed and is independent of either "bomb curve" [36]. A third approach is to measure two ^{14}C subsamples taken from adjacent positions along the growth axis of the sample of interest.

In most cases, and until the concentration of ^{14}C isotopes in the atmosphere reaches background levels, ^{14}C can date ivory samples to within a few years. Using accelerator mass spectrometry for ^{14}C dating, the carbon content of the sample should

be approximately 1 mg, which usually corresponds to approximately 10 mg of raw ivory. Thus, samples taken along the inner margin of ivory, along the tusk-pulp interface, can be used to determine the date of death. In certain cases where the problem of dual solutions of the ivory ^{14}C bomb curve needs to be resolved, measurement of ^{90}Sr or $^{228}Th/^{232}Th$ ratios or measurement of two ^{14}C subsamples taken from adjacent positions along the growth axis is required; for the measurement of ^{90}Sr or $^{228}Th/^{232}Th$ ratios, a minimum of 10 g of ivory is needed for these analyses [33, 37].

Table 2. Applicability of methods to determine age of ivory sample

Test	Tusk	Processed tusk	Tissue
Morphology, if there is an indication of aging or the sample appears very new	Whole structure	Whole structure Carving methods or styles	Not possible
Radio isotopes	Gives ± x years depending on combination of radionuclides (^{14}C, ^{90}Sr, $^{228}Th/^{232}Th$)		Only useful for tissue that is metabolically inert

14.3 Methods for the identification of geographical origin

Identifying the geographical origin of the ivory can provide intelligence to law enforcement officers and help detect poaching hotspots. The applicability of methods to determine the geographical origin of an ivory sample is outlined in table 3.

14.3.1 DNA analysis

There are three DNA regions that can be used for identification of geographical origin: mtDNA, Y-chromosome STRs and autosomal (nuclear) DNA microsatellites. DNA typing using microsatellites is a proven method for geographical assignment. Other genetic markers, such as the use of mitochondrial DNA, are possible, particularly if there is insufficient DNA for nuclear markers, and Y-linked microsatellites might be used. However, origin assignment using mtDNA and Y STRs are of lower power of discrimination compared to the use of autosomal nuclear microsatellite markers. They also require very comprehensive sampling because of their sex-specific transmission. Shared presence of a haplotype may not infer origin if that haplotype is present in unsampled populations from other locales. Similarly, absence of a haplotype from a location could result from incomplete sampling and thus does not necessarily imply that the sample came from elsewhere.

Microsatellites on the Y chromosome have a specific advantage, as they are only passed from the bull to male offspring. Both mitochondrial DNA typing and Y-STR markers have a significantly lower power of discrimination when compared to the markers described [38].

14.3.2 Isotope analysis

Stable isotope analysis is widely used to determine animal diets. Isotope maps of geographic distributions are used to determine geographical origins of plant, animals and processed materials [39]. Study of provenance of wildlife is based on the inheritance of natural isotope abundance ratios based on local food webs (carbon), ecology (nitrogen), geology (strontium), geography (sulphur, including marine aerosols) and elevation (oxygen and hydrogen). Taken together, a combination of isotopes can produce an intersection of space that is limited in geographic space. Bioapatite in ivory can be analysed for carbon, oxygen and strontium isotopes; collagen in ivory can be analysed for carbon, nitrogen, and sulphur isotopes; oxygen and hydrogen in collagen can be analysed if precautions are taken for exchangeable isotopes.

Van der Merwe et al. and Vogel et al. showed that stable isotopes (carbon and nitrogen) could be used to distinguish different African elephant populations [40, 41]; van der Merwe et al. and Vogel et al. showed that the heavy isotopes, such as $^{87}Sr/^{86}Sr$, which are related to the geological age of local bedrock, can also distinguish elephant populations [42, 43]. Work by Lee-Thorp et al., Hall-Martin et al. and Hart et al. [44-46] was built upon by Emslie et al. (2001) and Amin et al. [47-49] and found that stable isotope-ratio chemical composition of rhino horn also varied from area to area and from species to species, reflecting both geological and rainfall differences. More recently, Ehleringer et al., Valenzuela et al. and Chesson et al. have mapped isotopes in water and humans across North America and showed that coherent patterns emerge due to local geology, diet and meteorological patterns [50-52]. These are termed “isoscapes” [39] and are becoming widely used in various wildlife studies. Ziegler et al. have developed “isoscapes” of elephant ivory to assist in the determination of ivory origin [53]. Cerling et al. have applied carbon, oxygen and nitrogen isotopes to determine elephant “isoscapes” in Kenya [54], and Ziegler et al. have shown that the measurement of multiple isotopes in ivory greatly improves the predictive power in provenance studies [55].

Stable isotope analysis can be useful in answering specific compliance questions, such as whether a sample comes from a specific region. Depending on the nature of the sample and the reference database, stable isotope analysis may be able to identify an unknown sample with a precise geographic source. The precision of any discriminating tool depends on the number and variability of measures used as well as the comprehensiveness of the geographically specific reference sample map used to make the assignments, which can provide complementary information on the geographic origin of ivory.

Table 3. Applicability of methods to determine geographical assignment of ivory

Test	Tusk	Processed tusk	Tissue
Nuclear microsatellites	Possible for all sample types, provided ~ 1 ng of DNA can be isolated		
Mitochondrial DNA	Possible for all sample types, particularly if less than 500 pg of DNA is isolated		
Y microsatellites	Possible for all sample types, provided ~ 1 ng of DNA can be isolated		
Stable isotopes	Possible for tusk and bone material if at least 30 mg material is provided for multiple isotope testing		Possible if reference database is established

14.4 Methods for the determination of individual elephant numbers

Linking ivory to individual elephants allows scientists to estimate the number of elephants killed. The applicability of methods to link ivory to individual elephants is outlined in table 4.

The minimum number of elephants from which ivory may have originated can be determined simply by counting the number (n) of tusks and dividing by two if whole (or near whole) tusks are seized. However, this method may largely underestimate the real number of elephants killed when whole tusks are not present in pairs, and it is completely inapplicable when the tusks have been worked.

There is currently one scientific method available with the potential to determine individual elephant numbers, i.e. DNA typing (table 4) [56]. The microsatellites described in Wasser et al. and others can be used to link a seized sample to a carcass or to other seized samples in much the same way as DNA profiling is used in human identification [38, 57, 58]. Similarly, nuclear DNA typing using the RhoDIS system has been successfully used in rhino forensic investigations and as part of prosecutions in court [59]. Mitochondrial DNA—inherited by all, but passed down through the maternal lineage, and Y microsatellites—passed only through the paternal lineage, have a specific but complementary role in linking a seized sample to a carcass or other seized samples and can also play a valuable role in familial matching (matching tusks to their family members).

Table 4. Applicability of methods of individual identification from ivory samples

Test	Tusk	Processed tusk	Tissue
Minimum number of individuals	Possible if there are whole tusks, including reassembled pieces, using n/2	Not possible	Not possible
Microsatellites	Allows standard DNA assignment statistics and individual identification		
Mitochondrial DNA	Works on all samples, but only identifies the maternal lineage		
Y microsatellites	Works on male samples only and only identifies the paternal lineage		

14.5 Procedures

14.5.1 DNA analysis

Sample pre-treatment

Ideally all work should be performed in suitable facilities in order to minimize the opportunity for contamination of the samples, particularly from other ivory samples, from any elephant reference material and especially from polymerase chain reaction (PCR) products [60, 61].

There is limited DNA within ivory and potentially less from processed ivory. Human DNA is likely to be on the outer surface of the ivory. This human contaminant is best removed by washing the sample's surface, followed by swabbing with 10 % bleach (or with deionized water followed by 30 U/ml Benzonase® Nuclease). Once cleaned, it is important to handle the sample with gloves to prevent additional human DNA contamination. It is also necessary to avoid contaminating the material with DNA from other elephant material by processing at different times and places, or by ensuring a physical separation.

Sample preparation and extraction procedure for genomic and mitochondrial DNA

A standard method must be followed for DNA extraction. For DNA extraction from ivory, see Mailand and Wasser [62]. In addition, it is important to avoid overheating

the ivory when it is being pulverized for DNA extraction, as this can degrade the DNA. One way to avoid this is to use a freezer mill, which uses liquid nitrogen to freeze the ivory to –200°C in order to facilitate pulverizing while safeguarding the DNA in the process. Whether mechanical equipment or a manual approach is used, it is important to ensure that chances of contamination from one ivory extraction to another are minimized. The apparatus needs to be cleaned thoroughly between each sample. Cleaning using 10 % bleach and ultraviolet light are options.

Approximately 200 mg of powered ivory is needed per extract to ensure sufficient DNA at the end of the process. Ivory has relatively low amounts of elephant DNA encased in high amounts of minerals such as calcium. The ivory must thus be demineralized after being pulverized into a fine powder. Incubating the pulverized ivory powder in a high amount of ethylenediaminetetraacetic acid (EDTA) is a very effective way to remove calcium from solution. Incubate the powdered ivory overnight at 4°C in a solution of 0.5 M EDTA in a sterile tube, wash and repeat for another 24 hrs. It is ideal to perform two extracts on the same sample, because DNA is not evenly distributed in the tusk. Taking two extracts helps assure that you capture a reliable amount of DNA to maximize the chances of amplifying all the allele forms present in the sample [62, 63].

DNA is purified from the extraction buffer using a range of methods, including the use of commercially available products. Multiple extracts from the same sample can be pooled to increase the amount of DNA, but care must be taken to only combine samples that have been thoroughly demineralized.

DNA amplification by polymerase chain reaction

Some ivory samples can prove difficult to analyse for a variety of reasons. If both extracts completely fail to amplify DNA while other samples worked fine, it may be best to discard that sample from further analyses if many alternative samples are still available. If one of the two extracts work and the other does not, it may be worth adding a third extract from that sample. If the majority of samples fail, then it is important to review all steps to make sure they are each being properly performed.

If there is DNA present, but the sample fails to amplify, it is likely that the sample contains inhibitors that are preventing the DNA from amplifying. There are a variety of sample clean-up techniques available to remove inhibitors. Sometimes, diluting the sample helps because it reduces the number of inhibitors in the extract. In this case, the DNA is also diluted. However, as long as the DNA primers are able to find the DNA, it can be amplified. Concentrating the DNA is also an option. Sometimes, it is best to try both procedures to see which method helps most in the specific case.

Amplification of DNA by polymerase chain reaction (PCR) has the tremendous benefit of being highly sensitive, but this sensitivity can be a problem if not

performed in clean facilities in order to minimize the chance of contamination. Reference material should not be analysed in the same laboratory space as DNA from seized items. Clean gloves are needed, along with the use of negative and positive controls [60, 61]. Negative controls replace the DNA with sterile H_2O. (Blanks contain DNA-free, sterile water instead of the DNA solution.) For autosomal microsatellite amplification, DNA from one of the two African elephant species acts as a positive control. For mtDNA, DNA from each species acts as a positive control. In this case, the DNA from controls should also amplify the same product, which is identical to the known genotype of the added DNA from the control animal.

Because DNA is unevenly distributed in tusks, it is recommended that the microsatellite DNA is amplified from two or more extracts per sample, with each extract amplified in two separate reactions. Each heterozygous allele should be detected at least twice in order for the result to be confirmed. A homozygous allele should be detected at least three times in order for the result to be confirmed. This will help to prevent missing one of the two alleles per locus if the DNA is degraded (i.e. if it has a very small fluorescent peak on the genetic analyser, or a light band on a gel, multiple missing loci, and an excess of homozygous loci among those that do amplify). Further information on microsatellite markers can be found in Wasser et al. [38].

There are two main mitochondrial DNA loci used in species testing: either the cytochrome b (cyt b) or cytochrome oxidase 1 (COI) loci, as both have extensive reference data for comparisons. As an example, see Lee et al. [64]. These two loci have sufficient DNA sequences within the areas examined such that all the extant species of elephant can be separated from each other and from any other mammalian species, including the extinct mammoth. The work of the Barcode of Life Consortium* has standardized a section of the COI locus and combines species assignment by DNA typing with taxonomy and knowledge based on morphology to assist in accurate species assignment.

Primer sets for both loci are available in the published scientific literature that will amplify, for example, a 400 base pair fraction of the cyt b locus or 645 base pair fraction of the COI locus. These are standard sections of the loci with ample reference data available for comparison purposes. Full details can be found in Linacre and Lee [65].

Sequence alignment of any DNA data from ivory to reference data can be performed using free software (such as MEGA v4). Differences between the Asian, savannah, forest and mammoth elephants are all greater than 2.5 % for either locus. Dissimilarity less than 1.5 % when comparing two samples from the same species may be considered as intra-species variation [66].

*The Barcode of Life Consortium supports the development of DNA barcoding as a global standard for species identification. DNA barcoding is a method that uses a genetic marker in an organism's DNA to identify it as belonging to a particular species. For more information, see http://www.barcodeoflife.org/.

DNA fragment analysis procedure

The capillary electrophoresis equipment should be loaded with the software Genemapper or Genemarker. A size marker such as Rox500 should be used as an internal lane standard. The DNA profile should be from a single source and not show any indication of being a mixture from multiple individuals (e.g. detecting ≥ 3 alleles per locus in the same sample). The negative control should be clear of any DNA profile. The positive control should generate the correct DNA profile.

DNA data analysis

mtDNA sequence data analysis

Sequence alignment of any DNA data from ivory to reference data can be performed using free software (such as Molecular Evolutionary Genetics Analysis (MEGA), Arizona State University) [67]. Differences between the Asian, savannah, forest and mammoth elephants are all greater than 2.5 % for either locus. Dissimilarity less than 1.5 % when comparing two samples from the same species may be considered as intra-species variation.

Autosomal microsatellite DNA fragment analysis

Record the size in base pairs or data points and the height of each peak. The peak height data helps confirm that the data are reliable. The size data are essential for both individual assignment and geographical testing, for example, using the Smoothed Continuous Assignment Technique (SCAT) software.*

The SCAT is used to identify samples as forest, savannah or hybrid subspecies [38]. SCAT uses a Bayesian method implemented with Markov chain Monte Carlo spatial smoothing to simultaneously estimate allele frequencies at any location in Africa [68, 69]. Allele frequencies are assumed to depend on all reference samples with a spatial correlation that depends on distance between populations. SCAT is then used to assign population of origin to all pure (non-hybrid) samples using the estimated allele frequencies of the same subspecies, forest or savannah. Multiple samples from the same subspecies are assigned independently using a uniform prior over all parts of Africa, or as a group using a Voronoi prior that capitalizes on genetic similarities between samples [63]. Group assignment assumes the query samples were sampled uniformly from the same region consisting of one or more polygons, not necessarily adjacent, identified by a process known as Voronoi tessellation [63, 70].

Locus inclusion requires both alleles to be confirmed. Ideally, samples with less than 10 out of 16 confirmed loci should be excluded from the statistical analysis. However, the analysis can still be reliably performed with a minimum of 7 confirmed loci.

*Software freely available for Windows and Macintosh at http://conservationbiology.uw.edu/research-programs/tracking-poached-ivory/scat-win/ and http://stephenslab.uchicago.edu/software.html respectively.

14.5.2 Isotope analysis

Stable isotopes

Ivory fragments of at least 30 mg should be taken from different positions at the proximal end of the tusk by using a small hand saw, or alternatively, a pincer. Thus, as this is the youngest part of the tusk, it is assumed that the isotopic signal reflects the environment where the animal lived just before its death. Samples collected from the pulp cavity margin will give the last 6 to 12 months of geographic information. Fragments should be sealed in polyethylene bags until further analysis. Samples from processed tusks can also be taken, but then the determination of provenance where the elephant died is less certain because the time between the formation of that piece of ivory and the time the elephant died is unknown, and the animal could have dispersed far away in that interim. This concern is particularly significant for males.

After pulverization in a ball mill made of hardened steel with the grinding jar continually cooled with liquid nitrogen at –196°C, samples should be cleaned with dichloromethane to extract weakly bonded, adsorbed water on mineral and bone surfaces as well as apolar substances, such as tissue fat, and then allowed to air dry at 60°C for 36 hours. Samples should then be stored in a desiccator to avoid humidification. Isotopic measurements of subsamples (1-4.5 mg) of different stable isotope ratios of light elements should be carried out with high-precision continuous flow isotope ratio mass spectrometers (IRMS). Pulverized ivory can be measured directly, but conventional protocols suggest the separation of ivory into collagen and mineral components. This will allow for high-precision measurements of the isotope ratios for carbon and oxygen (bioapatite), carbon and nitrogen (collagen), hydrogen and oxygen (collagen), and sulphur (collagen). Stable isotope ratios (R) will be expressed in the delta notation (δ) in the conventional per mil (‰) unit, where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$, and is interpreted as the difference, in parts per thousand, between the sample and the international reference standard. Quality control should be ensured with control samples of known isotopic composition as described by Valengula et al [50]. In order to assess precision of the analyses, at least two replicate measurements should be performed for each sample. Isotope ratios can be used to assign ivory samples of unknown origin to areas of presumed provenance by using expert opinion or spatial reference data that will be stored in the Ivory ID database. This web-based database will initially store approximately 600 ivory reference samples of verified origin from 25 African and 6 Asian elephant range States.

Radionuclides

Samples should be collected from the proximal end of the tusk or the pulp cavity margin to estimate the year of death. Approximately 10 g of ivory is needed using conventional ^{14}C methods (e.g. LSC, ^{14}C); for the ^{14}C acceleration mass spectrometry (AMS) method, two samples totalling circa 20 mg of raw ivory are needed.

If the combined radioanalytical method is applied to determine the specific activity of $^{14}C/C$ and $^{90}Sr/Ca$ and $^{228}Th/^{232}Th$, a minimum of 10 g of ivory is necessary to realize sufficiently low values of lower limits of detection.

The radioanalytical method consists of ashing, radiochemical separation and preparation of suitable detection samples. The analysis methods must be executed as efficiently a nd undisturbed as possible, realizing very low limits of detection. It is necessary to separate and concentrate the elements of concern without significant losses first and purify them from possibly interfering radionuclides like ^{40}K and ^{137}Cs. Different low-level nuclear radiation detection methods are applied to determine the activities of the radionuclides of interest. These are liquid scintillation counting (LSC) to detect ^{14}C, beta counting by a gas-flow counter to detect ^{90}Sr and alpha spectrometry by a silicon-surface barrier junction detector to detect the radionuclides ^{228}Th and ^{232}Th. To fulfil the qualification, a well-appointed radioanalytical laboratory must be available in addition to well-trained staff and low-level nuclear detection devices [33, 37].

14.6 Proficiency testing or concordant studies

Laboratories performing forensic analysis should be part of a proficiency testing scheme. It is an integral part of the quality management system of the laboratory and a requirement for accreditation purposes. Proficiency testing helps to identify analytical problems and to support laboratories in their efforts to improve the quality of their analytical results. Samples of known or unknown origin are tested by the laboratory and results returned to an administrator to determine if the correct results were obtained. A number of proficiency tests are available.

DETECTION AND IDENTIFICATION OF ELEPHANT IVORY SOLD ONLINE

By Giavanna Grein and Crawford Allan



INCREASINGLY, IVORY AND LOOK-A-LIKE PRODUCTS ARE BEING SOLD THROUGH WEB-BASED PLATFORMS (referred to as *platforms* hereafter) such as e-commerce websites, search engines and social media applications. Identifying ivories through these platforms is more challenging as, unlike a physical item that can be closely examined, an item depicted online cannot be held and inspected. Often, information provided by an online seller or vendor, such as descriptions and images, can be insufficient to determine if a product is made from or contains elements of elephant ivory. However, there are some key indicators to look for in online monitoring to identify elephant ivory from other ivories and known substitutes or look-a-likes. It is important to note that not all of these indicators confirm authenticity, but rather contribute to evidence gathering in order to determine whether or not the product listing is a concern that merits further action.

PRIMARY INDICATOR: PRESENCE OF SCHREGER LINES

As demonstrated in the *Introduction* and section on *Elephant and Mammoth Tusks,* Schreger lines are physical properties unique to elephant and mammoth ivories. These may be visible in online images when the resolution is high enough and proper product angles are displayed. To look for Schreger lines, click on the product image to enlarge it, and use a zoom feature if present on the platform to closely observe cross-sections and rounded edges. See page 18 of this guide for examples. If these are visible in the product image, the item is likely to be either genuine elephant or mammoth ivory.

SECONDARY INDICATORS:

LANGUAGE - SEARCH WORD CLUES

'IVORY' LISTINGS

Some sellers will list their products as *ivory* or *elephant ivory* despite platform prohibitions of the products. This may be true even if the item is made from substitutes such as bone or plastic. If the listing states the item is *ivory*, it does not confirm authenticity; however, it may be worth a review.

KEY SEARCH WORDS

With increasing restrictions on elephant ivory products traded online, sellers are using text descriptions of the items for sale that attempt to mislead automated platform detection filters. The text descriptions may be in the relevant language of the platform or a different language and will still appeal to buyers who would understand what the product is and perhaps that it is authentic. For example, rather than listing a piece of ivory as an elephant product, the advertisement may say the product is made of *bone* and from a *large African mammal*. These workarounds are constantly evolving as automated filters pick up on known search words, though the text-based deceptions by sellers generally fall into the below categories:

- **AGE:** The trade in elephant ivory was first regulated under CITES in 1975 with Asian elephants listed in Appendix I, and subsequently with African elephants listed in Appendix II in 1977. An international commercial trade ban went into effect in 1990 when African elephants were uplisted to Appendix I (and the subsequent downlisting for populations in Botswana, Namibia, South Africa

and Zimbabwe to Appendix II in 1997). Sellers may try to indicate that the ivory they offer is old enough to be legal to trade without violating these regulations. Ivory claimed to be *pre-convention* would be any elephant ivory harvested prior to 1975 and elephant ivory claimed to be *pre-ban* would be African elephant ivory harvested prior to 1990. Sellers may also list an item as *antique,* though this is dependent on the definition of an ivory antique in the country where it is being traded (e.g., "over 100 years old"). It is common to see an advertisement referring to a time period, e.g., *Victorian*, *1930s*, *pre-ban*, *pre-convention*. Visually, it is almost impossible to be certain if the product is in fact from the time period referred to by the online seller. In rare occasions, a seller will include an appraisal or a copy of an official document like a CITES permit or sales certificate. However, there are many potential loopholes in the provision of such documents when trading online and original documents would need to be examined and verified.

SAMPLE KEYWORDS INCLUDE: *antique, pre-ban, pre-convention and vintage.*

- **AUTHENTICITY:** A seller may include words to indicate that the product is not made of elephant ivory but rather a known substitute or look-a-like to avoid platform regulations, assuming that a buyer would be able to identify the authentic product from other clues in the listing.

SAMPLE KEYWORDS INCLUDE: *faux, ivory-colored* and *ivory replica.*

- **RARITY:** Sellers may try to demonstrate the rarity of a product to indicate that it is authentic elephant ivory rather than the listed ivory substitute. In this case, the seller may not mention the material, but simply that the product is rare. **SAMPLE KEYWORDS INCLUDE** (followed by a substitute material e.g. bone)**: *no longer available, rare* and *one-of-a-kind*.**

- **SUBSTITUTES:** A seller may claim that the ivory for sale is another, legal material. This can be an alternate, naturally occurring ivory such as walrus or a known substitute material like plastic. **SAMPLE KEYWORDS INCLUDE: *Bone, resin* and *vegetable ivory (e.g. tagua nut)*.**

- **CROSS-HATCHING:** Some sellers may allude to the presence of Schreger lines without explicitly listing a product as elephant ivory. **SAMPLE KEYWORDS INCLUDE: *cross-hatching, natural graining* and *zoom in on all the angles*.**

- **HOT PIN TEST:** If a seller notes that an item would pass the *hot pin test*, this indicates that the product is a naturally occurring material such as ivory. When a needle or pin is heated and pressed against man-made materials like resin, it will melt and smell of burning plastic with the pin sinking into the surface of the product.

- **PROCESS AND PRODUCT:** Certain seller key words refer to the process by which the product was produced (e.g., *hand-carved*) or the final product itself which is commonly created using elephant ivory (e.g., *okimono*, *netsuke*).

- **PRIVATE MESSAGING:** Sellers may ask buyers to PM (private message) or DM (direct message) them for more details on product pricing and exchange. This may be because the product is authentic elephant ivory and therefore likely prohibited to trade on the platform.

- **USE OF EMOJIS:** While not thoroughly investigated to date, the use of emojis instead of text to depict live animals and animal products has been identified in some instances on social media and will require further investigation.

It is important to note that while the presence of these thematic search words and phrases may be an indicator that the seller is attempting to advertise authentic elephant ivory without alerting the platform's filters for prohibited items, it does not verify authenticity of those products. There are also many fraudulent claims to sell fake items as real, or the seller themselves may not really know what material the product is made from that they are trying to sell.

IMAGE CLUES

Increasingly, sellers are using less text description to accompany elephant ivory product listings, relying largely on images to communicate authenticity to buyers. This may be done by uploading image angles that clearly display Schreger lines, photographing products with tags or labels in the image that read *ivory* and include country of origin (e.g., ivory jewelry in a ring box or bag), or posting an image of elephant ivory on a scale to indicate that the product is more dense than substitute products like plastic. Examples of these image clues and associated online advertisement language are included in the table below. *Note the images and text have been pulled directly from real listings.*

TABLE 11: ONLINE ELEPHANT IVORY PRODUCT LISTINGS FROM 2017 TRAFFIC STUDY[2]

Picture from Platform Advertisement	Advertisement Language
	"Vintage ~ Carved Chinese Bone Bangle Bracelet ~ Ivory Color ~ w/Pouch ~ EUC Lovely vintage ivory color carved bangle bracelet from Hong Kong. I think this is Chinese bone...but I'm not 100% sure. In excellent condition ~ with pink pouch. Inside diameter is about 2 5/8" ~ the outer diameter is a bit over 3.25". Please view all photos and contact me with any questions. Thanks for looking" ***Why suspicious?*** *The product image contains the word 'ivory' while the seller lists the product as ivory-colored bone.*

[2]Kramer, R., Sawyer, R., Amato, S. and LaFontaine, P. (2017). The US elephant ivory market: a new baseline. TRAFFIC. Washington, DC.

Picture from Platform Advertisement	Advertisement Language
	"Vintage Asian hand carved horse sculpture figure ivory colored on wood base. Please zoom in on all pictures as it is part of the description. Highly detailed. They say a picture is worth a thousand words, so please look over the photos for more details." ***Why suspicious?*** *Schreger lines are visible in the image, which the seller hints at by asking the buyer to zoom in on all pictures as part of the description.*
	"Antique Chinese carved chopsticks with inscriptions: HERE I HAVE A PAIR OF CHINESE CARVED ANTIQUE CHOPSTICKS WITH INSCRIPTIONS OF A TIGER AND POEM.IT'S IN VERY GOOD CONDITION. NO CRACK AND NO SPLIT. Please look at photos." ***Why suspicious?*** *The seller indicates that further inspection of the images will show Schreger lines, which are visible when the images are zoomed in on.*
	"Early.. Hand Made CARVED Charm Bracelet..Pre-War with orig. Box..Faces J194. Vintage item from 1930s. Materials: sterling silver, ivory, paint. Early..Hand made CARVED Charm Bracelet.. Makino Brothers.....Rare" ***Why suspicious?*** *Ivory listed in the materials and the seller indicates that the product is rare.*

Picture from Platform Advertisement	Advertisement Language
	"IVORY MEDALLION PENDANT WITH TURNING CENTER - HONG KONG 1966. YOU ARE BIDDING ON A PRE-BAN (1989) ELEPHANT TUSK BEAUTIFULLY CARVED IVORY MEDALLION PENDANT MEASURING 2 1/4" DIAMETER. INSIDE THE DRAGON CARVING ON THE OUTER HOOP IS A DISC WITH A CHINESE CHARACTER CARVED THROUGH IT. THIS DISC IS ACTUALLY CARVED FROM THE LARGER PENDANT PIECE ALLOWING IT TO TURN OR SPIN AROUND, BUT NOT FALL OUT. (YOU CAN SEE HOW THIS TURNS BUT LOOKING AT THE SEQUENCE OF PHOTOS.) THE CROSS GRAINING ON THE PIECE IS VERY FAINT BUT SUBTLE. THE MEDALLION OS SECURED BY A TEAR DROP GOLD HOOP. THIS IS A MOST UNIQUE AND SPECIAL PIECE REFLECTING A HISTORY OF IVORY CARVING OVER SEVERAL DECADES." ***Why suspicious?*** *The seller lists the product as pre-ban elephant ivory and refers to Schreger lines as 'cross-graining'.*
	"This wonderful 1930's/40's ox bone necklace is a work of art. The bearded old man carries a staff which is connected to the figure at the back of the head. His kimono has a green pattern both front and back and the piece appears to have a signature on the foot. I am unsure of age but the beads (0.34") which are bone as well, are strung and knotted between each bead. This leads me to believe age. The attachment of the netsuke appears to be gold (plate?) with no tarnish. The necklace is 18". A beautiful piece in great condition." ***Why suspicious?*** *Schreger lines are present in the image. Additionally, netsuke are often carved from elephant ivory which warrants further review.*

Picture from Platform Advertisement	Advertisement Language
	"Ivory, faux antique salt and pepper shakers. Vintage item from the 1940s. Minimal wear...see pics..." ***Why suspicious?*** *Ivory is listed in the description along with a reference to the images which reveal Schreger lines.*

SHIPPING RESTRICTIONS

While not always an indicator of authenticity, if a seller restricts product shipping across international and/or relevant subnational borders (e.g., states or provinces within a country) it may be because the product is made from elephant ivory and does not meet the requirements of legal commercial shipment internationally under CITES/ across borders under regional and national laws.

PRICE

Product price used to be a more reliable indicator to distinguish authentic elephant ivory from other materials such as plastics online. However, with increased regulation of elephant ivory trade globally and a larger market of

sellers who may not know the value of the product being sold, ivory prices online are less stable than previously monitored. However, price may be a helpful indicator when comparing similar items. For example, a quality netsuke selling for 300 USD is more likely to be authentic compared to a similar listing for 20 USD.

CONCLUSION

Identifying authentic elephant ivory products online is a challenge given the inability to inspect the item up close, limited access to images, poor photo quality, confusing or limited text descriptions, and the ability to anonymize seller data. It is important to use the content from the morphological section of this guide to make a judgement from the photos accompanying these listings, and weigh information collected from secondary indicators to determine authenticity when images alone do not provide enough information. For additional information and assistance, contact your country's CITES authority which can be found on the CITES website (https://www.cites.org/cms/index.php/component/cp/).


© MARTIN HARVEY- WWF