Determination of Age and Geographical Origin of African Elephant Ivory

Project duration: 01 July 2010 – 31 Dec. 2016

Research project supported by the German Federal Agency for Nature Conservation and funded by the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety

Final report for the project part 'Geographical Origin'

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Summary

From 2010 to 2016, the Federal Agency for Nature Conservation (BfN) funded a research and development project with a grant from the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB) on the determination of origin of elephant ivory. WWF Germany was the lead executing partner and worked in the first half of the project duration in cooperation with the University of Mainz. The reference data set which has been compiled in this project consists of 714 ivory samples that were collected between 2009 and 2014 from 29 African and 6 Asian elephant range states from European museums and collections, zoological gardens, trophy hunters and via protected areas and CITES management authorities. Reference samples were analysed between January 2011 and September 2015 at the accredited Agroisolab Facility for Stable Isotope Research in Jülich, Germany. Continuous flow isotope ratio mass spectrometers measured five different stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, and $\delta^{34}S$). The statistics developed for the assignment of ivory uses the weighted *k*-Nearest Neighbor (NN) Classifier as a pattern classification algorithm.

The data show that isotopic profiling of African elephant ivory works confidentially on regional scales. In a cross check of the reference data it was possible to assign 50% of all samples within 381 km, and 75% within 1,154 km of their place of origin. The database was also subject to a blind testing exercise and the overall accuracy (no. of correctly assigned / no. of total samples) of the regional testing was calculated as 84.2%. For multiple isotope testing ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$) the test for Appendix I is 0.905 sensitive with a false positive rate of 0.135. Thus, the detection probability of the isotopic system that a randomly selected and positively tested tusk is from an Appendix I population is 98%.

The project also developed recommendations of how to measure powdered ivory. The project executant developed an open access database www.ivoryid.org ready to use, free of charge and accessible for everybody; including all information about the methods and information about certified laboratories throughout the world that are able to apply these methods. Since the onset of the project, the BfN has been contacted several times regarding the analysis of internationally seized samples using the developed forensic methodologies. In addition, the project actively contributed to the publication of the UNOCD Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis. Furthermore, the research was published in well-known and internationally peer-reviewed scientific journals and has gained public attention through several media releases and the participation at international conferences.

Zusammenfassung

Von 2010 bis 2016 förderte das Bundesamt für Naturschutz (BfN) ein Forschungs- und Entwicklungsprojekt zur Herkunftsbestimmung von Elefantenelfenbein mit einem Zuschuss des Bundesministeriums für Umwelt, Naturschutz, Bau und Reaktorsicherheit (BMUB). Der WWF Deutschland agierte als federführende Partner und arbeitete in der ersten Hälfte der Projektdauer mit der Universität Mainz zusammen. Der in diesem Projekt erstellte Referenzdatensatz besteht aus 714 Elfenbeinproben, die zwischen 2009 und 2014 aus 29 afrikanischen und 6 asiatischen Elefantenverbreitungsstaaten aus europäischen Museen und Sammlungen, zoologischen Gärten, über Trophäenjäger und Schutzgebiete sowie verschiedene CITES Managementbehörden zusammen getragen wurden. Die Referenzproben wurden zwischen Januar 2011 und September 2015 im akkreditierten Agroisolab Labor in Jülich untersucht und umfassen fünf verschiedene stabile Isotopenverhältnisse ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$). Die für die Zuordnung entwickelte Statistik verwendet den gewichteten *k*-Nearest Neighbor (NN) Klassifikator als Klassifikationsalgorithmus.

Die Daten zeigen, dass die isotopische Kennung von Elfenbein des afrikanischen Elefanten auf regionaler Ebene zuverlässig funktioniert. Bei einer Kreuzprobe der Referenzdaten war es möglich, 50% aller Proben innerhalb von 381 km und 75% innerhalb von 1.154 km ihres Ursprungsortes zuzuordnen. Die Datenbank wurde ebenfalls einem Blindtest unterzogen und die regionale Zuordnungsgüte lag bei 84,2%. Für multiple Isotopensignaturen ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$) ist der

Test für Proben aus dem CITES Anhang I 0.905 sensitiv mit einer falschpositiven Rate von 0.135. Die Erkennungswahrscheinlichkeit des hier vorgestellten Isotopensystems, dass ein zufällig ausgewählter und positiv getesteter Stoßzahn aus einer CITES Appendix I Population stammt, liegt bei 98%.

Das Projekt entwickelte ebenfalls Empfehlungen, wie zermahlenes Elfenbein idealerweise vermessen wird. Eine Open Access Datenbank www.ivoryid.org wurde erstellt, die kostenlos und für jedermann zugänglich ist und alle Informationen über Methoden sowie Informationen über zertifizierte Labore weltweit, die diese Methoden anwenden können, enthält. Seit Beginn des Projektes wurde das BfN mehrmals kontaktiert, um mit den entwickelten forensischen Methoden bei der Analyse von weltweiten Beschlagnahmen Hilfestellung zu leisten. Darüber hinaus hat das Projekt aktiv zur Ausarbeitung der UNOCD Leitlinien für Methoden und Verfahren zur Elfenbeinprobenahme und Laboranalyse beigetragen. Ebenfalls wurde die Forschung in renommierten und international begutachteten wissenschaftlichen Zeitschriften veröffentlicht. Mehrere Pressemitteilungen sowie die Präsentation des Projekts und der entwickelten Methoden auf internationalen Konferenzen haben zur weiten Anerkennung der entwickelten Verfahren beigetragen.

1. Introduction

The illicit trade of ivory and the associated poaching of African elephants has received international attention since several decades, peaking in the listing of the African elephant on CITES Appendix I in 1989 (CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora). This effectively cut off the legal supply of ivory to markets around the world. Since then, only two so-called one-off sales had been applied, allowing some countries to officially trade parts of their ivory stocks. Whether only these one-off sales led to an increase in poaching or not is still a matter of controversy. However, it is a matter of fact that after these sales, poaching of African elephants and illicit trade in ivory got into the focus of criminals, and therefore became increasingly professionalized. In 2011, an estimated number of 17,000 elephants were illegally killed to meet the increasing demand of ivory in Asian consumer countries (WASSER ET AL. 2008). The increasing illicit trade may be leading to a dramatic decline in some African elephant populations (WITTEMYER ET AL. 2014).

Poaching of African elephants and illicit trade in ivory has accelerated in some African sub regions during the recent years. Trafficking in this illegal wildlife products today can be considered professionalized as never been seen before. Well-organized and heavy-armed criminal bands do not only endanger elephant populations but also constitute a threat to regional stability, territorial integrity and sustainable social and economic developments of the countries concerned. International law enforcement, cross-border cooperation and effective forensic methods to uncover the structures and pathways of ivory smuggling and to differentiate illegal from legal ivory in trade are urgently needed.

Nowadays, *Loxodonta africana* can still be found in 38 range states in Sub-Saharan Africa, but certain populations, mostly in West and Central Africa, hardly exceed a few hundred individuals which are highly threatened by increased poaching. At the time of the last continent wide assessment in 2007, the African elephant population was calculated to be at least 472,000 individuals, with possible numbers exceeding 690,000 elephants (BLANC ET AL. 2007). A long-term preservation of the elephant populations, especially of Western and Central Africa and a prevention of criminal structures in wildlife trade will only be possible with a control mechanism that helps identify the geographical provenance of confiscated ivory. International law enforcement, cross-border cooperation and effective forensic methods to uncover the structures and pathways of ivory smuggling and to differentiate illegal from legal ivory in trade are urgently needed. At the 15th Conference of the Parties in 2010 the need of forensic methods for determining the age and origin of smuggled ivory was stressed explicitly.

Exact methods for the determination of age and geographical origin are essential to meet the unsolved problem of ivory smuggling and can help to avoid the intermixing of legal and illegal ivory, if decisions for a restricted legal trade will be taken in future. Long-term preservation of the constantly declining elephant population of Western and Central Africa will only be possible with a control mechanism that helps to identify the age and geographical provenance of confiscated ivory. Therefore, the African Elephant Action Plan by the African Elephant range states (CoP15 inf. 68) highlights the need for improved law enforcement and management by identifying the origin of seized ivory by using relevant analytic techniques (Activity 1.4.3. of Objective 1). At present a supporting instrument that meets court standards is not available for the CITES member states. Therefore, the development of a very exact method for age determination and the set-up of a database for the identification of the origin of ivory will help to better focus enforcement and conservation measures on an international level.

The German Federal Agency for Nature Conservation (BfN) responded to this need and initiated a research and development project, in co-operation with its executing partners, the WWF Germany and two German Universities (University of Regensburg, University of Mainz). The project was funded by the German Federal Ministry of Environment, Nature Conservation, Building and Nuclear Safety (BMUB) and started in July 2010 and has two parts:

I. The determination of the origin of ivory

Until the end of the project the following goals shall be reached:

Development of a reference database for ivory:

A method will be established that can be applied for the determination of the geographical origin and the validity of the geographical indication of ivory.

Application of the reference database for the enforcement:

The reference database for elephant ivory will be suggested to national authorities and the international community of states as a support to enforcement. The project also developed on online tool www.ivoryid.org for the source area determination of ivory samples of unknown origin.

II. The determination of the age of ivory.

The second part was completed in 2012, whereas the first part of the project has been extended until December 2016. The determination of age was also included in the online tool www.ivoryid.org in 2017.

2. Research and scientific background

2.1 Isotope analysis

Forensic analytical methods can play an important role in conserving wild populations as well as in the investigation of wildlife crime through identification and profiling of tissue samples (VOIGT ET AL. 2012, MONCADA ET AL. 2012). A definitive scientific method to determine sample origin and 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, which is characteristic of their half-life. Stable isotope analysis uses the natural abundance of isotope variation as geographic tracers, whereas radioisotopes can be used to determine the age of raw or worked elephant ivory.

Isotopes are everywhere in the environment and they are incorporated into the tissues of plants through soil and water and into animal tissues through eating, drinking and breathing (see Fig. 1), while undergoing certain changes in isotope ratios (HAYES 1982). Throughout its life time an organism

absorbs isotopes (air, water, food). They are deposited into all kind of tissues (e.g. skin, hair, bone) and usually are replaced within the cellular renewal. An exception from this rule is teeth: once formed, dental enamel does not remodel since it is metabolically inert. 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 (Fig. 1). The transverse growth rate is approximately 5 mm per year and the longitudinal growth rate is approximately 5 cm per year (UNO ET AL. 2013). Ivory is composed of around 70% bioapatite (containing 1% carbon) and 30% collagen (containing 45% carbon). The bioapatite and collagen in ivory do not exchange elements or isotopes once formed, and therefore they record the history of diet and provide a time sequence of the isotope ratios (CODRON ET AL. 2012).

Figure 1: Morphology and different layers of an elephant tusk.

Water isotopes undergo a fractionation process, which results in variable isotope ratios occurring in the different physical states of water and under the influence of various atmospheric conditions. H_2O with the heavy isotopes ^{2}H and ^{18}O does not evaporate as easily because the heavy isotopes require more energy for evaporation than the lighter ones. Molecular bonds holding lighter isotopes break more easily than those holding heavy isotopes, thus affecting reaction rates. An example of how natural processes influence the spatial isotopic signature can be illustrated with the ratio of heavy deuterium (^{2}H) and light hydrogen (^{1}H). The vaporisation of water leads to a process that is known as isotopic fractionation: heavy water – water that consists of a higher portion of deuterium – vaporises more slowly than light water. For this reason the vapour is enriched of hydrogen, whereas the remaining surface water is enriched in deuterium. Similar processes occur during the condensation, and therefore water in precipitation is usually lighter than the water of the ocean from which it originates. Figure 2 describes the relation of ^{2}H to ^{1}H in the atmospheric water throughout the African elephant range. The distinctive distribution of the hydrogen ratio is based on the presumption that the percentage of deuterium in the atmospheric water decreases with increasing distance to the equator, but also with increasing altitude and continental climate.

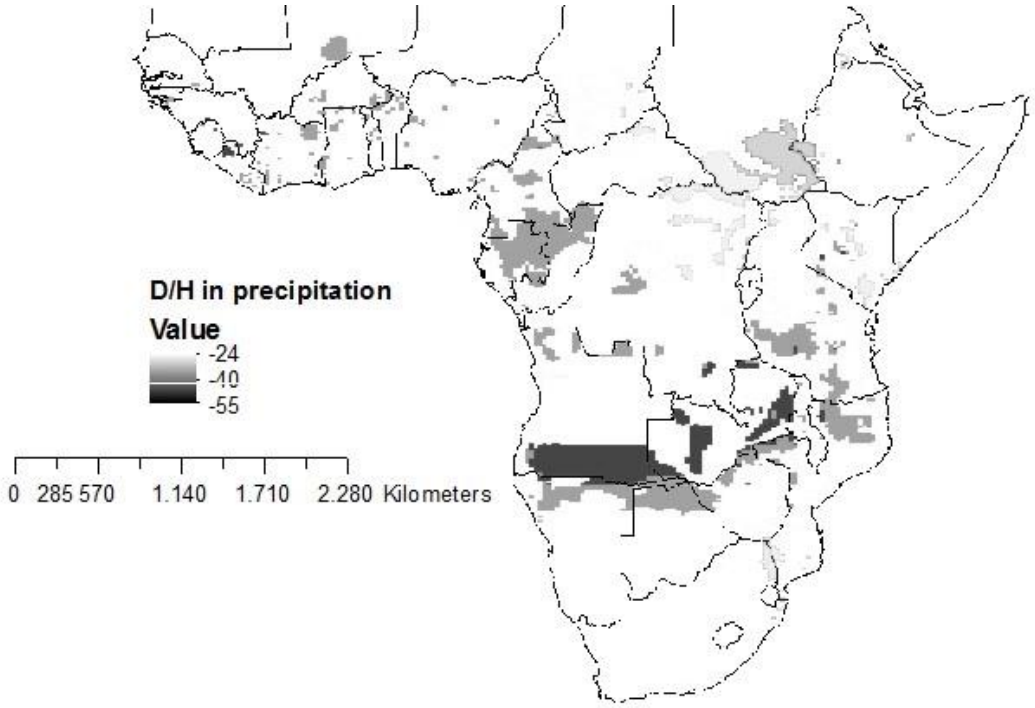

Figure 2: Spatial distribution of the $^{2}H/^{1}H$-values in the atmospheric water for the African elephant habitats. Areas range between values of -55‰ and -24‰.

Geochemical research indicates that $\delta^{34}S$-values are heavily dependent on geographical location, which is a reflection of the local geology and atmospheric sulfur composition of the area (FAURE 1977, KROUSE & HERBERT 1988).

2.2 Determination of provenance

Quantitative measurements of stable isotopes have been used extensively in the fields of biology, ecology, geology as well as in forensic investigation and identification (HOBSON 1999, EHLERINGER & MATHESON 2007). The use of natural abundance of isotope variation as geographic tracers has also been established to determine the provenance of food commodities, such as beef, dairy products and beverages (KELLY ET AL. 2005). Stable isotope analysis of light elements, especially of hydrogen, carbon and oxygen isotopic ratios is applied for food authenticity control for more than 20 years (BAUER-CHRISTOPH ET AL. 1997). Those methods rely on a database of authentic samples, which can be used for the comparison of data from commercial products with them.

Stable isotopes can play an important and efficient role in the investigation of wildlife crime (BOWEN ET AL. 2005). In the past 20 years, carbon, oxygen, nitrogen, strontium and lead isotopes in elephant tusks have been evaluated and used to determine the geographic source of ivory (VAN DER MERWE ET AL. 1988; CERLING ET AL. 1999, 2003, 2004, 2007). The geographical origin of ivory is determined by a combination of various geochemical routine analyses. Frequently applied in provancing is the determination of the isotopic composition of the element strontium (Sr). The element strontium in the food consists of the isotopes ^{87}Sr and ^{86}Sr that are combined in a distinct ratio that is related to the chemical composition of the geological sub-stratum: young volcanic regions such as the East African Rift are characterized by a low $^{87}Sr/^{86}Sr$ ratio, whereas older parts of the earth's crust have a high $^{87}Sr/^{86}Sr$ ratio. But the composition of the stable isotopes carbon (C), nitrogen (N), oxygen (O), hydrogen (H) and sulphur (S) also allows a reliable assessment of the provenance, as elephants ingest

these isotopes with the food they consume. Carbon and nitrogen isotopes can serve as indicator for the climate zone the elephant lived in. A very low $\delta^{13}C$ ratio indicates densely forested habitats, a high ratio is indicative of savannah landscapes. VAN DER MERWE ET AL. (1988), for example, firstly documented that carbon isotopic ratios in elephant ivory, expressed as $\delta^{13}C$, had a linear relationship with tree density, which can be used to distinguish forest elephants from savannah populations. In a similar way, a low $\delta^{15}N$ ratio suggests humid conditions, whereas in drier elephant habitats a rather high ratio can be expected. Hence a relatively correct determination of origin is possible by defining the chemical composition of the tusks.

Later, VAN DER MERWE ET AL. (1990) analysed more than 100 ivory samples from ten African countries and were able to distinguish most elephant populations by combining $\delta^{13}C$, $\delta^{15}N$ and $\delta^{87}Sr$ isotopic ratios. CERLING ET AL. (1999, 2003, 2004, 2007) combined $\delta^{13}C$- and $\delta^{18}O$-ratios of elephant ivory and distinguished different regions based on variable diet preferences and different sources of water. Lastly, ISHIBASHI ET AL. (1999) compared 163 ivory samples from 11 range states of *Loxodonta africana* and concluded that $\delta^{13}C$- and $\delta^{15}N$-ratios were useful for ivory sourcing. Although some of the tested isotopic systems showed a clear distinction between several different populations of the African elephant (VAN DER MERWE ET AL. 1990, CERLING ET AL. 1999, 2003), past sampling effort was reportedly low and the metrics did not fully incorporate the natural variability into the subsequent summary statistics (JACKSON ET AL. 2011).

3. Methodology

3.1 Sampling

The reference data set which has been compiled in this project currently consists of 714 ivory samples that were collected between 2009 and 2014 from 29 African and 6 Asian elephant range states from European museums and collections, zoological gardens, trophy hunters and via protected areas and CITES management authorities (Tab. 1). The reference data are stored in a database that is online accessible at www.ivoryid.org and can be used to determine the provenance of ivory of unknown origin (Appendix I).

Table 1: No. and origin of reference samples stored in the www.ivoryid.org reference database for elephant ivory.

No.	Region	Country	No. of samples	Museum	States	Private	Zoos
1	East	Ethiopia	1	-	-	1	
2	East	Eritrea	0	-	-	-	
3	East	Kenya	18	5	-	13	-
4	East	Rwanda	1	1	-	-	-
5	East	Somalia	3	-	-	3	-
6	East	Sudan	3	3	-	-	-
7	East	South Sudan	2			2	
8	East	Tanzania	26	12	-	12	2
9	East	Uganda	4	4	-	-	-
10	Southern	Angola	5	2	-	3	-
11	Southern	Botswana	101	-	99	1	1
12	Southern	Namibia	37	-	29	8	-
13	Southern	Malawi	35	1	33	1	-
14	Southern	Mozambique	38	8	29	1	-
15	Southern	Zambia	147	-	143	4	-
16	Southern	South Africa	58	3	55	-	-
17	Southern	Swaziland	0	-	-	-	-

18	Southern	Zimbabwe	19	-	-	19	-
19	West	Benin	0	-	-	-	-
20	West	Burkina Faso	52	-	50	2-	-
21	West	Côte d'Ivoire	4	-	-	4	-
22	West	Ghana	2	2	-	-	-
23	West	Guinea	0	-	-	-	-
24	West	Guinea Bissau	0	-	-	-	-
25	West	Mali	0	-	-	-	-
26	West	Niger	0	-	-	-	-
27	West	Nigeria	3	1	-	2	-
28	West	Liberia	3	1	-	1	-
29	West	Senegal	0	-	-	-	-
30	West	Sierra Leone	2	2	-	-	-
31	West	Togo	4	4	-	-	-
32	Central	Equatorial Guinea	0	-	-	-	-
33	Central	Democratic Republic Congo	83	80	-	3	-
34	Central	Gabon	6	1	-	5	-
35	Central	Cameroon	16	9	-	7	-
36	Central	Congo (Brazzaville)	9	5	-	4	-
37	Central	Chad	2	-	-	2	-
38	Central	Central African Republic	11	2	-	9	-
1	Asia	India	10	10			
2	Asia	Indonesia	5	5			
3	Asia	Malaysia	2	2			
4	Asia	Myanmar	1	1			
5	Asia	Nepal	1	1			
6	Asia	Sri Lanka	1	1			

Particularly the sampling of ivory references from the African elephant has been fairly comprehensive since we collected reference material from 29 range states (Tab.1). More than ten samples per country were collected from 13 range states which harbor almost 90% of all living elephants in Africa (Fig. 3). Although we were not able to compile ivory references from nine African elephant range states, we argue that those countries are of lower importance since they only harbor approx. 5,000 elephants which reflect less than 0.5% of the total African elephant population (Fig. 3). However, if additional samples could be added to the reference database, it is recommended to focus sampling effort to the triangle of Burkina Faso, Niger and Benin which harbor the most elephants in West Africa. Furthermore, ivory references from Gabon and Chad are still underrepresented in the database since these two countries comprise large areas of elephant habitat (approx. 370,000 km²).

Data quality of the reference database:

quality of the samples	Estimated number of elephants in these countries*	%
no samples	2,000	0,4
few samples	55,000	9.9
sufficient samples (≙ 10 samples/country)	500,000	89.7

*African Elephant Status Report of 2007 (BLANC ET AL. 2007)

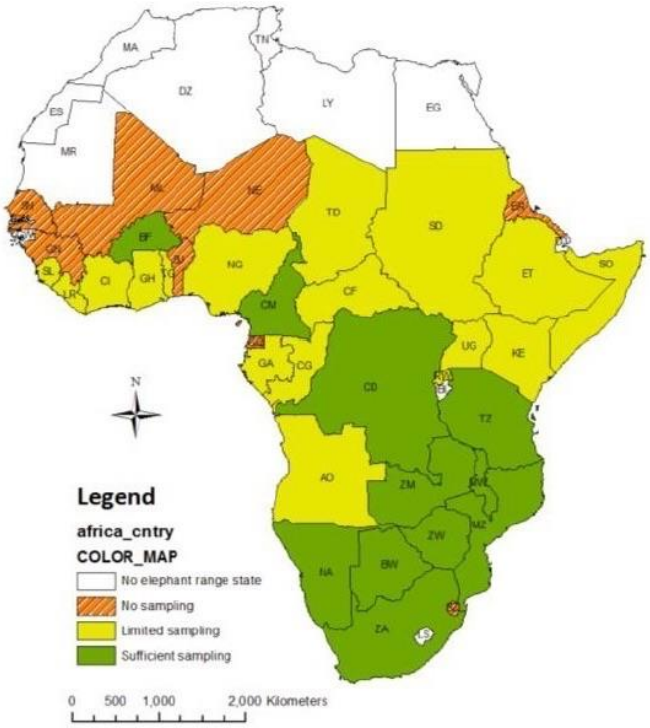

Figure 3: Coverage of reference samples in the www.ivoryid.org database from African elephant range states.

The exact provenance of sampled material was not always known, but geographic locations, such as proximity to a village or a river or coordinates were curated for most of the collected tusks from more than 380 reference sites in Africa with 1-28 samples (Fig. 4). It needs to be stated that limited sampling size bears the risk of cutting natural variation in the respective reference sites, so that samples may be traced to a completely different area.

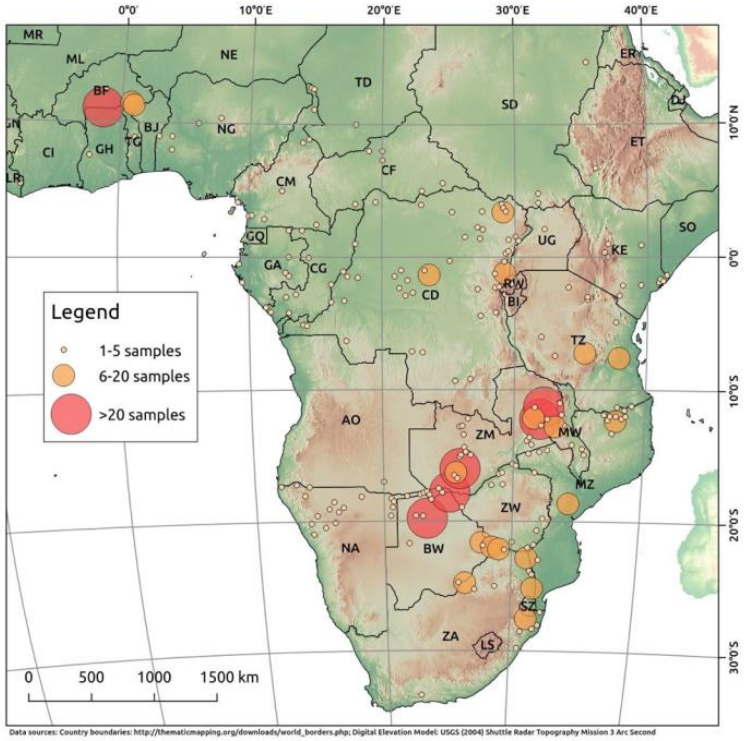

Figure 4: Reference sites and no. of samples per sites.

In most cases, ivory fragments of at least 30 mg and less than 2 mm thickness were taken from the most proximal end of the tusk by using a small handsaw or a pincer (Fig. 5). This section is largely composed of cementum, it is less than six months old and thus minimizes within-individual variation. As this is the most recently-formed and youngest part of the tusk, the isotopic signal reflects the most recent living environment. Thus, attempts to assign an individual elephant to the environment where it died should restrict sampling to the most recently-formed part of the tusks.

Figure 5: Sampling takes place at the most proximal end of the tusk.

3.2 Isotopic analyses

Reference samples were analysed between January 2011 and September 2015 at the accredited Agroisolab Facility for Stable Isotope Research in Jülich, Germany, according to DIN EN ISO/IEC 17025:2005 under the DAR-registration number D-PL-14370-01-00. After pulverization in a steel ball mill (Retsch MM200) with the grinding jar continually cooled with liquid nitrogen at -196 °C, samples were cleaned with dichloromethane for six hours to extract apolar substances, such as tissue fat, and then air-dried at 60 °C for 36 hours. The samples were then stored in a desiccator to avoid humidification. We initially tested the isotopic composition of the the isotopes ^{87}Sr and ^{86}Sr, but our results did not demonstrate a strong discriminatory power to this chemical element. This results and the relatively high costs of the strontium analysis, we therefore skipped this chemical element from further analysis and continued our isotope testing with carbon, nitrogen, oxygen, hydrogen and sulfur.

Subsamples of 1-4.5 mg were subjected to analysis by loading them into 4 x 6 mm tin capsules for carbon, nitrogen and sulfur isotopic measurements. Silver capsules (3.3 x 5 mm) were used for oxygen and hydrogen analysis of another split of the powdered samples. Continuous flow isotope ratio mass spectrometers measured five different stable isotope ratios (carbon and nitrogen: Nu Horizon; oxygen: Isoprime JB332; hydrogen: Isoprime JB102; sulfur: Optima A27). Results are reported relatively to the Vienna PeeDee Bemennite ($\delta^{13}C$), atmospheric N_2 ($\delta^{15}N$), Standard Mean Ocean Water ($\delta^{2}H$, $\delta^{18}O$), and Canyon Diablo Troilite ($\delta^{34}S$) respectively and measured isotopic ratios (R) are expressed in δ units in the conventional permil notation where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$. The samples were also measured against a set of secondary standards (carbon: IAEA-CH-6,

IAEA-CH-7; nitrogen: IAEA-N-1, IAEA-N-2; oxygen: IAEA-601; hydrogen: IAEA-CH-7; sulfur: IAEA-S-1; IAEA-S-2, IAEA-S-3) and laboratory standards (carbon and nitrogen: Leucin; oxygen and hydrogen: 1,4-Dihydroxyanthrachinon; sulfur: Cystein). In order to assess the precision of the analyses, at least two replicate measurements were performed for each sample. Analytical uncertainties were typically between 0.1‰ ($\delta^{13}C$, $\delta^{15}N$), 0.2‰ ($\delta^{34}S$), 0.4‰ ($\delta^{18}O$), and 2.3‰ ($\delta^{2}H$).

3.3 Statistics

All statistical analyses are conducted using the R environment for statistical computing and graphics. Reference (= learning set) and test values are normalized since no significant deviations from a normal distribution were detected in the data set. Then the Nearest Neighbor (NN) rule is applied, which builds on the rationale that samples with small Euclidian distance belong to the same class meaning that these ivory samples are likely derived from the same place of origin (FIX & HODGES 1989; see Fig. 6).

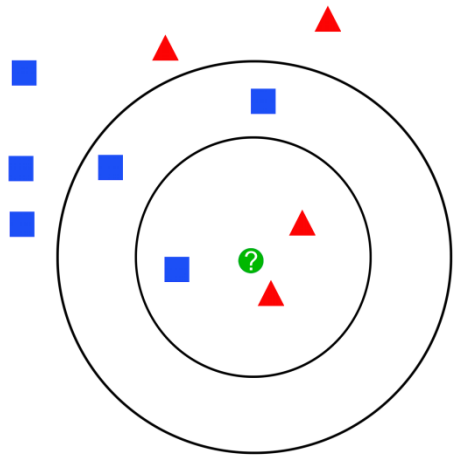

Figure 6: Principle of the Nearest Neighbor (NN) rule. The unknown sample (?) is assigned by majority vote to the class of blue squares with k = 5 and to the class of red triangles with k = 3.

The statistics developed for the assignment of ivory uses the weighted k-Nearest Neighbor (NN) Classifier (HECHENBICHLER & SCHLIEP 2004) as a pattern classification algorithm. We adapted the k-NN classifier and developed a script for R open source software (Appendix II). This extension is based on the idea that such observations within the learning set, which are particularly close to the new observation (= test value), should get a higher weight in the decision than such neighbors that are far away from the test observation. For each test set, the k-nearest training set vectors (according to Minkowski distance) are found, and the classification to site, country, region or CITES Appendix is done via the maximum of summed kernel densities with k values from 1 to 15.

The model applied is a nominal assignment model, and as such it is only capable to model the probability of all candidate geographic locations of origin (WUNDER 2012). Thus, any unknown sample will automatically be assigned to one classifier, i.e. CITES Appendix, region, country or coordinate. In order to assess whether this assignment is statistically robust, we capture the Euclidian distances of the unknown sample to its k nearest neighbors among the assigned classifier, or within a radius of 300 km for the classifier coordinate. We then regard each k nearest neighbor separately as test sample, calculate its Euclidian distance to its k-1 neighbors and deploy the Wilcoxon signed rank test for each pair of unknown sample and k nearest neighbors. If the p-value > 0.05, the test concludes

that the isotope signature of the unknown sample is similar to the respective nearest neighbor reference sample, meaning that the origin of the tested ivory likely (type I error: 5%) derives from the same place of origin as the respective reference sample(s).

We also apply a goodness of fit of the unknown sample while comparing several arrays of paired Euclidian distances and conclude:

- good fit: if $p > 0.05$ for at least two tested nearest neighbor reference samples;
- moderate fit: if $p > 0.05$ for at least one tested nearest neighbor reference sample;
- uncertain: if $p > 0.05$ for none of the *k* tested nearest neighbor reference samples.

4. Accuracy of methods

4.1 Assignment accuracy

We found that that assignment accuracy fluctuates widely between 0 km and 4,821 km, particularly from countries and sites where the number of reference samples is small, such as Ghana, Togo, Nigeria and Chad (Fig. 7).

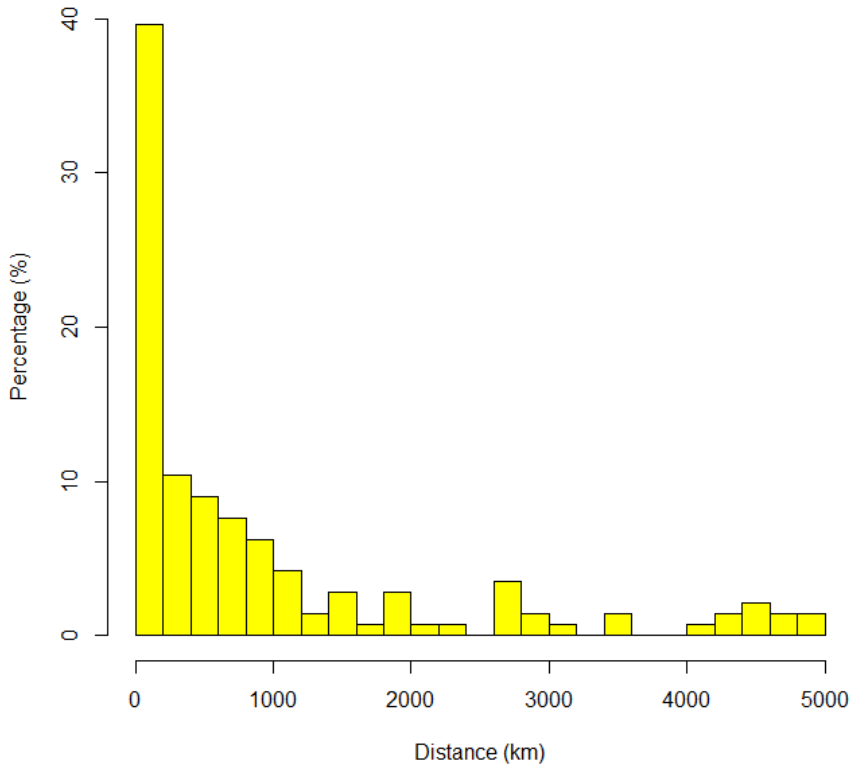

Figure 7: Average deviation (in km) of assigned locations for samples (n = 144) from their place of origin for the Africa dataset.

The data show that isotopic profiling of African elephant ivory works on regional scales, so that it is possible to assign 50% of all samples within 381 km, and 75% within 1,154 km of their place of origin. This assumption may not hold for tusks of unknown origin which may derive from any location in the elephant's range. The lack of on-site neighbors can be compensated through the assignment

framework of candidate locations although median assignment accuracy increased to 876 km if all reference samples for assigning from a sampling location were removed.

4.2 Cross-validation

The five stable isotopic measurements of our data can be described as a vector in a five-dimensional space. Euclidian distance between vectors was calculated by using R open source software and formed the basis for our statistical model. In order to cross-validate the data, we applied the nearest neighbor (NN) rule, which was first developed by FIX & HODGES (1951, 1989) as the pattern classification algorithm. The basic rationale for the NN rule is that samples with low Euclidian distance belong to the same class meaning that those samples are likely derived from the same place of origin. Given the huge spatial range and the ecological heterogeneity of the African and Asian elephant habitat, we assumed that the pattern classes do overlap to some extent, rendering the NN rule sub-optimal. Thus, we also performed the *k*-nearest neighbor (*k*-NN) rule that classifies the vector to the class that appears most frequently among its *k* nearest neighbors and performed the rule with *k*=5.

In order to evaluate the performance of the predictive model, we applied cross-validation across all reference samples, which involved the determination of classification accuracy for multiple partitions of the input samples used in training. We first partitioned the reference samples into regional training categories and built two classifiers, representing elephant populations, which are currently listed on Appendix I and Appendix II in CITES. We developed an R-script that run leave-one-out cross-validation by using a single observation from the original data set as the validation data, and the remaining observations as the training data. This step was repeated until each observation in the sample was used once as the validation data. That way, we evaluated to what extent the combination of different stable isotopes increases the conditional probability with which reference samples were correctly assigned. The combination with the best performance in terms of correct assignments was used to calculate sensitivity and the false positive rate of the ivory reference database as a predictor to distinguish the provenance of ivory. A perfect predictor would be described as 100% sensitivity (i.e. all ivory samples from Appendix I populations are assigned to Appendix I populations) and a false positive rate of zero (i.e. no samples from Appendix II populations are assigned to Appendix I populations). The results are presented in Table 2.

Table 2: Results of isotope combinations with cross-validation across all reference samples which involved the determination of classification (populations of Appendix I vs. Appendix II). **Sensitivity**: no. of correctly assigned Appendix I samples / total Appendix I samples; **False positive rate**: 1 – no. of correctly assigned Appendix II samples / total Appendix II samples; **Accuracy**: [no. of correctly assigned Appendix I samples + no. of correctly assigned Appendix II samples] / no. of reference samples. Costs are estimated prices for stable isotope analysis at a certified commercial laboratory within the European Union.

Isotope combination	Sensitivity (%)	False positive rate	Accuracy (%)	Estimated costs (US$)
$\delta^{13}C$	74.3	0.253	74.5	65
$\delta^{15}N$	75.0	0.354	71.1	65
$\delta^{34}S$	92.2	0.275	84.8	130
$\delta^{18}O$	68.2	0.315	68.4	130
$\delta^{2}H$	80.1	0.556	66.7	130
$\delta^{13}C$, $\delta^{15}N$	80.7	0.213	80.0	110
$\delta^{18}O$, $\delta^{2}H$	77.4	0.545	65.4	225
$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$	82.1	0.202	81.2	360
$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	89.5	0.146	88.0	240
$\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$	91.9	0.236	86.1	360
$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$	90.5	0.135	89.0	450

The interpretation of the results suggests that the combination of isotopic parameters has the potential to provide predictable and complementary markers for estimating the origin of seized

elephant ivory. The database for ivory can be used as a reference to predict the provenance of ivory of unknown origin. For example, with this new approach it is possible to distinguish between Appendix I and Appendix II populations. We cross-validated the reference database and carried out assignment simulations. Up to 92.2% of all ivory reference samples deriving from elephant populations listed in Appendix I of CITES were correctly assigned to their region of origin (Table 2). Approximately 13.5% of all ivory samples from Botswana, Namibia, South Africa and Zimbabwe, whose elephant populations are listed in Appendix II of CITES, were misclassified as Appendix I populations. However, we are confident that the so-called false positive rate can be reduced if more references samples are made available; particularly from elephant range states which are still under-represented in the database.

We used Bayes' theorem to calculate the probability that a randomly selected and positively Appendix I tested tusk really derives from an Appendix I population. The $\delta^{34}S$ testing for CITES Appendix I is 92.2% sensitive and 72.5% specific; thus the false positive rate is 0.275. Since we do not know the true proportion of Appendix I or II tusks in the illegal trade, we assume a 50% partition. Thus, if we only test $\delta^{34}S$, the probability that a positively tested tusk is from an Appendix I population is:

$$P(\delta^{34}S) = 0.922 * 0.5 / (0.922 * 0.5 + 0.275 * 0.5) = 77\%$$

For multiple isotope testing ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$) the test for Appendix I is 0.905 sensitive with a false positive rate of 0.135. Thus, the probability that a randomly selected and positively tested tusk is from an Appendix I population is:

$$P(\delta^{13}C, \delta^{15}N, \delta^{18}O, \delta^{2}H, \delta^{34}S) = 0.905 * 0.5 / (0.905 * 0.5 + 0.135 * 0.5) = 87\%$$

The detection probability increases / decreases according to the base rate of Appendix I or Appendix II tusks in (illegal) trade. If 90% of all traded tusks are from Appendix I populations, the probability that a positively tested tusk is Appendix I increases to 98%. However, in a trade regime that is dominated (say 90%) by tusks from Appendix II populations, the probability to detect a tusk from an Appendix I population decreases to 42%. Although this probability appears to be low, it is important to keep in mind that, in such circumstances, the prognosis to detect fraudulent Appendix II claims is more than four times higher than the random case. This illustrates the importance of base rates, and how the formation of policy can be egregiously misguided if base rates are neglected.

The false positive rate can be reduced through the combination of stable isotopes which has direct cost implications (Tab. 2). The lowest false positive rate in our cross-validation exercise yields the combination of all five stable isotopes ratios. However, though the false positive rate for the combination of carbon, nitrogen and sulfur is one percent higher, measuring these isotopic markers can be done at a reduced rate and might be a cost efficient alternative without compromising the accuracy of the methodology.

4.3 Blind testing

The BfN took 19 samples from the reference sample pool and sent them onto the Agroisolab facility for stable isotope research where the isotope ratios of $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, and $\delta^{34}S$ samples were measured in continuous flow isotope ratio mass spectrometers (IRMS). Five samples where from Democratic Republic of Congo, two samples from Kenya, five samples from Botswana, six samples from Malawi and one sample from Zimbabwe. Neither the Agroisolab nor the statistical research staff was aware of the origin of the test samples.

For the statistical testing purpose, the ivory reference database was partitioned into regional training categories: (i) Central, (ii) East, (iii) Southern, and (iv) West, based on the regional structure of the

African Elephant Database (BLANC ET AL. 2007). Isotopic data deriving from Asian elephant ivory formed a fifth class: (v) Asia. We then applied the *k*-NN rule (*k*=5) and assigned one regional class label to each of the 19 blind tested samples. The results are displayed in the contingency tables below (Tab. 3). The statistical testing classifies all five samples from Democratic Republic of Congo correctly to the Central African region. One of the Kenyan samples was misclassified to Southern Africa; two test samples from the Southern African group were misclassified to the East and Central Africa class, but ten samples were correctly assigned. No test sample was assigned to West Africa or Asia. Thus, the overall accuracy (no. of correctly assigned / no. of total samples) of the regional testing is 84.2%.

Table 3: Contingency table of predicted (horizontal) and real (vertical) origin of test samples (n=19), classified by regions.

		Central	East	Southern	West	Asia
predicted	Central	5	-	1	-	-
	East	-	1	1	-	-
	Southern	-	1	10	-	-
	West	-	-	-	-	-
	Asia	-	-	-	-	-

Since the material for the blind testing and the reference was taken from different fractions of the tusk, we also found misassignments at local level, which possibly can be explained by elephant migration. For example, three samples from Malawi (no. 1, 9, 17; see Fig. 8) were assigned to sites in neighboring Mozambique within a radius of 300 km.

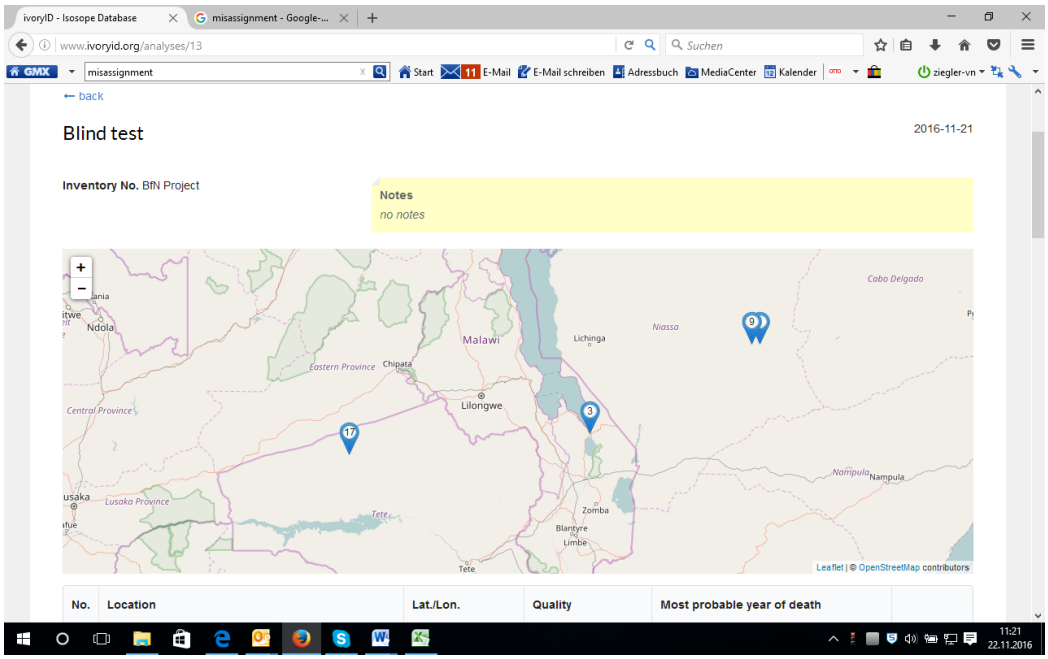

Figure 8: Assignments of samples from Malawi to neighboring sites in Mozambique.

5. Practical application: seizures

Detailed information on all seizures listed in this report is available at:
http://www.ivoryid.org/en/analyses/public

5.1 Leipzig

In spring 2011, the customs department at the DHL freight centre in Leipzig, Germany seized a shipment of total 35 kg ivory (674 pieces), declared as plastic goods, which was posted in Nigeria and designated for import into Hong Kong. Out of this consignment, 40 ivory bracelets were chosen for isotope analysis to find out from which geographical area the carved ivory might derive from. Each ivory bracelet was defined as one test sample and treated individually. The assignment simulations provided overwhelming evidence that the majority of the seized ivory derives from elephant populations in Central Africa. 31 out of 40 test samples were assigned to Central Africa with high probability. The frequency of locations in Cameroon and D.R. Congo is evident, and we conclude with high probability that the bulk of ivory that was seized in Leipzig does originally derive from this part of Africa.

Figure 9: Assumed area of origin of 40 ivory samples from a seizure at the DHL freight center in Leipzig, Germany in 2011.
Source: http://www.ivoryid.org/en/analyses/14/public

5.2 China

This case refers to a shipment exported illegally from Dar es Salaam, Tanzania. It was seized in China in early 2012, when the customs department in Tianjin discovered 931.7 kg (363 pieces) of ivory. Out of this seizure 10 samples were selected and analyzed to find out from which geographical area the carved ivory might derive from. Each ivory piece was defined as one test sample. The simulations assigned the samples to an area in East Africa which is prone to intensive elephant poaching activities (Tanzania, Zambia Mozambique). According to our simulations the provenance area covers a vast geographical range which stretches more than 1,500 km throughout East and eastern Southern Africa (Figure 10). This is, however, not surprising for a relatively large consignment of illegal ivory.

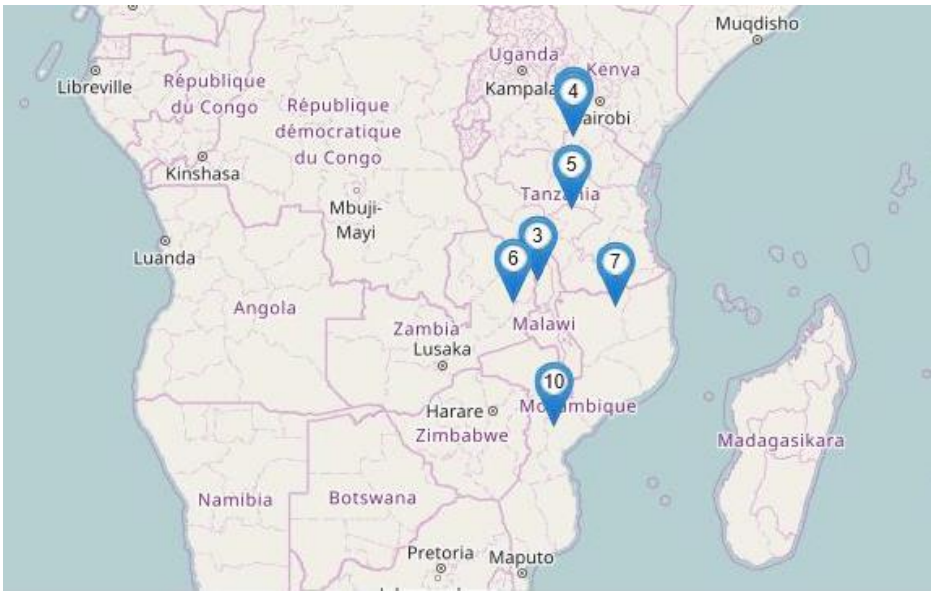

Figure 10: Assumed area of origin of 10 ivory samples from a seizure in China in 2012.
Source: http://www.ivoryid.org/en/analyses/4/public

5.3 Sri Lanka

In summer 2013, the Sri Lankan customs department seized a shipment of whole ivory tusks, which was presumably shipped from Africa. A survey team from Interpol was sent to Sri Lanka to take samples of the seizure. A subset of 60 samples from the consignment were selected for stable isotope analysis, The isotopic profiling of the samples leads to the presumption that the illegal shipment derives from different geo-locations throughout the range of the African elephant. According to the applied test statistic, sites in southeast and southern Africa and in the north of the Democratic Republic of Congo appeared most frequently as assumed greater areas of provenance.

Figure 11: Assumed area of origin of 60 ivory samples from a seizure in Sri Lanka in 2013.
Source: http://www.ivoryid.org/en/analyses/16/public

5.4 Togo

Sampling of the tusks was conducted by a forensic team of Interpol in Lomé, Togo in early 2014. In total 200 samples were sent to BfN for isotope analysis of which 40 samples were randomly selected for further stable isotope analysis. The isotopic profiling of the samples from Togo leads to the presumption that the majority of tusks derive from sites within the Congo Basin. Notably, D.R. Congo appears most frequently as the alleged countries of provenance. According to their isotope profile, a few tusks seem to derive from different regions of Africa. A high degree of similarity with reference patterns have also been found for seized tusks in Botswana, South Sudan and Burkina Faso.

Figure 12: Assumed area of origin of 40 ivory samples from a seizure in Togo in 2014. Source: http://www.ivoryid.org/en/analyses/15/public

5.5 United Arabic Emirates

The isotopic profiling of 20 samples of seized elephant ivory from the port of Jebel Ali in the United Arab Emirates leads to the presumption that the illegal shipment derives from different geo-locations throughout the range of the African elephant. Notably, countries in Southern Africa and to a lesser extent Kenya, Cameroon and Gabon occur as presumed country of origin. Only three tusks were found to derive from Kenya despite the fact that Mombasa was the port of embarkment of the containers in which the ivory was concealed. Remarkably, 13 tusks appear to derive from CITES Appendix II populations in Botswana, Namibia and South Africa. 10 samples were assigned to an area of approx. 12,000 km² in east Botswana, near the international border with South Africa and Zimbabwe. This could be either linked to leakage of stockpiled ivory onto the market or may reflect migration of elephants across international borders from elephant sites in neighboring.

Figure 13: Assumed area of origin of 20 ivory samples from a seizure in UAE. Source: http://www.ivoryid.org/en/analyses/8/public

5.6 Europe

The isotopic profiling of 15 samples of seized elephant ivory from Czech Republic leads to the presumption that the illegal shipment derives from different geo-locations throughout the range of the African elephant. Notably, Democratic Republic of Congo and Zambia occur frequently as presumed country of origin. Analyzed samples from the Czech seizure hint to the fact that several tusks derive from the Congo Basin, or the few remaining rain forest areas of West Africa where browsing of C_3-vegetation forms the prominent part of the elephants' diet. Furthermore, on tusk appear to derive from the CITES Appendix II population in Namibia. In such places where elephant populations migrate across international borders, the assignment simulations are useful in indicating greater areas of provenance, but supplementary information, such as poaching incidents or other forensic details, is needed to pinpoint exactly the origin of seized material.

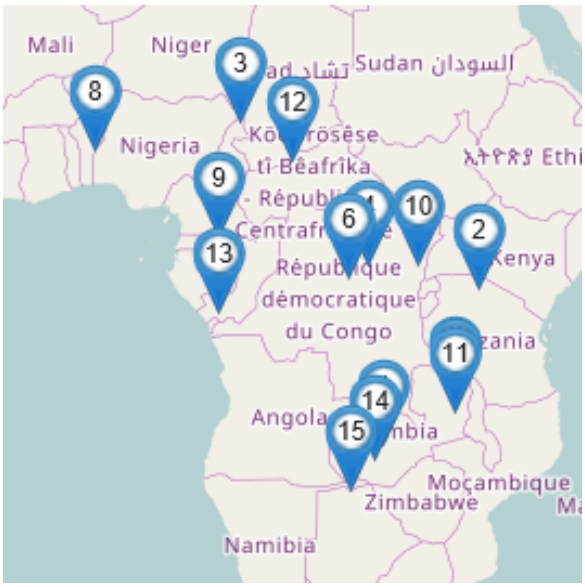

Figure 14: Assumed area of origin of 15 ivory samples from a seizure in Europe.
Source: http://www.ivoryid.org/en/analyses/3/public

6. Further output of the project

6.1 Recommendations for isotopic analysis of ivory

The project also developed recommendations of how to measure powdered ivory. The direct measurement of ivory is particularly challenging for sulfur, oxygen and hydrogen measurement due to the fact that a high amount of anorganic (Calcium) could have a negative impact on the combustion. Furthermore the high amount of phosphate is hard to handle. It is normally increasing the maintenance interval of the IRMS.

The further suggestion should help to measure ivory:

Isotopic ratios of sulfur:

Set-up: EA with one tube filling of wolfram oxide and copper. Water trap (Magnesiumperchlorate) directly at the heated connector.

1) An addition of 1 mg V_2O_5 to round about 4 to 6 mg of ivory is very helpful to enhance the combustion.

2) Samples should be ball-milled to gain an optimal combustion.

3) Furthermore a 2/4 valve is helpful to protect the IRMS of contamination-

High temperature pyrolysis for $^{18}O/^{16}O$ and D/H measurement

Set-up: High temperature furnace (Blisotec): 1550° C with Silicium-Carbide tube (Hekatech) filled with glassy carbon and 500 mg of coal (powder). To protect the system for aggressive gases, Agroisolab is using always elementary calcium in a 10 cm tube connected to the end of the pyrolysis tube. Furthermore to avoid any humidity of the sample, Agroisolab is using a new generation of auto sampler (Zero-Blank-Auto sampler, Blisotec). Every sample is heated up (65° C) for 10 minutes in a separate chamber and flushed with dried Helium to gain an optimally dried sample.

Isotopic ratios of hydrogen:

It is strongly recommended to use a temperature higher than 1450° C to avoid any creation of methane.

Isotopic ratios of oxygen:

Because of the high amount of calcium there is always the problem of incomplete pyrolysis or trapping of oxygen (Calciumoxide). To avoid it Agroisolab is working with a temperature of 1550° C. Unfortunately, a measurement of high numbers of ivory samples is always decreasing the sensitivity of the IRMS. It could be assumed that the phosphor can hardly be controlled. Normally the source of the IRMS has to be maintained after 100 samples measured.

6.2 Online database www.ivoryid.org

One of the goals of the project was to make available the developed tools as well as the collected isotope data to national authorities and the international community to support law enforcement. In order to fulfil this goal, the project executant developed an open access database ready to use, free of charge and accessible for everybody; including all information about the methods and information about certified laboratories throughout the world that are able to apply these methods. How to use the online tool is described in a manual (Appendix I).

6.3 Proficiency testing

In order to assess as to whether the obtained isotopic values of the reference samples measured at the Agroisolab facility can be directly compared with samples that are measured elsewhere, we conducted an interlaboratory comparison. Such procedures are used to monitor laboratories' continuing performance. Proficiency testing compares the measuring results obtained by different laboratories. The reason behind is that ivory is a complex matrix of inorganic and organic components, and that there is no guarantee that different laboratories achieve identical results if the same sample is measured. This is due to different sample preparation and storage, different incineration temperature and different atmospheric conditions in the respective facilities.

Ivory material from one tusk was pulverized and homogenized. This material was sent to n = 10 participating laboratories. Each laboratory measured the sample according to a given set of instructions (see chapter 6.1) and reported the results back to the reference laboratory at Jülich. Laboratories were anonymized since the initial aim of the test was to compare results and give an indication as to whether the reference data stored in www.ivoryid.org can be used by all interested isotope facilities, or whether a correction factor needs to be established.

We measured the so-called "Trueness" which refers to the closeness of agreement between the arithmetic mean of a number of test results and the true or accepted reference value which was established by Agroisolab. None of the participating laboratories was able to measure all five stable isotopes. Although all facilities analysed $\delta^{13}C$ and $\delta^{15}N$, coverage for the remaining isotopes was lower. The laboratories taking part in this proficiency test in general could perform most of the tested $\delta^{13}C$ and $\delta^{15}N$ ratios. This is demonstrated by the 80% fraction of accepted results for these two isotope ratios. Results for $\delta^{18}O$ and $\delta^{34}S$ only achieved a 66% proportion of accepted results whereas this fraction was even further reduced to 33% for $\delta^{2}H$.

One important finding of this proficiency test is the identification of the performance of certain laboratories as well as the uncertainty to reliably measure water isotopes and $\delta^{2}H$ in particular. This is due to different incineration temperature in the furnace. Furthermore, the $\delta^{2}H$ of vapor for

different laboratories around the world varies by more than about 150 ‰. Thus, the identical analysis procedure in Jülich will give different values than in other laboratories with different site coordinates. The analytical uncertainty introduced from exchangeable hydrogen is caused by the highly hygroscopic collagen and if the samples are not air-dried for three hours at 103°C before analysis. Thus, improvements may include the application of necessary method specific corrections, quality control mechanisms and method validation so that all available reference data in the database can be used by all interested parties. Currently, we can only recommend this for $\delta^{13}C$ and $\delta^{15}N$ with a specific correction factor that needs to be determined by further proficiency testing.

Table 4: Result of the interlaboratory comparison (n = 11) with trueness as tested parameter. A1: |Value (reported) - Value (target established by Agroisolab)|; A2: 2.58 x √[sd(target value)^² + sd(reported value)^²]. In theory, a laboratory would receive a pass if A1 < A2 (green color) and a fail if A1 > A2 (red color).

Laboratory	**$\delta^{18}O$**	**$\delta^{2}H$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{34}S$**	**$\delta^{18}O$**		**$\delta^{2}H$**		**$\delta^{13}C$**		**$\delta^{15}N$**		**$\delta^{34}S$**	
no.	mean	mean	mean	mean	mean	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
1			-19.2	5.3	7.1					0.00	0.36	0.10	0.82	1.00	1.39
2	18.1	-58.1	-19.3	5.7		1.70	2.31	2.50	9.30	0.10	0.58	0.30	0.58	-	-
Agroisolab	16.4	-55.6	-19.2	5.4	8.1					**Reference laboratory**					
4			-19.6	5.6						0.40	0.36	0.20	0.36	-	-
5		-40.2	-19	5.6				15.40	9.30	0.20	0.58	0.20	0.82	-	-
6		-63	-19.2	5.7				7.40	13.89	0.00	0.58	0.30	0.58	-	-
7	3.7	-74	-18.8	5.7	6.6	12.70	3.72	18.40	12.00	0.40	0.26	0.30	0.26	1.50	0.58
8		-91	-19	5.3	8			35.40	9.30	0.20	0.36	0.10	0.58	0.10	0.93
9	14.1	-45.1	-19.2	6		2.30	2.92	10.50	6.45	0.00	0.36	0.60	0.26	-	-
10			-19.2	5.5						0.00	0.26	0.10	0.26	-	-
11			-19.3	5.6						0.10	0.36	0.20	0.58	-	-

6.4 Publications and conference talks

Ziegler, S., Merker, S., Streit, B., Boner, M., Jacob, D.E., 2016. Towards understanding isotope variability in elephant ivory to establish isotopic profiling and source-area determination. Biological Conservation 197: 154-163, http://dx.doi.org/10.1016/j.biocon.2016.03.008.

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7. Critical review and outlook

Sampling of the elephant ivory material in this project was neither random nor systematic, but determined by the samples that were made available for this study. Thus, there is a risk that this opportunistic sampling may have introduced a bias. However, while earlier studies were often restricted by low numbers of samples and hardly exceeded ten elephant range states (see chapter 1), this study goes far beyond the geographical scope of previous studies and provides isotopic signatures from 714 ivory samples from 29 African and six Asian elephant range states. Nonetheless, given the size of known and possible elephant habitat, number of respective elephant sub-populations (BLANC ET AL. 2007) as well as the number of climate classes according to the Köppen-Geiger climate classification system (KOTTEK ET AL. 2006), we acknowledge that the sample population for Gabon and Chad is still comparatively low; thus sampling bias could be stronger for these countries, which harbour large elephant populations.

We measured pulverised ivory powder directly and did not follow the standard protocol where mineral parts and collagen in ivory are studied separately. We think that this approach is easier for laboratories, particularly in elephant range states, to replicate due to less complicate sample preparation and cost constraints. Furthermore, our initial aim was to assess the accuracy of isotopic fingerprinting and to evaluate its forensic potential and limitations. Whether separate analysis of different stable isotope reservoirs in bioapatite or collagen will improve the assignments is subject to additional research. However, we also propose a systematic correction with which our carbon and nitrogen isotopic data can be directly compared with literature data. Values of δ^2H can be compared with other laboratories if a site-related effect of different δ^2H of vapor at different latitudes is taken

into account. $\delta^{18}O_{bulk}$ would not be comparable to any published $\delta^{18}O$ on either bioapatite or collagen, whereas $\delta^{34}S$ in collagen might be compared with our data, but to our knowledge no such studies have been carried out on elephant ivory. All corrections factors have been published in ZIEGLER ET AL. 2016.

This research and development project demonstrates that stable isotope analysis can be used for geographic assignments within a probability context and exclude unlikely areas of origin. African elephant ivory from south of 15°S is significantly enriched in ^{34}S, possibly caused by geological factors. This effect has potential to distinguish CITES Appendix I from Appendix II elephant populations if current trade prohibition was eased in the future. Therefore, our isotope-based results are promising for the aim of classifying elephant ivory according to its regional provenance under current CITES listing of elephant populations. In cases where knowledge of isotopic differences between elephant ivory is important for conservation activities and to better control of contra banded ivory trade, cost-effective isotopic markers and the statistical methods examined here may be used.

Since the onset of the project, the BfN has been contacted several times regarding the analysis of seized samples using the developed forensic methodologies. In addition, the project actively contributed to the publication of "Guidelines on Methods and Procedures for Ivory Sampling and Laboratory Analysis" prepared by the United Nations Office on Drugs and Crime; thus the method has become state-of-the-art in the forensic analysis of ivory (UNOCD 2014). Furthermore, the research was published in well-known and internationally peer-reviewed scientific journals and has gained public attention through several media releases and the participation at international conferences (HEIM & BÖCHER 2016). The reference data base for elephant ivory can be accessed via an online platform free of charge, and there is a tool available that allows for comparison of isotope data from seizures all over the world. The administration of the database, IvoryID (www.ivoryid.org), is intended to be passed on to a UN organization.

This research and development project confirmed that isotopic profiling can be very useful in answering specific compliance questions whether a sample comes from a specific region. Thus, the methodology and tools developed may help supporting a management regime that informs authorities and enforcement staff to focus and deploy law-enforcement efforts to alleged poaching hotspots. In addition to stable isotope work, molecular approaches to track the provenance of elephant ivory have been developed throughout the last ten years and include analyses of microsatellite DNA markers (WASSER ET AL. 2004, 2008) and mitochondrial DNA (ISHIDA ET AL. 2013). Based on elephant relatedness rather than habitat signatures, these methods do not necessarily seem to allow a more precise and accurate assignment of individual samples to its region of origin. None of these methodologies, however, is flawless, their singular application results in potentially unacceptable miss-assignment rates. Given the different evolutionary trajectories of nuclear and mitochondrial DNA (ROCA & O'BRIEN 2005) and the still different rationale for stable isotope rates, we strongly suggest subjecting samples to a combination of all three types of provenance testing, or do identify geographical areas where one methodology might perform better than the others. The overall accuracy of such a procedure remains to be determined but it is foreseeable that error probabilities can be decreased to levels acceptable for decision makers and law enforcement.

8. Acknowledgements

The research team is thankful to the CITES Management Authorities and national park services of Botswana, Burkina Faso, Malawi, Mozambique, Namibia, South Africa, Tanzania and Zambia as well as to the Museums of Natural History in Berlin, Frankfurt, Helsinki, Paris, Stockholm, the Bavarian State Collection of Zoology, and the Museum of Zoology at Hamburg University for the provision of ivory samples. We are particularly grateful to the Royal Museum for Central Africa, Tervuren, the London Museum of Natural History and the State Museum of Natural History Stuttgart where we

were given permission to extensively sample their ivory collections. We sincerely thank R. Baldus and the International Council for Game and Wildlife Conservation (CIC) for promoting the project among the hunting community. Our thanks go to numerous trophy hunters who made samples available to our research. Our project is funded through the Federal Agency for Nature Conservation (BfN) with a grant from the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB).

9. References

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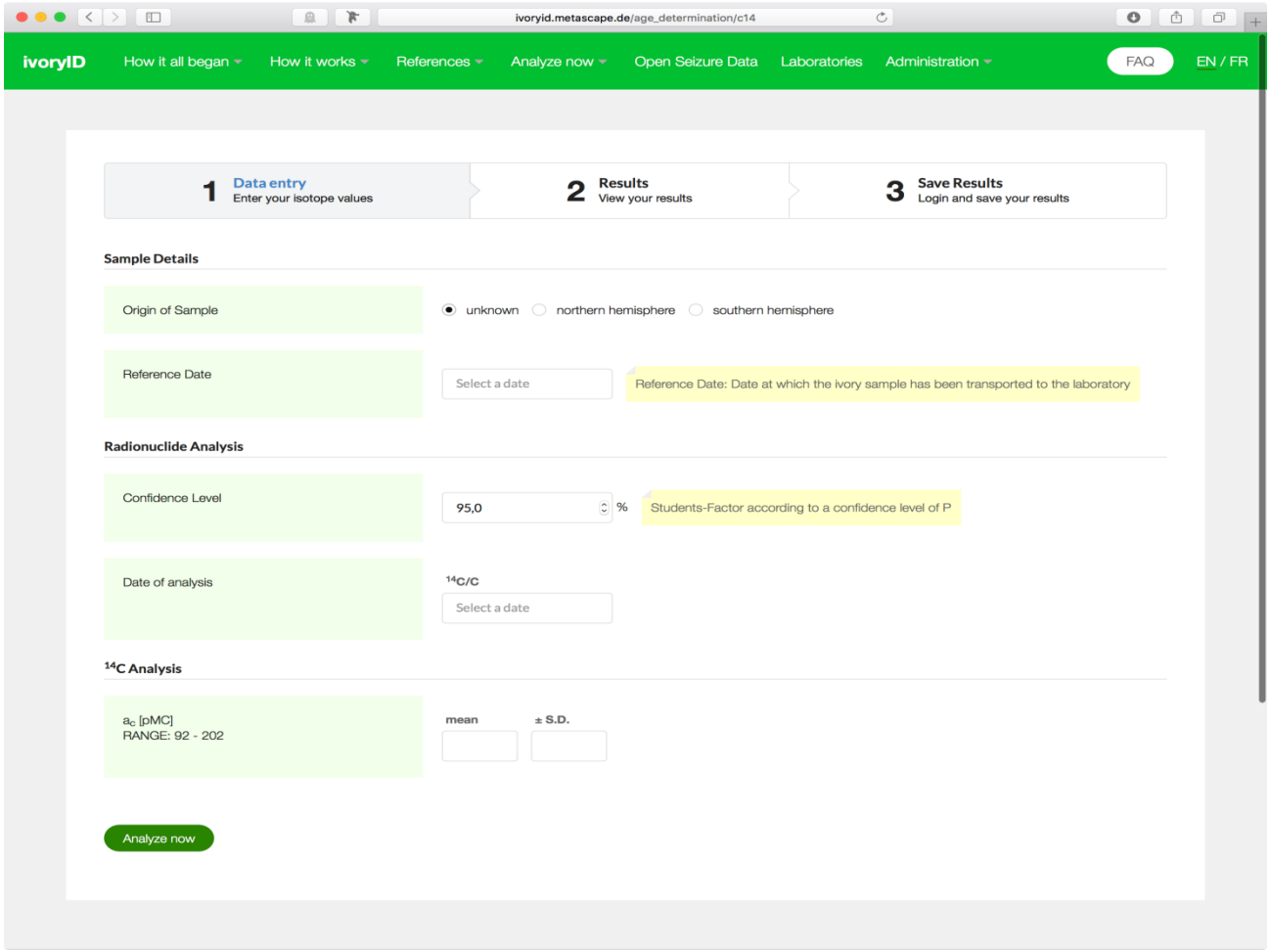
Appendix I

ivoryID Manual

How to use the Database?

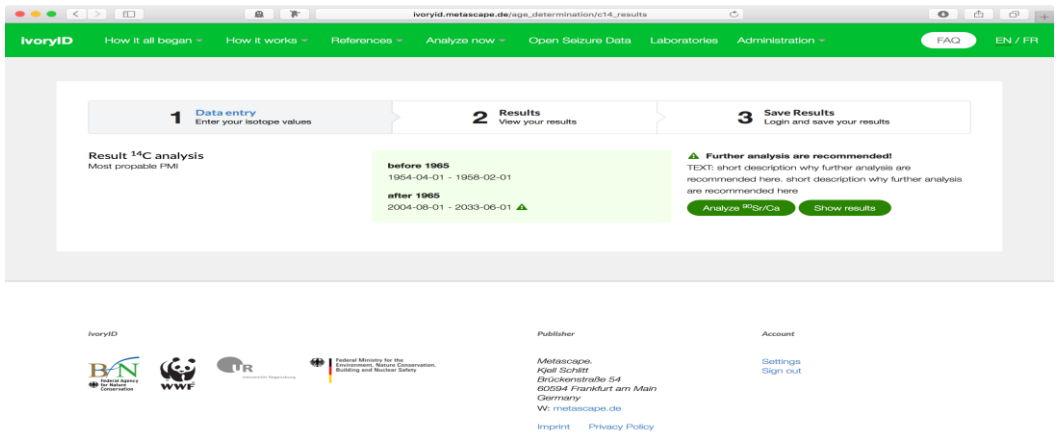
1. Age Determination



1.1 ^{14}C data input

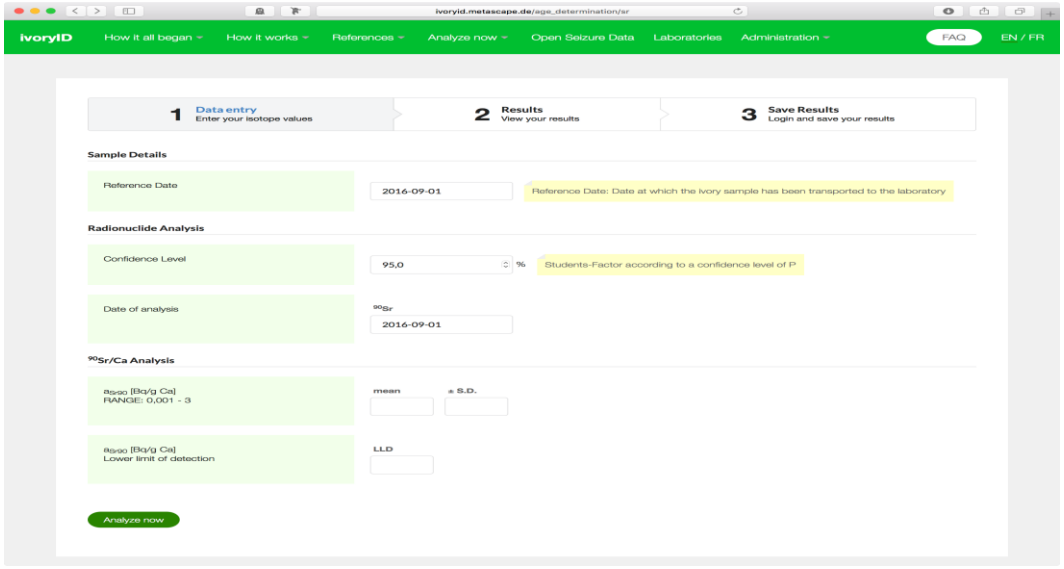
This section starts with a check box from which hemisphere the sample derives from. The default value is **unknown**.

Measured ^{14}C concentrations which are detected by a low-level scintillation counter should be inserted in the respective field. It is also required to insert **dates** when the sample was **transported to the laboratory** and when the **analysis took place**.



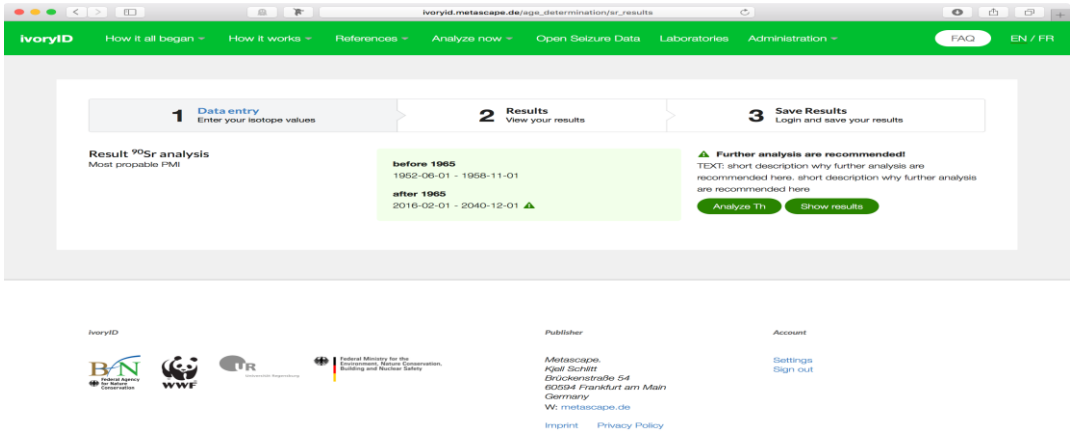
1.2 Results of ^{14}C analysis

The "bomb-curve" had a rapid rise, peaking in ca. 1964, and 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 solutions of age** may be obtained (one between 1955 to 1965; the other 1965 to present). 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 is needed In these cases, please **proceed** with **further analysis**.



1.3 ^{90}Sr data input

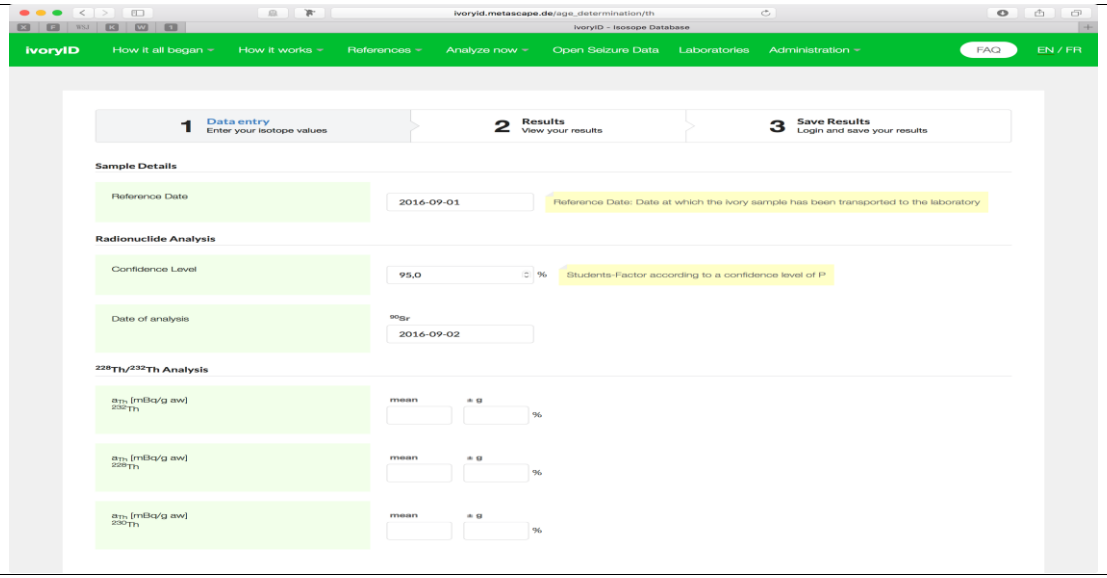
^{90}Sr is one such isotope: this is based on its production with ^{14}C due to nuclear weapons testing, but with a different rate of removal from the atmosphere (within less than a decade). Thus, the sample is compared with the calibrated "^{90}Sr bomb-curve". Please **insert** the **respective ^{90}Sr values**.



1.4 Results of ^{90}Sr analysis

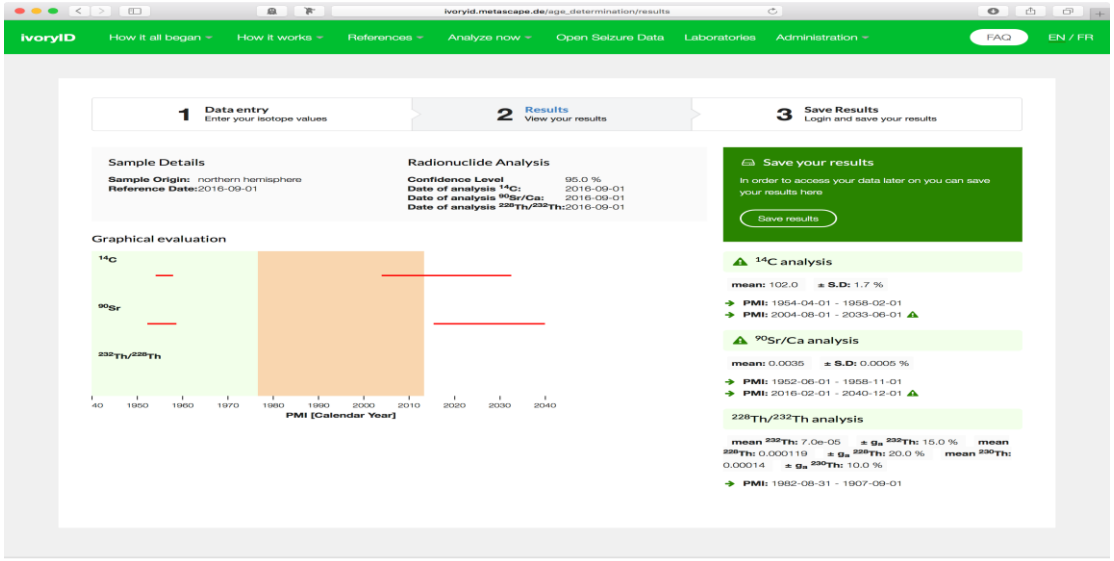
An unambiguous dating is enabled at following findings. If ^{90}Sr/Ca is lower than 0.003 Bq/g Ca the death occurred before about 1958. At values above 0.4 Bq/g Ca the time of death can be assumed to be occurred during about 1960 and 1970. If the interpretation is ambiguous, analysing thorium is a further possibility.

You can either visualize the results of the analysis by clicking on the respective button, or proceed with the Th analysis if the result is ambiguous.



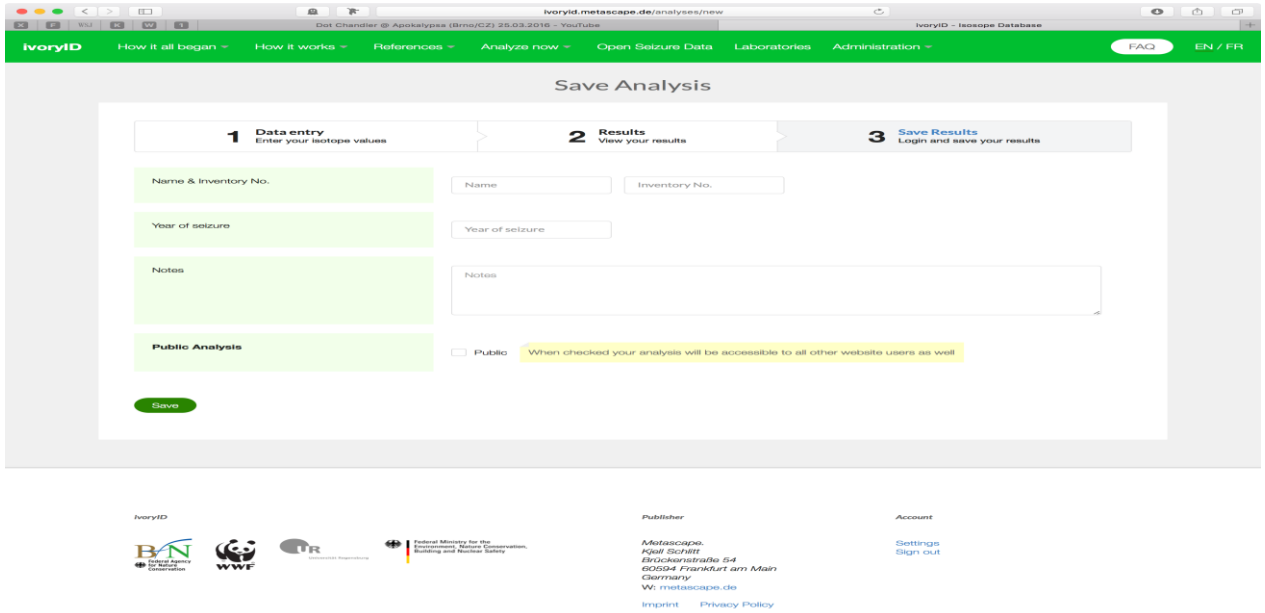
1.5 Th data input

An additional method is based on the relative uptake of radium (bio-available) compared to thorium (not bioavailable); this uses the in-growth of ^{228}Th from ^{228}Ra (half-life 5.7 years), resulting in a ratio of ^{228}Th/^{232}Th above that of secular equilibrium over time. This method will determine the absolute time since the tissue was formed and is independent of either "bomb-curve". With increasing time from death to analysis the ratio of activity of ^{228}Th/ ^{232}Th decreases from about 40 to about 1 if death occurred before 1968.



1.6 Overview of all results of age determination

This output summarizes the results of all tested radionuclides and indicates the most likely data of death.

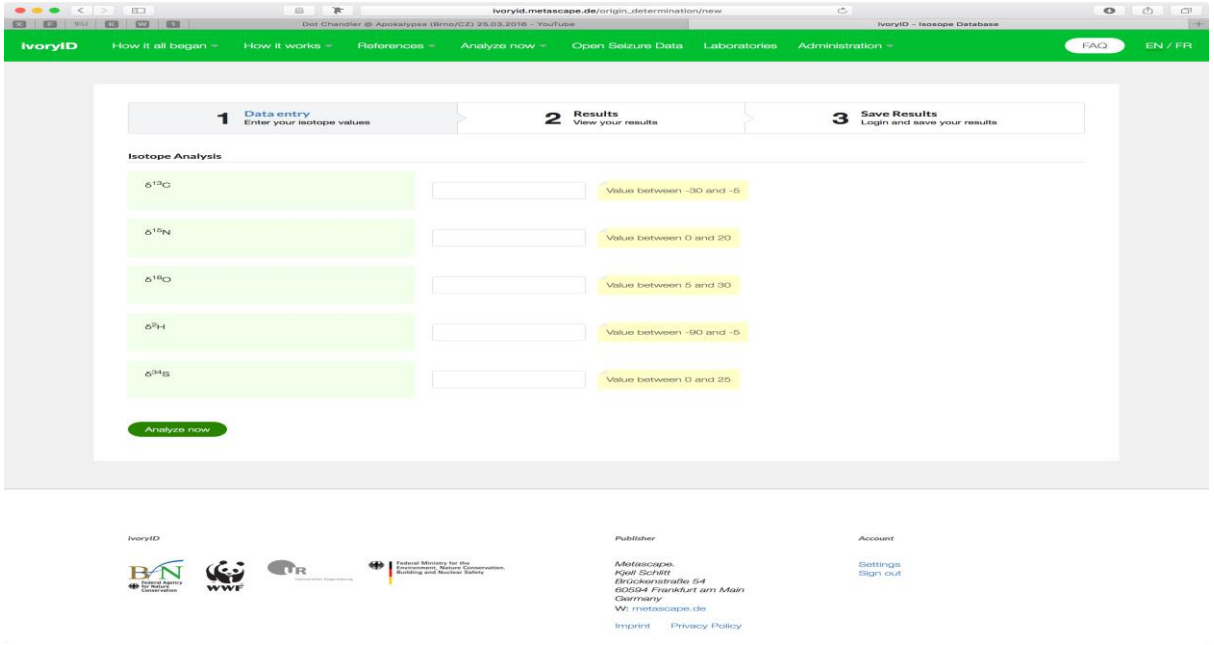


1.7 Save your Data

If you want to save your results, you have to create your own account while using the **log on button.** Thus, you can include additional data later on or you can research your data.

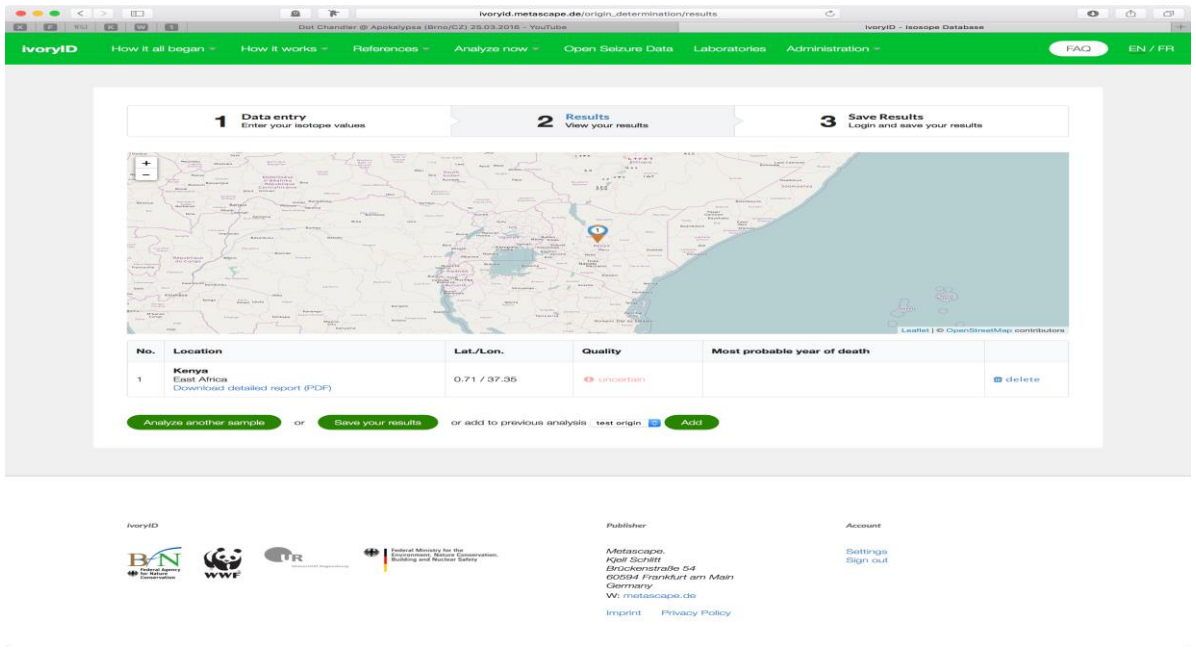
There is also an option as to whether your data are classified as public and can be seen and researched into by other users.

2. Origin Determination



2.1 Enter your isotope values

The database is set to run with five stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$), but the determination of origin also runs with less than five values. However, it should be pointed out that accuracy of the assignment is higher the more isotope values are inserted.

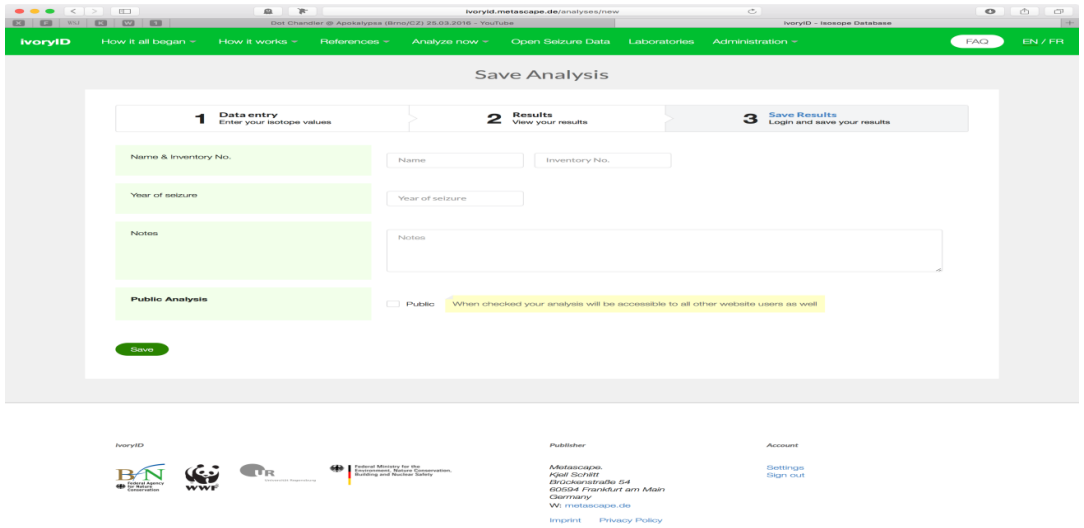


2.2 View your results

- The test statistics plots the reference sample to one of the 386 geographical locations whose isotope signature is most similar to the test sample. The quality of the assignment is also assessed.

- You can also open and download a detailed report in pdf format, which provides more information about the statistics applied and the accuracy of the assignment.

- You can also add another sample from your seizure, which is added on the map.

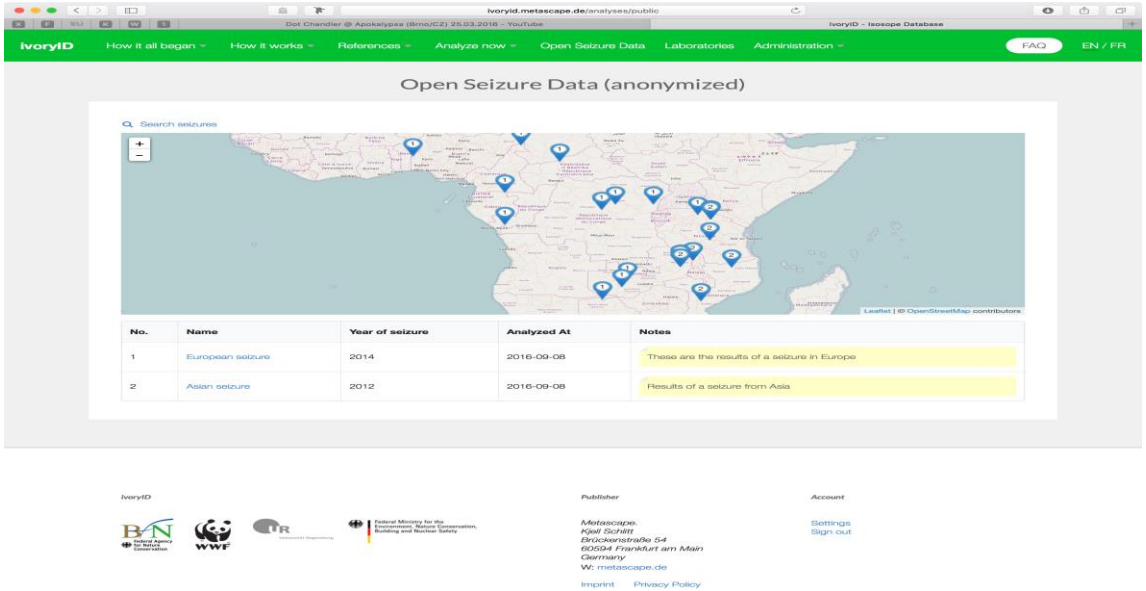


2.3 Save your results

If you want to save your results, you have to create your own account while using the **log on button.** Thus, you can include additional data later on or you can research your data.

There is also an option as to whether your data are classified as public and can be seen and researched into by other users.

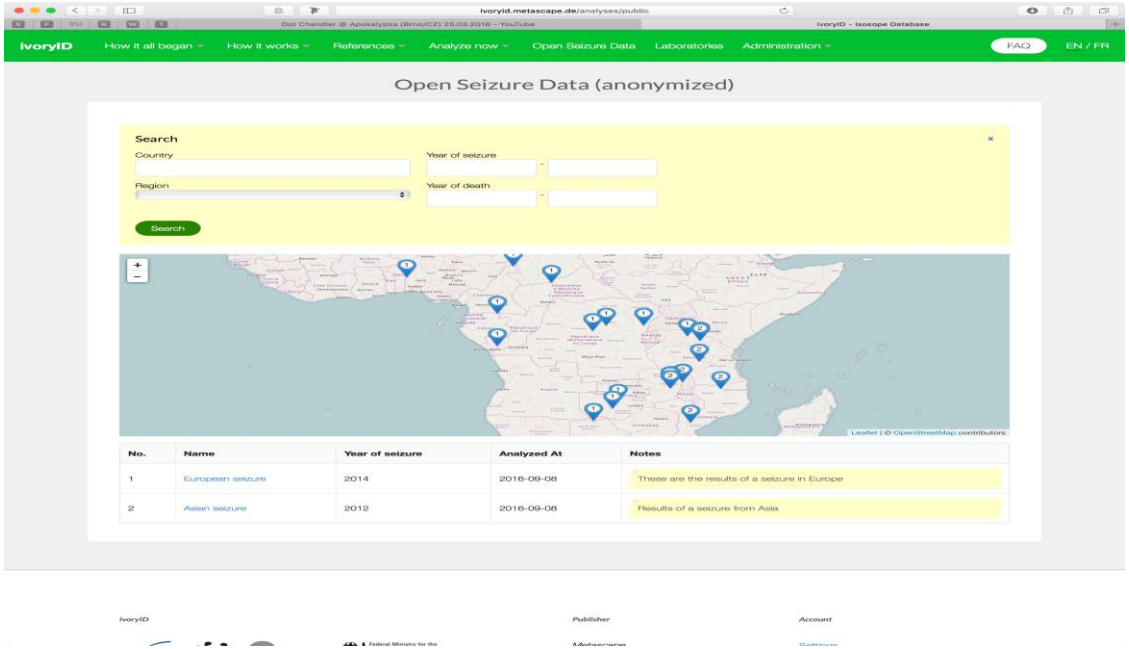
3. Open Seizure Data



3.1 Open seizure data

If you have classified data as public, they can be seen and researched into by other users.

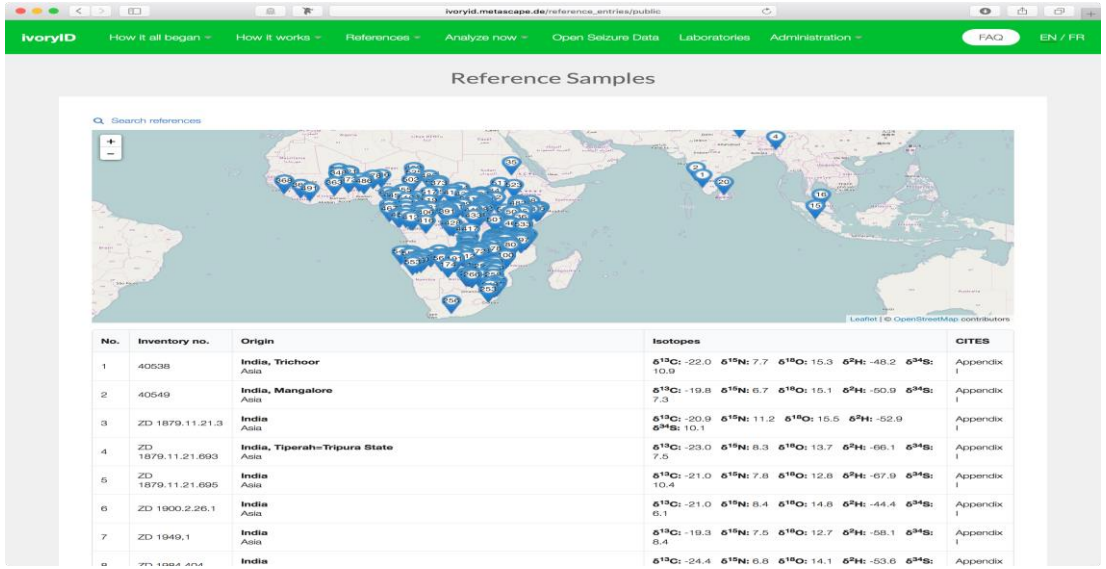
Results of the determination of origin which are categorized as a seizure are displayed on a map.



3.2 Search seizures

By clicking on the **Search seizures** button, open seizure data can be searched for variables, such as **Country**, **Region**, **Year of seizure**, **Year of death**.

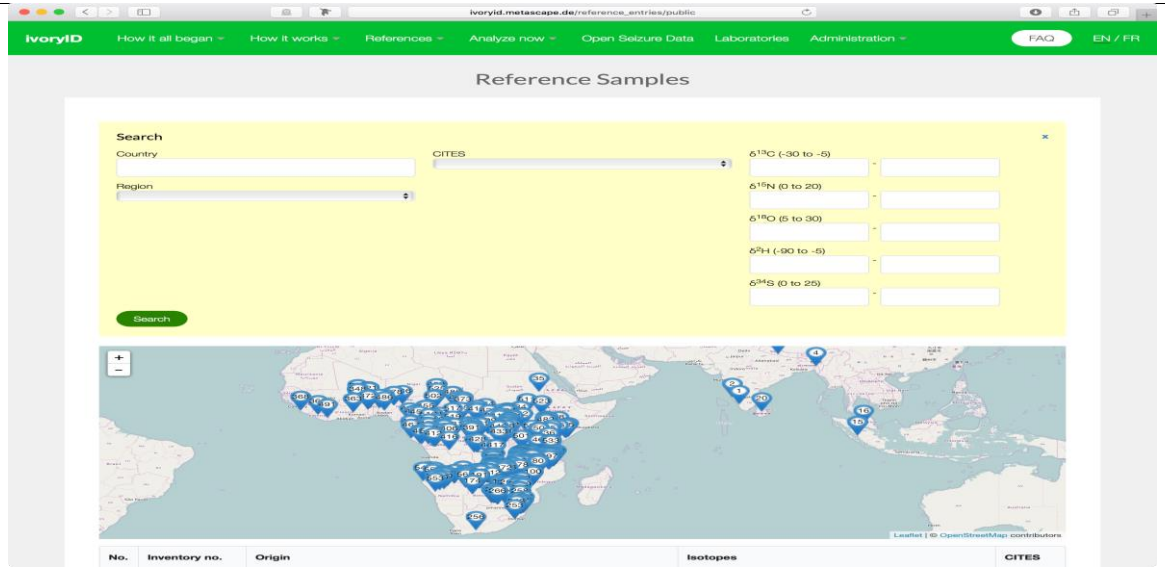
4. Reference Samples (Origin)



No.	Inventory no.	Origin	Isotopes	CITES
1	40538	**India, Trichoor** Asia	$\delta^{13}C$: -22.0 $\delta^{15}N$: 7.7 $\delta^{18}O$: 15.3 $\delta^{2}H$: -48.2 $\delta^{34}S$: 10.9	Appendix I
2	40549	**India, Mangalore** Asia	$\delta^{13}C$: -19.8 $\delta^{15}N$: 6.7 $\delta^{18}O$: 15.1 $\delta^{2}H$: -50.9 $\delta^{34}S$: 7.3	Appendix I
3	ZD 1879.11.21.3	**India** Asia	$\delta^{13}C$: -20.9 $\delta^{15}N$: 11.2 $\delta^{18}O$: 15.5 $\delta^{2}H$: -52.9 $\delta^{34}S$: 10.1	Appendix I
4	ZD 1879.11.21.693	**India, Tiperah=Tripura State** Asia	$\delta^{13}C$: -23.0 $\delta^{15}N$: 8.3 $\delta^{18}O$: 13.7 $\delta^{2}H$: -66.1 $\delta^{34}S$: 7.5	Appendix I
5	ZD 1879.11.21.695	**India** Asia	$\delta^{13}C$: -21.0 $\delta^{15}N$: 7.8 $\delta^{18}O$: 12.8 $\delta^{2}H$: -67.9 $\delta^{34}S$: 10.4	Appendix I
6	ZD 1900.2.26.1	**India** Asia	$\delta^{13}C$: -21.0 $\delta^{15}N$: 8.4 $\delta^{18}O$: 14.8 $\delta^{2}H$: -44.4 $\delta^{34}S$: 6.1	Appendix I
7	ZD 1949.1	**India** Asia	$\delta^{13}C$: -19.3 $\delta^{15}N$: 7.5 $\delta^{18}O$: 12.7 $\delta^{2}H$: -58.1 $\delta^{34}S$: 8.4	Appendix I
8	ZD 1984.404	**India**	$\delta^{13}C$: -24.4 $\delta^{15}N$: 6.8 $\delta^{18}O$: 14.1 $\delta^{2}H$: -53.6 $\delta^{34}S$:	Appendix

4.1 Reference Samples

The database holds isotope values of more than 700 reference samples from more 386 different locations throughout the ranges of distribution of the African and Asian elephant.



4.2 Search Reference Samples

By using the **Search references** button, the reference samples can be searched for isotope values (single and combined), Countries of origin, Region (West Africa, Central Africa, East Africa, Southern Africa, Asia) and CITES Appendices,

Appendix II

R-script for statistical analysis of isotope values

 # Assignment of stable isotope values

 # Thomas Ziegler, Stefan Ziegler, November 2016

 # Input: isotope values

 # Output: Map of best fitted reference sample, incl. accuracy check

```
calculate_confiscation <- function(asset_n = NA, asset_c = NA, asset_o = NA, asset_dh = NA, asset_s = NA,
outcome = "coordinates") { ## Here the default outcome is defined
    suppressMessages(library(kknn))
    suppressMessages(library (FNN))
    suppressMessages(require("RPostgreSQL"))
    suppressMessages(library(rgeos))
    suppressMessages(library(sp))
    suppressMessages(library(dplyr))
    suppressMessages(library(mapview))

    ## read all sites data
    #reference_entries <- read.csv("LatLon.csv",header=TRUE,sep=";")

    ## ---- Loading from database ----
    drv <- dbDriver("PostgreSQL")
    con <- dbConnect(drv, dbname = "ivoryid_production", host = "localhost", user = "thomas", password = "")
    strSQL <- paste("SELECT id, location, isotope_n, isotope_c, isotope_o, val_d_h, isotope_s,
postgis.st_x(postgis.geometry(coordinates)) as lon, postgis.st_y(postgis.geometry(coordinates)) as lat, ",
outcome, "::text", " FROM view_valid_reference_entries_ranked ORDER BY id", sep = "")
    reference_entries = dbGetQuery(con, strSQL)

    ## Formating
    ## str(reference_entries)
    ## If data where loaded from DB formatting is necessury!!
    #reference_entries[, outcome] <- as.factor(reference_entries[, outcome])
    ## ---- END ----

    ## Instead of adding isotope ratios of test data to csv use rbind as follows:
    ## Create a one-row matrix the same length as data
    temprow <- matrix(c(rep.int(NA,length(reference_entries))),nrow=1,ncol=length(reference_entries))
    ## Make it a data.frame and give cols the same names as data
    newrow <- data.frame(temprow)

    ## Assign column names to newrow
    colnames(newrow) <- colnames(reference_entries)

    # Assign given values to newrow
    newrow$isotope_n <- asset_n
    newrow$isotope_c <- asset_c
    newrow$isotope_o <- asset_o
    newrow$val_d_h <- asset_dh
    newrow$isotope_s <- asset_s

    # rbind the empty row to data
    xdat <- rbind(reference_entries, newrow)
```

```
###---------- LEARNING ----------###
## Standardize values via custom standardize function
# scaled_references <- standardize(xdat)
# colnames(scaled_references)[1] <- "id" ## reset the id column name to "id" instead of "V1"
# colnames(scaled_references)[7] <- outcome

## Alternative: Standardize values via dplyr scale function
scaled_references <- xdat %>% mutate_each_(funs(scale),vars=c("isotope_n","isotope_c", "isotope_o",
"val_d_h", "isotope_s"))


## Get original data frame without new row but now standardized
reference_end <- nrow(scaled_references)-1
scaled.references.without.test <- scaled_references[c(1:reference_end),]

## Define the training and testing data
#ivory.train <- scaled.references.complete[, c("isotope_n","isotope_c", "isotope_o", "val_d_h",
"isotope_s", outcome)]
ivory.train <- scaled.references.without.test[, c("isotope_n","isotope_c", "isotope_o", "val_d_h",
"isotope_s", outcome)]
ivory.train <- ivory.train[complete.cases(ivory.train),]
#ivory.train <- as.data.frame(ivory.train) ## Has to be a DF and not a matrix for model building
ivory.train[, outcome] <- as.factor(ivory.train[, outcome])

ivory.test <- scaled_references[reference_end + 1,]
ivory.test <- ivory.test[, c("isotope_n","isotope_c", "isotope_o", "val_d_h", "isotope_s", outcome)]

## Get only column names where there is no NA
valid_clms <- names(ivory.test[colSums(!is.na(ivory.test)) > 0])
clms <- paste(valid_clms, collapse=" + ")

## Build the dynamic formular
fmla <- as.formula(paste(outcome, "~", clms))

## Build the model
knl <- c("rectangular", "triangular", "epanechnikov", "gaussian", "rank", "optimal")
kknn.model <- train.kknn(fmla, data = ivory.train, kmax = 15, kernel = knl, distance = 2)
print(kknn.model)

##---------- TESTING ----------###
## Prediction is performed with the model we just built on the test data, to determine
## how many times this succeeds in predicting
result <- kknn(fmla, ivory.train, ivory.test, distance = 2, kernel = kknn.model$best.parameters$kernel,
k=kknn.model$best.parameters$k)

## Vector of highest prediction
fit <- fitted(result)

## Sort probability of the k nearest neighbors.
knn.prop.sorted <- sort(result$prob, decreasing = TRUE)

## Eucidian distance of the k=15 nearest neighbours
best.params <- knn.prop.sorted[1:kknn.model$best.parameters$k]


## Matrix of distances of the k nearest neighbors
eu.test <- result$D
```

```
    ## Matrix of indices of the k nearest neighbors
    result$C

    ## Get rownames instead of indices because only this gets the correct id if lines were omited because of
missing values
    rowname.vector <- c()
    for (i in result$C) {
        rowname.vector <- append(rowname.vector, rownames(ivory.train)[i])
    }

    ## Return the IDs of the nearest entries
    id.vector <- c()
    for (i in 1:length(rowname.vector)) {
        id.vector[i] <- scaled_references[rowname.vector[i],1]
    }


    # ----- Build df with Index and ID to identify result items -----
    result_table <- ""
    result_table <- as.data.frame(result$C)          ## "C" = Matrix of indices of the k nearest neighbors.
    result_table <- cbind(result_table, result$D)    ## "D" = Matrix of distances of the k nearest neighbors.
    result_table <- cbind(result_table, result$W[1,]) ## "W" = Matrix of weights of the k nearest neighbors.
    names(result_table)[3] <- "result$W"
    result_table <- cbind(result_table, result$CL[1,]) ## "CL" = Matrix of classes of the k nearest neighbors.
    names(result_table)[4] <- "result$CL"
    result_table <- cbind(result_table, id.vector)   ## ids of the reference entries
    # ----- END -----


    # Add geo data to df
    tmp.lon <- c()
    tmp.lat <- c()
    tmp.location <- c()
    tmp.isotope_n <- c()
    tmp.isotope_c <- c()
    tmp.isotope_o <- c()
    tmp.isotope_h <- c()
    tmp.isotope_s <- c()

    # Function creating vectors for later appending to df
    fun <- function(x) {
        tmp.lon <<- append(tmp.lon, filter(reference_entries, id %in% as.integer(x['id.vector']))$lon)
        tmp.lat <<- append(tmp.lat, filter(reference_entries, id %in% as.integer(x['id.vector']))$lat)
        tmp.location <<- append(tmp.location, as.character(filter(reference_entries, id %in%
as.integer(x['id.vector']))$location))
        tmp.isotope_c <<- append(tmp.isotope_c, filter(reference_entries, id %in%
as.integer(x['id.vector']))$isotope_c)
        tmp.isotope_n <<- append(tmp.isotope_n, filter(reference_entries, id %in%
as.integer(x['id.vector']))$isotope_n)
        tmp.isotope_o <<- append(tmp.isotope_o, filter(reference_entries, id %in%
as.integer(x['id.vector']))$isotope_o)
        tmp.isotope_h <<- append(tmp.isotope_h, filter(reference_entries, id %in%
as.integer(x['id.vector']))$val_d_h)
        tmp.isotope_s <<- append(tmp.isotope_s, filter(reference_entries, id %in%
as.integer(x['id.vector']))$isotope_s)
    }
```

```
    apply(result_table,1, fun)

    # Append data within lon, lat, location
    result_table <- cbind(result_table, tmp.lon)
    result_table <- cbind(result_table, tmp.lat)
    result_table <- cbind(result_table, tmp.location)
    result_table <- cbind(result_table, tmp.isotope_c)
    result_table <- cbind(result_table, tmp.isotope_n)
    result_table <- cbind(result_table, tmp.isotope_o)
    result_table <- cbind(result_table, tmp.isotope_h)
    result_table <- cbind(result_table, tmp.isotope_s)

    # ----- Select only samples within the k-nearest neigbours that match the Classificator -----
    if (outcome == "country_code" || outcome == "region" || outcome == "cites") {
        # Filter all entries with the same country as rank 1 or "fit" value
        nearest.tab <- filter(result_table, result$CL %in% fit)
    } else {
        # Filter all entries within 300 km radius
        # Create geom matrix
        ll <- as.matrix(cbind(result_table$tmp.lon, result_table$tmp.lat))
        # Use spDistsN1 from sp package to return ids in 300 km radius
        km <- spDistsN1(ll, ll[1,], longlat=TRUE)

        # Get indexes of nearest items
        idx.nearest_outcome <- which(km <= 300)
        nearest.tab <- result_table[idx.nearest_outcome, ]
    }
    # ----- END -----


    # ----- Wilcoxon test -----
    if (length(nearest.tab$id.vector) >= 2) {
      ## Apply the Wilcoxon Test
      p3.wilcox <- c()
      ## Get the ids with the nearest ids only
      nearest.entry.ids <- nearest.tab$id.vector

      for (i in nearest.entry.ids[1:length(nearest.entry.ids)]) {
        test.eval <- subset(scaled_references, id== i)
        test.eval <- test.eval[, c("isotope_n","isotope_c", "isotope_o", "val_d_h", "isotope_s", outcome)]
        result.eval <- kknn(fmla, ivory.train, test.eval, distance = 2, kernel =
kknn.model$best.parameters$kernel, k=kknn.model$best.parameters$k)
        eu.eval <- result.eval$D[result.eval$D !=0] ## Remove the zero distance of the item to itself
        p3.wilcox <- append(p3.wilcox, wilcox.test(eu.test, eu.eval)$p.value)
      }
      ## Sort P values of Wilcoxon test desc
      p3.wilcox <- sort(p3.wilcox, decreasing = TRUE)

      ## Classifying the goodness of the P values in Wilcoxon test (subjective classifying)
      if(sum(p3.wilcox > 0.05) >= 2) { ## if 2 or all values out of 3 have this condition and return TRUE
        fit.classifyer <- "good fit"
      } else if (sum(p3.wilcox > 0.05) == 1) { ## if only 1 value out of 3 return TRUE
        fit.classifyer <- "moderate fit"
      } else if (sum(p3.wilcox > 0.05) == 0) { ## if none of the 3 values is > 0.05
        fit.classifyer <- "uncertain"
      }
    } else {
      ## Get the ids with the nearest ids only
```

```
    nearest.entry.ids <- nearest.tab$id.vector
    # if only one nearest.entry.ids is available
    p3.wilcox <- "Attention: only 1 reference site. Wilcoxon Test is uncerton."
    fit.classifyer <- "uncertain"
  }
  # ----- END -----

  # ----- Report generation -----
  ## Load all necessury libraries
  suppressMessages(library(leaflet))
  suppressMessages(library(htmlwidgets))
  suppressMessages(library(webshot))
  suppressMessages(library(knitr)) # for calling rmarkdown renderer
  suppressMessages(library(xtable))

  ## Get the nearest reference entry
  #print(nearest.entry.ids[1])
  strSQL <- paste("SELECT *, postgis.st_x(postgis.geometry(coordinates)) as lon,
postgis.st_y(postgis.geometry(coordinates)) as lat FROM reference_entries WHERE id = ", nearest.entry.ids[1],
sep = "")
  nearest.reference <- dbGetQuery(con, strSQL)
  lon <- nearest.reference$lon
  lat <- nearest.reference$lat

  ## Create the map here
  m <- leaflet(nearest.tab) %>%
      addTiles() %>%  # Add default OpenStreetMap map tiles
      addMouseCoordinates() %>%
      #setView(lng= tmp.lon, lat = tmp.lat, zoom = 4) %>%
      addMarkers(~tmp.lon, ~tmp.lat, popup = ~tmp.location, clusterOptions = markerClusterOptions())
  m

  ## Generate temporary file identifyier
  tmp.identifier <- floor(runif(1, min=0, max=1000001))
  tmp.filepath <- ""
  tmp.filename <- paste(tmp.filepath,tmp.identifier, "_map", ".png", sep = "")
  ## Save html to png
  saveWidget(m, "temp.html", selfcontained = FALSE)
  webshot("temp.html", file = tmp.filename,
      cliprect = "viewport")


  ## Rename variables to avoid problems
  newrow <- newrow[,c("isotope_n","isotope_c", "isotope_o", "val_d_h", "isotope_s", outcome)]
  names(newrow) <- c("15N/14N", "13C/12C", " 18O/16O ", "2H/1H", "34S/32S", outcome)
  # Reorder by column name
  newrow <- newrow[c("13C/12C", "15N/14N", " 18O/16O ", "2H/1H", "34S/32S", outcome)]

  # Trim the string length in column tmp.location
  nearest.tab$tmp.location <- strtrim(nearest.tab$tmp.location, 40)

  ## Call renderer and foreward params
  fullpath <- rmarkdown::render('~/ivoryid/calculate_confiscation.Rmd', 'pdf_document',
   params = list(
     newrow = newrow,
     outcome = outcome,
     nearest.tab = nearest.tab,
     best.params = best.params,
```

```
            p3.wilcox = p3.wilcox,
            fit.classifyer = fit.classifyer,
            kknn.model = kknn.model,
            nearest.reference = nearest.reference,
            tmp.filepath = tmp.filepath,
            tmp.identifier = tmp.identifier
          ),
          output_file = paste(tmp.filepath, tmp.identifier, '.pdf', sep='') # dynamic filename for temporary
generated pdf
        )

        ## Disconnect from database
        dbDisconnect(con)

        return(fit.classifyer)
}
```