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The study of morphological, structure of art and DNA of antique carved rhinoceros horns

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ABSTRACT

This article aimed to study the art history to analyze antique carved rhinoceros horns combined with the morphology and DNA testing cytochrome b DNA barcoding using the PCR technique. The hypothesis is that DNA extracted from ancient rhinoceros horn tissue has complete DNA strand integrity and could be amplified to create a DNA profile. The results of the study found that art history and morphology analysis can be examined in antique carved rhinoceros horn considering, the structure of art such as lines, form, color, and space, because the carvings are designed and the shape of the artwork is adjusted according to the rhinoceros horn morphology. Meanwhile, the symbolic interpretations of antique carved rhinoceros horn can explain the lifestyles of the people at the time they were created. Including being able to explain changes and developments in culture, traditions, and beliefs in each era. Next, the DNA analysis of antique rhinoceros horns carved using DNA barcode techniques from 8 samples was able to confirm only 1 sample as white rhinoceros DNA, which is 12.5% of the total sample. Therefore, the research results can be concluded to reject the hypothesis because the tissue from the ancient carved rhinoceros horn could not be tested for DNA.

Keywords: Morphological; Art History; Antique Carved Rhinoceros Horns.

1. Introduction

Rhinoceros horn is considered a valuable and rare item, which has a long-standing belief among the Orientals that it has been used in traditional Chinese and Vietnamese medicine for centuries. The ancient Chinese believed that rhinos eat all kinds of poisonous vegetables, so the horn of the rhinoceros can neutralize the poison. (But, Lai-Ching, & Yan-Kit, 1990: 158; Dang Vu & Nielsen, 2021: 390) Today, the belief in its medicinal use still exists, especially in Vietnam (Vu, Nielsen, & Jacobsen, 2022) but on the other hand, rhinoceros horns, especially carved rhinoceros horns, have been used as investment assets, symbolizing the wealth of the owner, and as religious and cultural artifacts of the upper class, especially in Asian countries. (Hübschle, 2020: 199) The trade in rhinoceros horns in the art and antiques markets has been cited as a threat to living rhinos, with some evidence showing that new horns are being made

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to look old and sold as antiques at auctions this issue needs serious investigation, as does the continued illegal trade in rhinoceros horn in other forms, such as online black markets. (Gao, Stoner, Lee, & Clark, 2016: 345)

The examination of antique artwork is very important to solve this problem at the same time, it is also very important in terms to prevent the destruction of valuable works due to misunderstandings if they are not carefully examined, and prevent the use of imitation antiques to be carved instead of rhinoceros horns, which would be a shameful destruction of cultural heritage and the historical value of artwork. Therefore, it is also very important in terms of the value of antique artwork to prevent the destruction of valuable works due to misunderstandings if they are not carefully examined and to prevent the use of imitation antiques to replace rhinoceros horns, which would unfortunately destroy cultural heritage and the historical value of artwork.

The examination of antique artwork at present is an examination by experts such as archaeologists, art appraisers, art historians to know the type of object, its age or the period it was produced by analyzing the shape, form and structure of art from different eras, a variety of theories are used in the process, such as symbolic analysis, aesthetic analysis, and historical interpretation. Each work of art contains cultural symbols and meanings that reflect the social and cultural context of each era very well. This allows us to know the period in which the work was produced and the age of the artwork.

However, expert assessments may have human errors due to misinterpretation of data or biases using feelings or personal opinions currently, the examination of antique artifacts is difficult; scientific methods are used, such as X-ray fluorescence (XRF) or spectroscopy, radioactive-carbon dating, or carbon-14 dating, and DNA testing, etc. Similarly, using Morphological analysis is an analysis of shape, form, color, light, shadow, and various lines that are external elements, emphasizing objectivity studying the external appearance and structural properties of living things, such as color, structure, size, shape, and form, without giving importance to the personal feelings of the analyst, will help promote the analysis of the structure of art, which is different because it emphasizes the analysis of the relationship and elements of art, arrangement, balance, or things inside, emphasizing the subjective aspect that is reflected from that piece of artwork.

This study focuses on the analysis of morphology and structure, using the concept of art history as a framework for analysis, along with DNA examination for greater depth and coverage to identify the carved rhinoceros horn artworks into different eras, the hypothesis is DNA extracted from ancient rhinoceros horn tissue was complete DNA strand integrity and could be amplified to create a DNA profile.

2. Literature review

2.1 Art history

The study of art history was initially a discipline that focused on the origins of works of art, such as who produced what, when and how, and later became interested in the study of symbolism in works of art, communication through symbols, the analysis of social history and the social situation at the time the works of art were produced and the period in which they were consumed (Harris, J. (2006: 23-24). In addition, the analysis of style or form, which is the specific expertise of the artist who produced the work, is one of the methods that art historians often use to examine, considering the linear style of art, proportion, composition, space or space, and the creation of connected patterns of the work. Art historians can use this style or form of art as a criterion to determine the age of the work, as well as to determine the source of the work and investigate the relationship between antique art objects and monuments, allowing them to know the innovation and working process of the artist or group of artists through that piece of work (Hartwig, 2011: 313). Art history is thus the study of the history of created objects, presumably having a certain visual content. The task of art historians is to explain why such objects are the way they are, taking into account wider issues of social context, cause, effect, and value of works of art. (Pooke, & Newall, 2008: 20)

2.2 Morphology

Morphology is concerned mainly with the study of the form and structure of living things (Arnold, 1983: 348). It deals with the materiality of tissues, organs, and organisms, the processes by which these are shaped, the nature of biological materials and their physical and chemical constraints, and the

modification of form in successive generations of organisms (Wake, 1982: 606). Morphology is the study of structure at various levels, from the molecular level to the holistic level (Koehl, 1996: 502). The principle of morphology is the study of the organ systems of living things, the level of organization from simple to complex, symmetry, genetic variation and cell division, division of labor between cells, and similarity. Therefore, it is the basis for interpreting and understanding other aspects of biology such as physiology, genetics, ecology, and taxonomy (Chadwick, 1995: 14-15). Therefore, the analysis of morphology in art will make it possible to understand the form, structure of art in various shapes, the arrangement of shapes, shapes, and be able to analyze and interpret art through the design of art, which is the external appearance.

2.3 Related research

Morphological analysis is a method based on the external characteristics that can be observed, as mentioned above. However, when the object has similar external characteristics, naked eye examination cannot distinguish or classify the type of the object which organism it comes from, biological evidence or DNA testing can help in confirming the type of organism and scientific processes provide a high level of credibility because they are more verifiable than social sciences, such as the dating of ancient artifacts, carbon dating, or skeletal remains. Quartieri (2011) studied the application of synchrotron radiation (SR) to the analysis of archaeological and cultural heritage, was found to have been used since the 1980s and is now used. Synchrotron radiation is a small, bright X-ray beam and a concentrated X-ray beam. It can be used for diffractometric surveys, spectroscopy, and photography of archaeological objects and art. It is effective in inspecting fragile objects to prevent damage. Research related to the use of scientific technology to detect forgery in art by de Manincor (2024) analysis of Lazzaro Bastiani's artwork using infrared light, Infrared Reflectography (IRR) is a non-invasive imaging technique based on the ability of the Near-Infrared (NIR) radiation to penetrate the paint layers, down to the preparatory ground, thanks to the partial transparency of most of the pigments in this spectral region, allowing us to detect features beneath the visible paint surface. The technique of Medieval and Renaissance paintings is particularly suitable for studying the underdrawings through of the IRR technique because of it contrast between the preparatory drawing, sketched in a dark absorbent. A study by Stirpe and Thieme (2024) is a case of using scientific tools to analyze a work of art to determine whether it is an original work of art or a copy. They studied Brueghel's masterpiece, Christ and the Woman Taken in Adultery. Previously, art historian Maurizio Seracini used radiocarbon dating and found that the painting was painted between 1679 and 1939, after Brueghel died in 1638. Stirpe and Thieme's method of analysis used submicron spatial resolution beamlines of submicron resolution X-ray spectroscopy (SRX) at Brookhaven National Laboratory (BNL) to perform XRF microscopy on paint fragments from the artwork. PyXRF software identified the elements based on the emission spectrum, leading to the discovery of titanium in the painting. The presence of titanium, an element mixed in pigments since 1921, indicates that the artwork may be modern. The application of synchrotron radiation to XRF at higher energies than Seracini's experiments allowed for the discovery of more elements within the artwork. The following elements were identified in the paint parts: arsenic, calcium, cobalt, chromium, copper, iron, mercury, potassium, manganese, lead, titanium, and zinc. In particular, high levels of cobalt were found on the exterior of the paint parts, while arsenic, calcium, and lead were found in the glaze, calcium, dark, and white coats, respectively.

3. Data and methodology

This research used 8 samples of ancient carved rhinoceros horns. The morphology of antique carved rhinoceros horns is the study of the shape and form of the carved rhinoceros horn, the composition and details of the carved rhinoceros horn, and then analyzed using the concept of art history.

DNA Testing through DNA Barcode Techniques. The method was started by extracting DNA from rhinoceros horn using Geneaid's Genomic DNA Mini Kit (Tissue) by scraping the outside of the rhinoceros horn piece into a 1.5mL tube. Then, add 200 µl of GT Buffer and 20 µl of Proteinase K and grind the sample with a plastic grinding stick. Then, incubate at 60 °C for 30 minutes, shaking the tube every 5 minutes. Then, add 200 µl of GBT Buffer, vortex for 5 seconds, and incubate the tube at 60 °C for 20 minutes or

until the solution in the tube is clear. Every 5 minutes, shake the tube while waiting for the preheating of Elution Buffer solution at 60 °C, after the time, the sample was eluted from RNA by adding 4 µl of RNase A (10 mg/ml) and mixing thoroughly with a vortex mixer, then left at room temperature for 5 minutes. Then, 200 µl of absolute ethanol was added and the entire solution was placed in a GS Column in a 2 ml Collection Tube, then centrifuged at 14,000-16,000 g for 2 minutes. Then, the 2 ml collection tube was discarded and the GS column was placed in a new 2 ml collection tube. The purging was started by adding 400 µl of W1 buffer to the GS column and centrifuged at 14,000-16,000 g for 30 seconds. When the time is up, discard the supernatant in the 2 ml collection tube, then place the GS column back into the 2 ml collection tube and add 600 µl of Wash Buffer to the GS Column and centrifuge at 14,000-16,000 g for 30 seconds. Then discard the solution in 2 ml collection tube and dry the column by placing the GS column back into the 2 ml collection tube and centrifuging at 14,000-16,000 g for 3 minutes. DNA elution is initiated by placing the GS column into a new 1.5 mL tube. Add 50-100 µl of Pre Heated Elution Buffer to the center of the GS column and leave it at room temperature for 5 minutes. Then centrifuge at 14,000-16,000 g for 30 seconds. The clear solution in the tube is the extracted DNA. The extracted DNA is tested for quality on a 1% agarose gel and quantified with a nanodrop spectrophotometer. The extracted DNA is then stored at -20 °C for use in the next step.

The DNA of all living things begins to degrade immediately after the cells die. Cell death by autolysis occurs once a cell is no longer in contact with the body's circulating oxygen supply. (Latham, & Miller, 2019; Otagiri et al., 2024) Therefore, this research has two limitations: first, rhinoceros horn is a protein called keratin, dead tissue, a protein similar to nails or hair, and is of inferior quality to other types. Second, the ancient rhinoceros horn carvings are more than a hundred years old, which may cause the quality of the tissue to deteriorate.

4. Results and discussion

4.1 Morphology of Rhinoceros Horns

The morphology of the rhinoceros horn is considered a type of horn, but it is different from other animal horns in that it does not have a bone core. The horn is attached to the dermis layer that covers the forehead and nosal bones, often with prominent wrinkles. The rhinoceros horn is a derivative of the epidermis, which is composed of keratinized cell tubes, classified as scleroproteins, i.e., insoluble in water, swollen to a limited extent, creating two molecules that are arranged to form intermediate filaments (IFs). Each tube grows from the creation of cells of the epidermis that cover the skin, resulting in a knob with a hard texture that is resistant to bending and tearing. The horn is therefore a composition of dead tissue, with no living cells (Hieronymus, Witmer, & Ridgely, 2006: 172; Jha, Kshetry, Pokharel, Panday, & Aryal, 2015).

The morphology of rhinoceros horn is not entirely conical, but there is a clear change in slope, with a rounded base and a downward curve at the tip, slender and conical. The color of the rhinoceros horn ranges from black, brown, gray, and golden yellow. The surface is rough, especially at the base, and the underside of the base is porous and concave. (Hieronymus, Witmer, & Ridgely, 2006: 175-176) The surface of the rhinoceros horn has a hair pattern structure, and the inclined part is rough and slightly bumpy, and there is melanin on the surface. In addition, the light can pass through well and there is a slight fishy smell. The hair pattern structure can be observed, with the naked eye or through a microscope, which is a characteristic that is different from other horns, including the infrared spectrum of the rhinoceros horn, which is also very different from the horns of other animals (Li, Zu, & Liu, 2011: 637, 639). The unique shape of the rhinoceros horn is due to the gradient density of non-keratinous components, namely calcium, and melanin, which are concentrated near the center or core. Due to these differences in concentration, the outer layer of the rhinoceros horn erodes faster than the core, which is made up of keratin. As a result of this continuous erosion, the structure of the rhinoceros horn has a pointed structure, despite the absence of a bony core. The density of rhinoceros horn ranged from 0.465 to 6.047 g/cm3 with the average value of 0.794 g/cm3 ± 0.22. The density of rhinoceros horn fibers is 7 mm-2 and the average fiber diameter is 100 micrometers. (Yang, 2011)

The carving of rhinoceros horn into various shapes is mostly divided into 2 parts: the tip and the base of the horn. The tip of the rhinoceros horn, which is small, curved, slender, and pointed, is cut off to carve a small deity to hang around the neck or to make a small seal. The base may include the area almost

reaching the tip of the horn or may slightly extend beyond the middle of the horn, depending on the shape that the craftsman it is carves into a goblet, which is a container used to hold drinks or a champagne cup because of its suitable structure. It is also carved into various antique cups. Most of them are containers that are used in the daily life of the upper class because it is believed that the rhinoceros horn can protect against poison. The rhizome or the base of the horn is cut-off to make the horn smooth and beautiful. The cut off part is used as an ingredient in making medicine to treat various diseases.

5. The art structure of antique carved rhinoceros horns

5.1 Ming dynasty

The art structure of rhinoceros horns of the Ming dynasty is as follows:

1. *Antique rhinoceros horn carving techniques*, the techniques; used in carving rhinoceros horns in each era are difficult to imitate. The arrangement of artistic elements that are unique to the craftsman, as well as the access to the spirit of the artwork, the carving techniques in the Ming Dynasty were influenced by the Song and Yuan Dynasties, which were social and historical contexts, because before that, China was ruled by the Yuan Dynasty, which was Mongolian. When the Ming Dynasty, which was originally Han Chinese, was restored, there was an attempt to revive ancient Chinese art again. However, the Ming Dynasty was famous for producing pottery, porcelain, and silk, which were exported to Europe and were in demand all over the world. Rhinoceros horn carving emphasized simplicity, but was popular for fretwork, especially carving ornaments, mostly made for the royal family or the upper class in the palace.

2. *Line*, the lines or patterns are curved, showing grace but simplicity.

3. *Forms* are mainly influenced by the art styles of the Tang, and Song dynasties. The shapes, and forms are therefore landscapes, flowers and birds. Symbolic shapes such as dragons, swans and Fretwork are popular.

4. *Space* there is a balance between space and patterns.

5. *Color*, the antique carved rhinoceros horns of both dynasties (Ming and Qing Dynasties) are not different because it depends on the species of rhinoceros horn that is carved. However, black rhinoceros horns are mostly ground into medicine and made into small ornaments such as combs or hairpins because it is believed that they have better medicinal properties than other colors of rhinoceros horns.

6. *Textures* are coatings, and polishing often use lacquer, which is called "Qi" in Chinese, to make it shiny and durable. It is applied to the surface of the carving, and then polished or rubbed with a cloth or animal hair brush to make it shiny and smooth. Lacquer carving was first developed in the Yuan Dynasty and continued to the Ming and Qing Dynasties. Lacquer can be applied to the surface of diaoqi, qiangjin, disotian, enameled, lacquerware, silver, ivory, and pearl jewelry. The most popular lacquer is red lacquer, and it is considered the highest quality. It has the properties of toughness and fineness. The color popularly used to coat carved rhinoceros horns is reddish brown.

5.2 Qing Dynasty

The art structure of rhinoceros horns of the Qing dynasty is as follows:

1. Antique rhinoceros horn carving technique, the unique characteristic of the antique rhinoceros horn carving of the Qing Dynasty is the seal under the base of every piece of art, especially during the Qianlong Emperor era, using the multi-layer carving technique with different depths and shallowness to design as a high-relief image, emphasizing the exquisiteness and luxury.

2. Lines are more detailed and refined due to the development of various techniques from the past, especially carving natural shapes, western patterns, poems, poetry, which are characters, so the use of lines with a variety of characteristics according to the characteristics of Chinese character patterns, which must be especially graceful, including curved lines, straight lines, diagonal lines, thick lines, thin lines, and continuous lines.

3. Forms, carved rhinoceros horns are made into new shapes that are different from the past, such as geometric shapes, carved into everyday objects, liquor cups, tea cups in various forms, tall and short, according to the size of the rhinoceros horn, and are more realistic and natural than any previous era, which is different from the previous dynasty that liked to carve bracelets, bracelets, and other types

of jewelry. In addition, auspicious animals were carved to decorate the prestige of mythical animals that were gods.

4. Space there is a balance between space and lines, but more importance is given to emphasizing a more natural balance, imitating nature, and being more realistic.

5. Color is no different from the Ming Dynasty.

6. Texture is no different from the Ming Dynasty, but it is more developed. The Qing Dynasty established many factories for lacquerware in carvings.

No. 1: a cup carved from a rhinoceros horn in the Ming Dynasty

No. 2-4: a cup carved from a rhinoceros horn in the Qing Dynasty (no. 2: a carved in imitation of the Han Dynasty, no. 3: a carved in imitation of the Song Dynasty, no. 4: a carved pattern in the Qing Dynasty)

No. 5: a seal carved from the tip of a rhinoceros horn

Figure 1. Antique carved rhinoceros horns.

6. DNA testing

The results of DNA extraction found that the concentration was in the range of 3.7–14.2 ng/µl and the A260/280 ratio was between 1.02–1.32, indicating that the extracted DNA was contaminated with protein. When the DNA was tested with the cyt b primer, it was found that samples RH3 and RH7 could amplify DNA with the primer.

Table 1.

DNA concentrations of the tested samples and the results of DNA amplification.

No.	Sample ID	Concentration (ng/µl)	260/280	260/230	PCR amplification
1	RH1	7.7	1.32	0.49	No amplification
2	RH2	14.2	1.04	0.51	No amplification
3	RH3	3.8	1.02	0.93	amplify
4	RH4	6.5	1.30	0.67	No amplification
5	RH5	6.8	1.15	0.51	No amplification
6	RH6	6.3	1.21	0.85	No amplification
7	RH7	3.7	1.06	0.96	amplify
8	RH8	4.0	1.30	0.64	No amplification

The DNA was tested with cyt b gene barcode DNA using PCR technique, which has a volume of 20 µl, consisting of 25 ng of DNA, L14724 (F): CGAAGCTTGATATGAAAAACCATCGTTG and H15149 (R): AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA each 0.2 µM, dNTP 0.2 mM, 1× PCR buffer, 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase and the following PCR cycles were used: denaturation 94°C 2 min, denaturation 94°C 45 sec, Annealing (by choosing the appropriate temperature for each primer pair) 45

sec, and extension 72°C 1 min, repeated for 30 cycles, then final extension 72°C 5 min. The PCR products were separated by size on a 1% agarose gel, stained with safe dye, and recorded under UV light.

When the DNA was tested with the cytb primer, it was found that only 2 samples RH3 and RH7

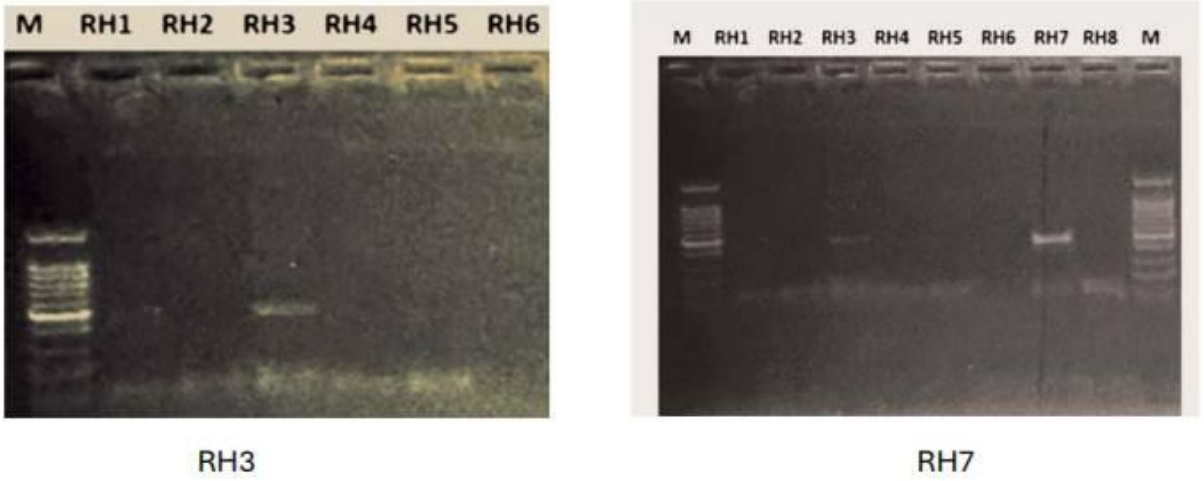

Figure 2. PCR amplification product and Cyt b gene electrophored in 1% agarose gel.

sample could be amplified with the primer as shown in figure 2. However, when it was tested to determine the species of rhinoceros, RH7 decomposed. Only RH3 could be sent for DNA testing and was found to be Ceratotherium simum. Nucleotide sequence of the Cyt b gene of RH3: GTAAATCCCACCCACT AATCAAAATTATCAACCACTCATTCATCGATCTGCCCACCCCATCAAACATCTCAGCCTAGTGAAATTTTGGCTCCC TGCTAGGAATGTGCTTAATCTTACAAATTCTAACCGGACTATTCCTTGCCATACACTACACACCAGACACAATAAC TGCCTTCTCATCTGTCGCCCATATCTGTCGAGACGTGAATTACGGCTGAATTATCCGCTATCTCCATGCCAACGGAG CATCCATATTCTTTATCTGCCTATTCATCCACGTAGGACGCGGTATCTATTACGGATCATATACCTTCCTAGAAACC TGAAACATCGGAGTTATCTTACTATTCACTCTAATAGCCACCGCATTCATACGCTACGTCCTACCATGAGGACAAA TATCAT

7. Conclusion

This research has the hypothesis is DNA extracted from ancient rhinoceros horn tissue has complete DNA strand integrity and could be amplified to create a DNA profile. From the research results, ancient rhinoceros horn tissue can be tested for DNA; only one piece it was 12.5%, ancient rhinoceros horn carvings older than 200 years can not able to be tested for DNA, is a greater than 50%, or a low probability, and therefore this hypothesis can be rejected.

Antique carved rhinoceros horns are not suitable for DNA testing and still need to rely on expert knowledge in analyzing the shape and art. Analyzing art history, morphology and structure is important in deciding which rhinoceros horn is an antique carved rhinoceros horn, which will be more extensive and comprehensive than just testing whether it is a real or fake rhinoceros horn because experts will be able to tell which art form of the ancient carved rhinoceros horn is from which era and when it was made.

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