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From Highways to Biological Collections: Plastination of Wild Animals Victims of Roadkill in the Sooretama Biological Reserve, Brazil

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HIGHLIGHTS

- Room temperature shows advantages to plastinate birds.
- The run over animals can compose a collection of high quality plastinated specimens.
- There are necessary adaptations in plastination for each class of vertebrates, but the standard protocol can produce good specimens.
- Run over animals plastinated can be used in environmental and scientific education.

Abstract: Roadkill is one of the most direct impacts on fauna, leading to numerous direct and indirect impacts. Road-killed animals could serve as a resource for research and teaching purposes, but first, they need to have their tissues preserved by some technique. Plastination is one of the most modern techniques for the conservation of biological tissues, its principle is the replacement of body fluids by a curable polymer, resulting in a non-toxic, odorless, and manipulable material. So the main aim of this work was to compose a collection of plastinated roadkill victims from the Biological Reserve Sooretama region, adapting the standard protocol to the different taxa worked here (*Avialae*, *Reptilia*, *Amphibia*, and *Mammalia*). For this, 42 animals were used, previously frozen and stored in the Sooretama Biological Reserve and Vale Natural Reserve until they were sent for processing. Upon arriving at the Plastination laboratory at the Federal University of Espírito Santo, the animals underwent the 4 stages of plastination: fixation, dehydration, forced impregnation, and chemical cure; some adaptations were made to improve the final specimens. The results proved that the plastination of these animals is extremely viable in generating satisfactory results. Given the results obtained,

it becomes clear how plastination can become an alternative to implement in the composition of biological collections using roadkill animals. The animals prepared in this work comprise an unprecedented collection available for different applications, such as exhibitions, practical classes, and research.

Keywords: Plastination; Roadkill; Conservation; Biological collections.

INTRODUCTION

Roads and highways are the most used land transport in the Neotropics [1], and are extremely important for economic development of those countries. However, highways are among the main drivers of biodiversity loss worldwide [2]. According to some studies by the Brazilian Center for Studies in Road Ecology (Centro Brasileiro de Estudos em Ecologia de Estradas - CBEE), about 450 million wild animals die each year on the highways that cross the country [3].

These roadkill animals could act as a reservoir of information, helping to preserve and mitigate impacts on fauna in places where highways are the main precursors of direct and indirect impacts. Using carcasses from roadkill victims enables the composition of valuable biological collections [4]. However, to study these animals, it is necessary to have adequate conservation techniques to prevent autolysis and decomposition of biological tissues.

Plastination is one of the most modern techniques for the conservation of biological tissues, being called by some authors the anatomical technique of the century [5]. Developed by the German physician Gunther von Hagens in 1977, this technique has as its principle the replacement of water, the main constituent of biological tissues, and body fluids with a curable polymer [6]. This technique produces specimens with no odors, no toxicity, flexibility, easy maintenance, and storage, and is extremely durable. Plastinated specimens can last for decades, probably centuries, with proper care, preserving with excellent fidelity the original gross and microscopic characteristics of the tissues [7].

Currently, Brazil has been standing out on the international scene with the Plastination Laboratory of the Federal University of Espírito Santo (UFES), which has been developing research and carrying out the plastination of different specimens of wild animals from the Atlantic Forest and human cadaveric specimens, being the first laboratory to create an exhibition collection available to the non-academical and academical public at the national level and one of the first to host an International Interim Conference on Plastination in 2015, gathering some international names of Plastination in Brazil to teach, learn and disseminate the technique.

Thus, the aim of this work was to create a biological collection of plastinated animals from Atlantic Rainforest victims of trampling, to have a powerful tool to promote research, scientific and environmental education, beyond scientific dissemination about the biodiversity of the Atlantic Rainforest animals, zoology, anatomy, and related topics.

MATERIAL AND METHODS

A total of 42 animals were prepared during the last three years (2019-2021). Final specimens were qualitatively evaluated, using as parameters for comparative effect: initial and final visual aspects; the presence of visual changes/distortions of structures and tissues; and final aesthetic characteristics, such as the appearance of coat/feather and eyes, colors of the different biological tissues and maintenance of the desired position. For further comparison, all specimens were photographed before and after being plastinated.

The specimens used in this work came from a collaboration with the Project "Collection of biological samples of run over, hunted and killed wild vertebrates, from the Sooretama Biological Reserve (ReBio) and Vale Natural Reserve, Linhares - ES", with SISBIO license /ICMBIO number 31762-6, coordinated and executed by UFES. All the animals were run over on the Federal highway BR-101, in the stretch that crosses Sooretama Biological Reserve and Vale Natural Reserve, in the north of Espírito Santo state. This study was also approved by the Ethics Committee on the Use of Animals - UFES, number 31/2019.

Due to a large number of specimens received, processing was performed in small groups with approximately 8 - 10 specimens per round. The first step was to perform thawing of the animal carcass at room temperature (24 hours), followed by a biometrical evaluation. After this process, animals were photographed and performed identification of lesions or deformations that could mischaracterize or disfigure the specimen were, and whenever possible, made sutures and adjustments to enhance the results.

For processing, due to the large volume of specimens, the animals were arranged in chronological order and divided into 6 groups (Table 1). During the plastination of the specimens, the standard protocol was followed, divided into four steps: fixation, dehydration, forced impregnation, and cure [8] (Figure 1).

Table 1. Groups and their respective species are separated for processing, arranged in chronological order.

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
<i>Cuniculus paca</i> ²	<i>Tapirus terrestris</i> ¹³		<i>Leopardus pardalis</i> ¹³		<i>Sapajus robustus</i> ³
<i>Cerdocyon thous</i> ¹²	<i>Artibeus lituratus</i> ¹³	<i>Boa constrictor</i> ³	<i>Galictis vittata</i> ¹³		<i>Didelphis aurita</i> ³
<i>Tapirus terrestris</i> ²	<i>Callithrix geoffroyi</i> ³	<i>Salvator merianae</i> ³	<i>Salvator merianae</i> ¹³		<i>Cavia fulgida</i> ³
<i>Procyon cancrivorous</i> ¹²	<i>Porphyrio martinicus</i> ³	<i>Erythrolamprus miliaris</i> ³	<i>Alouatta guariba</i> ¹³	<i>Bothrops jararaca</i> ²	<i>Tamandua tetradactyla</i> ¹³
<i>Nasua nasua</i> ²	<i>Cerdocyon thous</i> ¹³	<i>Ophiodes cf. fragilis</i> ³	<i>Galictis cuja</i> ³	<i>Amazona amazonica</i> ³	<i>Colaptes campestris</i> ¹³
<i>Picumnus cirratus</i> ²	<i>Eupsittula aurea</i> ³	<i>Micrurus corallinus</i> ³	<i>Callithrix geoffroyi</i> ¹³	<i>Leptodactylus latrans</i> ¹²	<i>Alouatta guariba</i> ¹³
<i>Leopardus wiedii</i> ²	<i>Sciurus aestuans</i> ³	<i>Oxyrhopus petolarius</i> ³	<i>Leopardus wiedii</i> ¹³		<i>Alouatta guariba</i> ¹³
<i>Bradypus variegatus</i> ²	<i>Pteroglossus aracari</i> ¹³	<i>Tyto furcata</i> ³	<i>Artibeus lituratus</i> ¹³		<i>Potos flavus</i> ¹³
		<i>Siphlophis compressus</i> ²	<i>Coendou insidiosus</i> ¹³		

Legend: ¹- Underwent dissection; ² - Room temperature impregnation; ³ - Cold temperature impregnation.

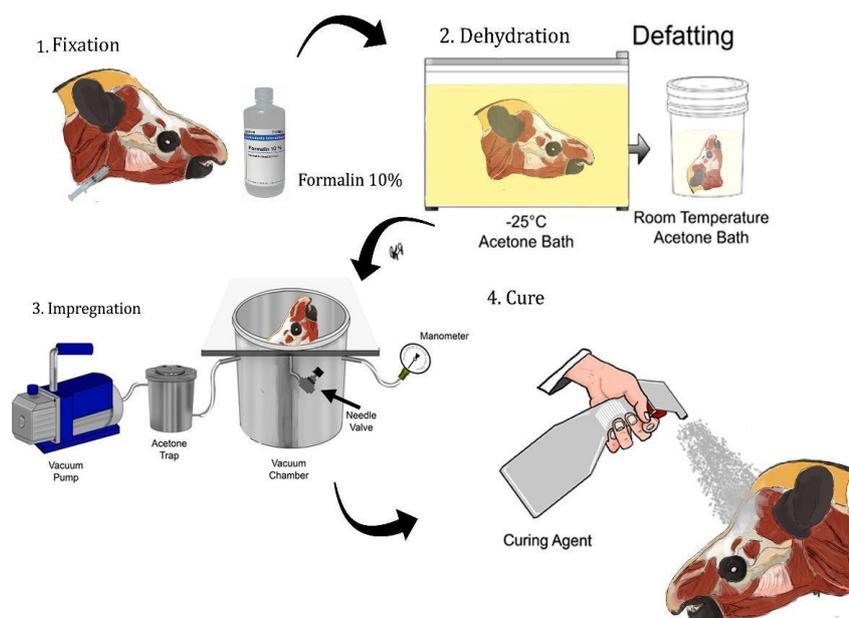


Figure 1. Plastination process followed by the standard protocols proposed by Hagens et al., (1987) [8] and Raof et al., (2007) [9].

Note. Adapted by authors from Visible Heart® Laboratories from the University of Minnesota website.

Fixation

Chemical fixation was performed with 10% formalin, through direct tissue infiltration, followed by immersion in the formalin with the same concentration, remaining there for at least 2 months. For exhibition purposes, the fixation process requires a previous positioning of the animals; therefore, bases were made on which they were fixed with the help of screws, wires, and gauze, ensuring that by the end of the fixation, it would maintain the desired positions, based on photographs and records of alive animals.

After fixation, some specimens were dissected, while others remained intact. In the animals to be dissected, a superficial dissection was preferred, with evidence of muscle groups and removal of the subcutaneous tissue, since the main use of the animals was to prepare the animals for use in exhibitions at the Museum of Life Sciences at the Federal University of Espirito Santo exploring the topographic anatomy.

Some adjustments were necessary, such as suturing wounds and lacerations from collisions, repositioning injured limbs, and replacing the eyeball with a mold to maintain the eye's original shape. Criteria for dissection were the number of individuals from the same species; the presence of injuries; animal integrity; type of coat/feather; the presence of species-specific anatomical structures; and mounting for comparison between certain specimens. Regarding integrity, very damaged specimens with apparent lesions were preferably made entirely without dissections, with only abdominal and thoracic incisions being made to facilitate the penetration of silicone into the body cavities.

Dehydration

Two months after fixation and after dissection, the animals went through the plastination process following the basic protocol proposed by von Hagens, Tiedemann and Kriz (1987) at cold temperatures (CT) and described by Raof, Henry, and Reed [9] at room temperatures (RT). First, animals went through the dehydration stage, in which the specimens were submitted to 4 successive immersive baths of acetone, with concentrations of 95%, 95%, 100%, and 100% (v/v), with a minimum duration of 7 days bath in each solution (Figure 1). By the end of the process, the concentration of acetone in the last bath was greater than 99%, a parameter by which the dehydration was considered complete.

Forced impregnation

After dehydration, forced polymer impregnation was started. This step took place inside a chamber, where, with the action of a vacuum, the acetone present in the animals' tissues was vaporized and slowly replaced by the silicone polymer (Figure 1). Specimens from groups 2, 3, 4, and 6 were impregnated at a cold temperature of -18 ± 2 °C and from groups 1 and 5 at room temperature. The criteria used in designating the adopted temperature were the size of the specimens and for comparative testing of similar coats and

plumage between the two methods. Animals impregnated at cold temperatures were made with the reactive mixture of silicone Polydimethylsiloxane (PMDS) and its catalyst dibutyl tin laurate (DBTL) at a ratio of 100:1 (m/m). And the specimens prepared at room temperature were carried out with the silicone PMDS and the crosslinker Tetraethyl Silicate (TES) in a ratio of 100:8 (m/m). The duration of the process varied from 10 - 25 days, during which the vacuum was applied slowly and gradually, with small daily adjustments until the bubbles stopped. After the impregnation period, the specimens were removed from the chamber and distributed under papers to start the mechanical drainage of excessive silicone.

Drainage and Chemical cure

The process of manual removal of excessive silicone, in the case of mammals, was carried out by scraping with the use of a knife or spatula blade in the region where the coat was kept, and manual removal with the absorbent paper was necessary to repeat this process over the drain time. In reptiles and birds, only absorbent paper was used to avoid damage to the skin and loss of feathers' disposition.

After the end of drainage and the manual removal of excessive silicone, the final stage of chemical curing started. However, prior to this step, it was necessary to reposition the animals to recover the desired position, which sometimes may change during dehydration and impregnation. The process varies according to the temperature used during the impregnation: in specimens impregnated at cold temperature, the TES crosslinker was vaporized in a closed plastic chamber, and in those impregnated at room temperature, DBTL catalyst was applied on the surface of the specimens, using brushes. After three days, the cure was complete, and the specimens were ready. Some animals went through replacing eyes with replicas before and others after the chemical cure, seeking to establish which protocol would be more appropriate.

RESULTS

A total of 42 animals were prepared, covering four Classes (Mammalia, Reptilia, Aves, and Amphibia), 14 Orders, and 27 Families, which were used in different methods of preparation and finalization, especially for the temperature used during impregnation and during the removal of excess silicone prior to cure.

At the end of

the process, in general, the specimens maintained their initial characteristics, such as post-fixation color, maintenance of anatomical structures, and the natural disposition of the coat, in the case of mammals, resulting in high-quality specimens in most cases (Figure 2). Figure 3 shows dissected specimens in one antimer to highlight the main superficial muscle groups and then plastinated.

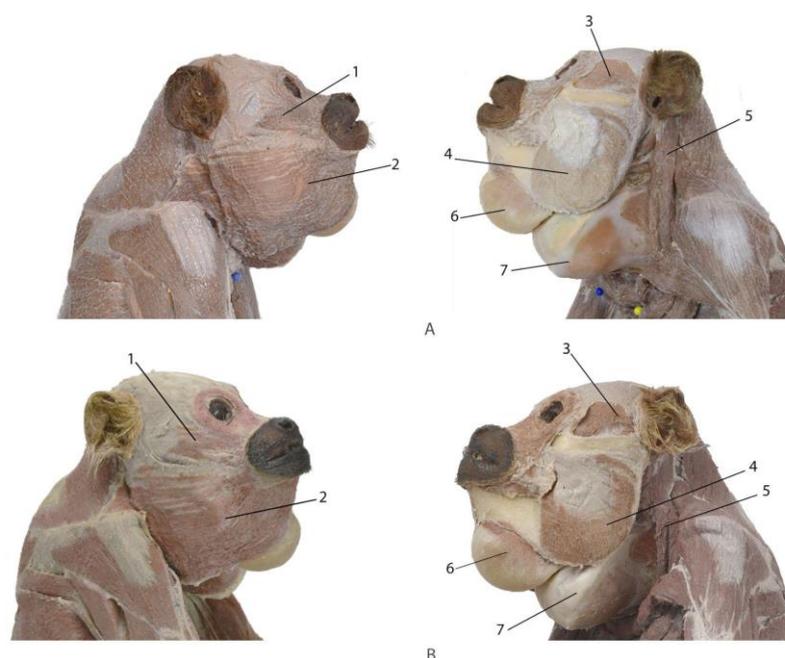


Figure 2. Deep and superficial craniofacial and neck muscles from *Alouatta guariba* before (A) and after (B) plastination at cold temperature (CT). **Legend:** 1. *m. zygomaticus major*; 2. *M. platysma*; 3. *m. temporalis*; 4. *m. masseter*; 5. *m. sternocleidomastoideus*; 6. *Hyoid bone*; 7. *Thyroid cartilage*.



Figure 3. A) A Kinkajou (*Potos flavus*) and B) an Ocelot (*Leopardus pardalis*) plastinated at cold temperature (CT) with dissection to evidence the main superficial muscle groups.

After the process, the animals showed a reduction in the initial weight and an increase in their stiffness, with little or absent malleabilities. Mechanical removal of residual silicone, which preceded the cure, was essential, as it helped to minimize the wet aspect of impregnated the integumentary system (Figure 4).

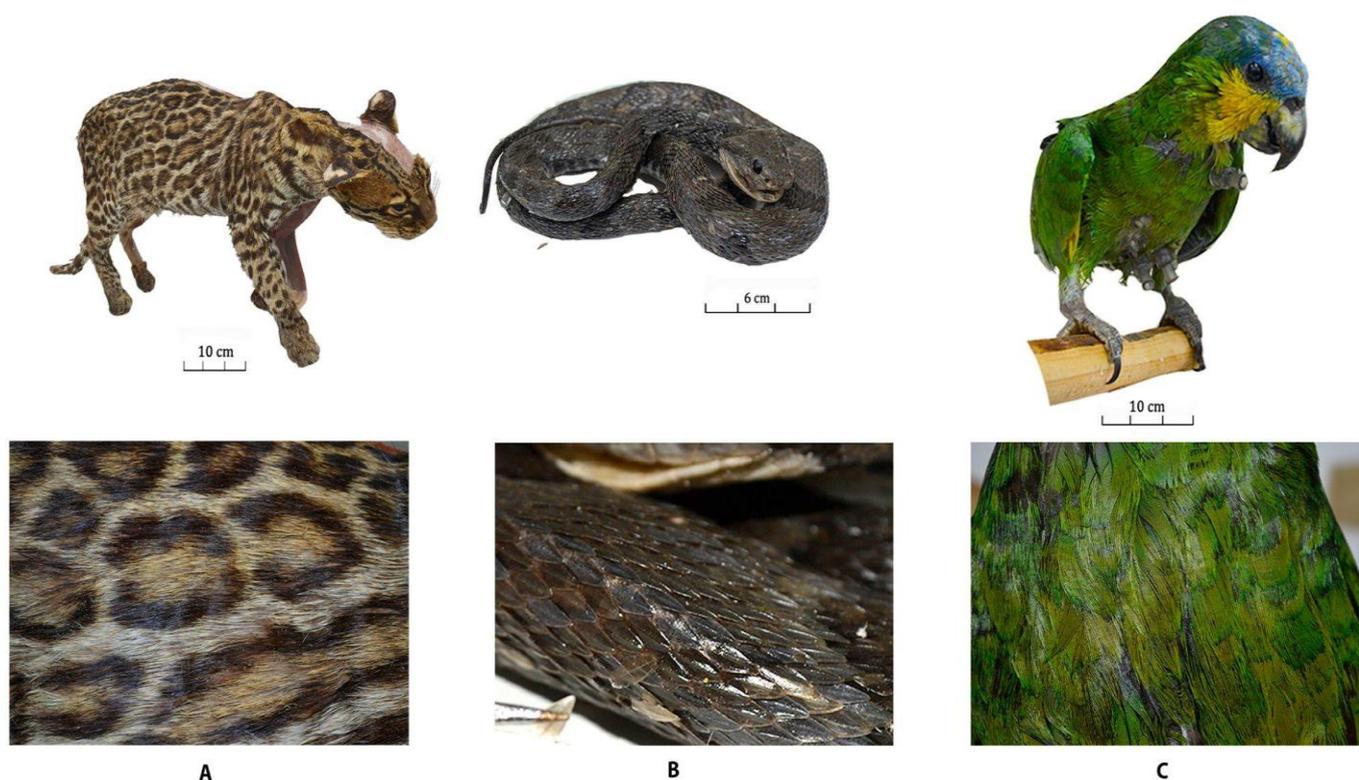


Figure 4. Different integumentary systems and their respective results after impregnation with silicone at cold temperature (CT) and room temperature (RT). A) *Leopardus pardalis* fur (CT). B) *Bothrops jararaca* scales (CT). C) *Amazona amazonica* feathers (RT).

In the case of mammals, the type of coat was crucial for silicone drainage before curing. The shortest and thickest hair was the easiest to work with, while the longest and thinnest or furry ones presented a greater difficulty in maintaining the natural state, however, satisfactory results were found in the standard protocol at cold temperature and at room temperature (Figure 5).

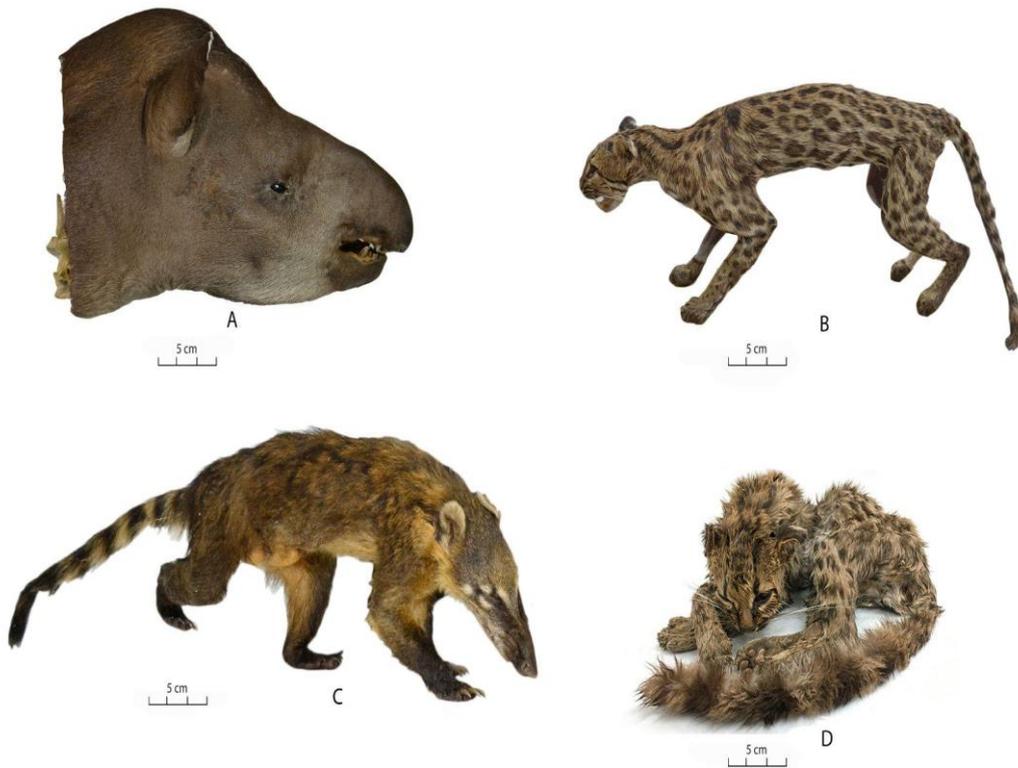


Figure 5. Mammals with distinct types of furs impregnated with silicone at cold temperature and at room temperature: A) *Tapirus terrestris* (CT); B) *Leopardus wiedii* (CT); C) *Nasua nasua* (RT); D) *Leopardus wiedii* (RT).

In general, feathers have a high tendency for silicone adhesion, especially to barbs and barbules, which is why greater care and time are needed during drainage. Birds impregnated at room temperature showed promising results due to the longer drainage time (Figure 6).

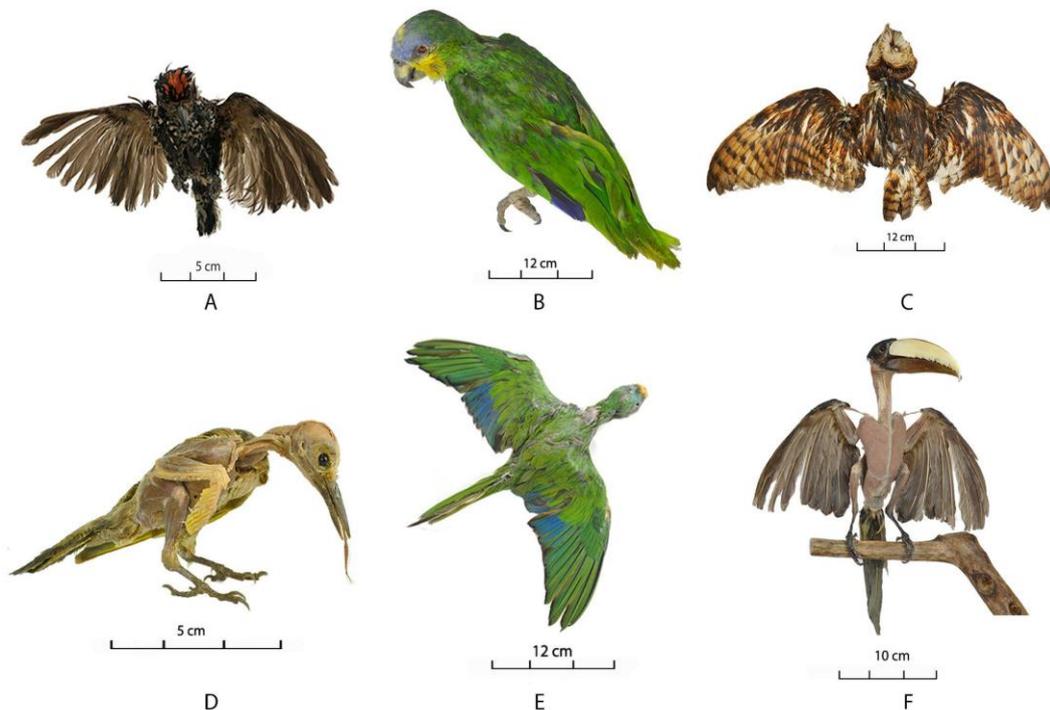


Figure 6. Birds with distinct feathers patterns impregnated with silicone at room temperature (RT) and at cold temperature (CT): A) *Picumnus cirratus* (RT); B) *Amazona amazonica* (RT); C) *Tyto furcata* (CT); D) *Colaptes campestris* (CT); F) *Eupsittula aurea* (CT); E) *Pteroglossus aracari* (CT).

In reptiles, some individuals had lost a cuticle that covers the scales, requiring manual removal of these cuticles. In addition, during fixation and dehydration, there was a significant loss of pigment in some snakes, especially red and green (Figure 7A and 7B). One way to solve the loss of pigment was to carry out the fixation and dehydration steps simultaneously, as proposed by Ekim (2017) [10] (Figure 7C).

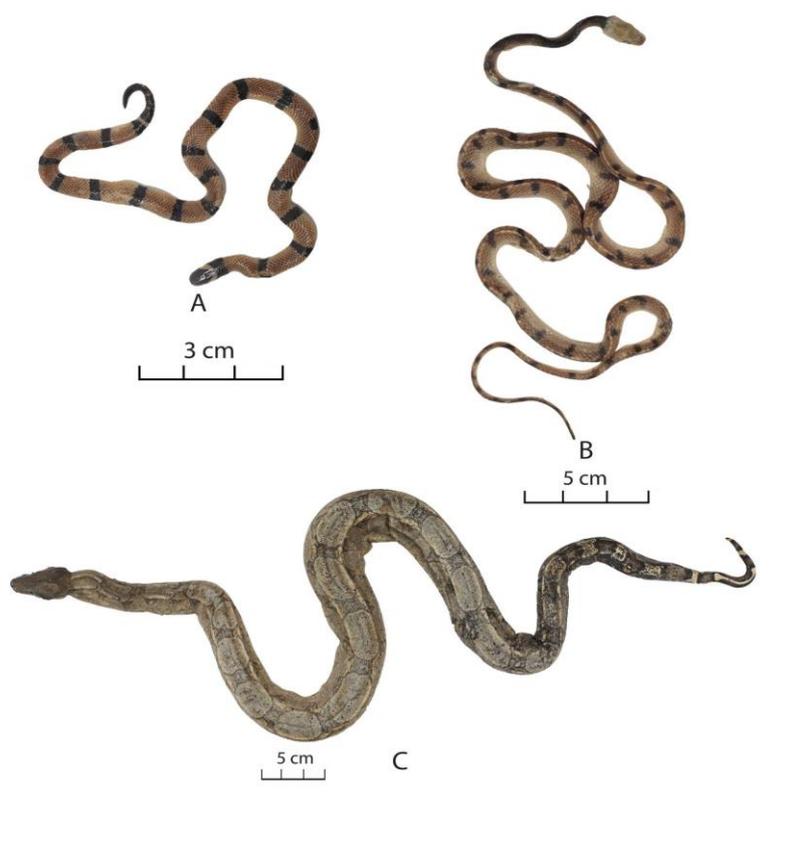


Figure 7. Snakes plastinated at cold temperature (CT) following the standard protocol proposed by Hagens et al. (1977). A) *Micrurus corallinus* (CT); B) *Siphlophis compressus* (CT); C) *Boa constrictor* (CT).

The eyeball tissues suffered a loss of natural pigments during processing, losing their natural appearance and shape. Therefore, at the end of processing, the eyes, previously replaced by spheres, could give way to replicas, thus ensuring a more natural appearance. The animals that had the eyeball replaced before fixation had a better result since the shape and volume of the eyes were maintained because of the plastic spheres (Figure 8).



Figure 8. Tapir head plastinated at cold temperature before and after replacement of the original eye by a synthetic eye.

The color of the tissues, especially the muscles, was changed during the fixation process, in which formaldehyde inevitably ends up causing the denaturation of the proteins that are responsible for these colors, promoting tissue darkening over time [11].

Most of these animals were victims of being run over and, despite that, they could all be plastinated, even with some injuries and lacerations evident in many cases. For example, when thawing the capuchin monkey (*Sapajus robustus*) was noted lesions in the hind limbs with an open fracture of the femur, even though the result was satisfactory (Figure 9).



Figure 9. A capuchin monkey (*Sapajus robustus*) victim of roadkills. A) Before the plastination process, evidencing a femur injury; B) After the plastination process at cold temperature.

After processing, the animals were properly photographed and stored on shelves in the Plastination Laboratory without any type of maintenance, where they could be covered with plastics or fabrics to avoid the deposit of dust or residues over time, making them available for future uses and to compose the exhibition collection at the Museum of Life Sciences, linked to UFES.

DISCUSSION

During the plastination of the groups, there was a progressive evolution in the quality of the specimens in positioning, finalization, and replacement of the eyes and coats and plumage finalization. There are no descriptive studies in the literature on the plastination of animals with an evaluation of coat and plumage treatment techniques for a more natural appearance. At the end of preparing these animals, some questions were raised related to the specificities of each group worked during their preparation. The different classes presented their singularities that are reflected in the adaptations of the standard protocol and, despite this, little can be found in the literature about the nuances of each one, since vertebrates are a vast and heterogeneous group in terms of sizes, shapes, and types of integuments, an attempt was made to use a robust and diverse sample number in classes and orders.

Due to the diversity of characteristics found in the integumentary system of the vertebrates, such as fur, feathers, and scales, greater care was needed in draining excess silicone to avoid phenotypic alterations, since these can cause problems in future analysis. Draining was extremely important in minimizing these changes and maintaining the initial characteristics of the worked specimens.

In the case of mammals, the process of manually removing the silicone by scraping was extremely important, because, additionally to maintaining the disposition of the hair in the natural position, it significantly removed silicone excess, helping to reduce the silicone drainage time. In addition, the use of brushes and combs also helped when used after curing, removing its opaque appearance and reclaiming volume, returning it to an appearance close to natural.

Mammals impregnated at room temperature showed an advantage over silicone drainage, allowing it to remain for a long time without going through the curing process, which results in better drainage since the impregnation mixture at room temperature did not undergo polymerization before the addition of the catalyst. However, the need to brush the entire specimen with the catalyst (DBTL) proved to be a hindrance in large specimens. The cold temperature impregnation mixture, on the other hand, has a shorter working time, reducing the draining time (before curing). This occurs because the addition of the DBTL catalyst to silicone initiates the polymerization, increasing the viscosity of the mixture until the formation of the gel state (a state that precedes the solidification of the polymer) which makes its complete removal practically impossible.

In the case of birds, mechanical removal of silicone is limited due to the risks of losing the original arrangement of the barbs and barbules. An alternative in the protocol could be a combination of plastination

and taxidermy, preserving the feathers most susceptible to deformation separately and reapplying them in some cases. Using less viscous silicone and impregnation at room temperature seemed to help in the drainage process, preventing the accumulation of silicone. Despite *Tyto furcata* having presented a negative result, *Porphyrio martinicus* and *Eupsittula aurea* presented satisfactory results in the impregnation at cold temperature. *Amazona amazonica*, impregnated at room temperature with a low-viscosity silicone, remained draining for a longer time, thus giving an appearance with few changes and extremely satisfactory (Figure 6). The neck region is difficult to work with due to the grouping of the feathers when soaked with silicone.

In the case of non-avian reptiles, plastination proved to be extremely viable, especially in specimens with more neutral pigments, such as tegu. The removal of silicone from this integument occurred easily. However, it was noted that some pigments had changed during fixation and dehydration, therefore the exposure of these specimens to formaldehyde and acetone for a long period should be avoided, as it has solvent effects on pigments and on some anatomical structures formed by adipose tissue, being this shorter exposure time by these animals to acetone recommended by some studies [10].

In addition to changes in pigments, some reptiles, especially snakes, had a marked tissue retraction, demonstrating the need for internal filling, as Eikim and coauthors (2017) [10] demonstrated in their work. In our work, we chose to open incisions in the ventral region of these animals, followed by the addition of silicone in the abdominal cavity in the reptiles that presented this retraction, thus generating a more natural appearance, but increasing the drain time.

In general, abdominal and thoracic incisions were important during the processing of these animals, as it seems to have helped the penetration of the silicone and the cross-linker, opening a space for the polymer to enter the tissues both internally and externally.

Given the inherent characteristics of plastinated specimens, they are excellent sources of information, serving as an available and long-lasting material, since silicone is a polymer that is hardly susceptible to microbial and fungal infestation [12], and even if it happens, it can be easily removed with a damp cloth with 70% ethyl alcohol.

When compared to other conservation techniques available, such as taxidermy and conservation in immersion liquid solutions, plastination has numerous advantages, since complex structures can be preserved, such as muscles, vessels, nerves, and organs in their most natural state (Figure 2), without showing toxicity and dry to the touch. In addition, several analyzes are already possible on plastinated specimens.

The main advantage of plastination consists in the final nature of the specimens produced, even escaping from the drawers of collections for museum exhibitions, which thus constitutes a repository for easy exposure in spaces such as natural history museums and can serve as a collection for use in theoretical-practical disciplines [13]. It can act as an alternative to conventional techniques. In the case of run over animals, plastination can be extremely viable, even in cases with open injuries and fractures, which can be solved with sutures and dissections.

Currently, it is possible to extract DNA samples from plastinated tissues embedded with silicone that have not undergone the formalin fixation process. The protocol, described by Ottone and coauthors (2020), allows analysis of plastinated materials submitted to the de-plastination process through PCR tests, thus generating new applications for plastinated materials in general, since these are materials with long-lasting biosafety indefinitely, guaranteeing the possibility of molecular analysis for a longer time [14]

Another possibility is histological analysis, Ramos and coauthors (2018) compared some ways to prepare histological slides with plastinated materials. The results revealed that plastinated specimens can be used in the assembly of histological slides directly, without any preparation, such as de-plastination [15].

Despite the numerous advantages, some limitations/disadvantages must be considered when choosing plastination as a method of conservation, such as loss of malleability, a small percentage of tissue retraction, the impossibility of dissection after curing the specimens, processing time, and high initial investment for setting up a suitable laboratory, so plastination requires a small initial investment. About the high initial cost, plastination is very cost-effective, since plastinated specimens do not need maintenance over time and are very resistant (inert), unlike other techniques that require replacement of conservative solutions and degrade with time and insect action, furthermore, show similar features with wet specimens in terms of anatomical structures [16]. So, despite the processing time, plastinated specimens can solve a lot of maintenance problems, such as formalin acquisition and disposal, and longing for decades.

In some cases, plastinated specimens can show dark spots, deformations, and high levels of shrinkage. But the most common failures are results of errors during the processing or little experience in plastination by itself [17]. So, independent of the specimen's origin and nature, during plastination, unexpected or unintended results may occur.

During preparation, it was noticed that the state of the carcass interferes with the result, as plastination itself produces few changes. The morpho-anatomical characteristics are little changed during the process. The major changes, especially in tissue colors, occur during fixation, once they are evidenced in the dissection and mainly in impregnation when shrinkage occurs [6]. Therefore, in some cases, other conventional techniques may prove to be more viable, thus avoiding undesired results, especially in specimens in an advanced state of decomposition.

In cases of carcasses in good condition, plastination may be the best option given its long-term benefits and possibilities for dissections. Sometimes the roadkill can cause injuries and/or lacerations that make dissection unfeasible. A solution is to prepare these animals with the maintenance of the coat and suture of the wounds to hide the lesions and improve the final specimens (Figure 8). In specimens with an advanced state of decomposition, osteotechnics become the best option [18]

The main challenge was to process such different groups with many peculiarities, such as scales, feathers, and furs. The roadkill by itself can make it unfeasible, but in general cases, these animals from roadkill can result in very good specimens, with many applications.

The use of plastinated animals is not restricted to research and biological analysis. When implemented in collections, it becomes a formidable tool in the composition of environmental education actions. This is one of the possibilities of using these listed animals, serving as a small sample of what exists in the zoological diversity for the population that, many times, does not have access to specimens that compose the fauna of the biomes in which they reside, a work already widely done using taxidermied animals, with expressive results [19].

In 2018, the Life Sciences Museum held an exhibition in which some plastinated animals were used, receiving thousands of visitors who had the opportunity to see and recognize some specimens that make up the fauna of the Atlantic Forest. Not only serve the external public, but these animals also have a direct application in teaching, with numerous works in the literature reporting the use of plastinated animals and their relevance in teaching [5]. In addition, the Plastination Laboratory at the Federal University of Espírito Santo (LabPlast) has been standing out in the processing of Brazilian wild animals internationally, with presentations of lectures and works at scientific events and invitations to exhibit specimens in other locations.

CONCLUSION

The plastinated animals produced in this work established an unprecedented biological collection, especially for being a technique little applied in this area of conservation biology.

This work showed that plastination applies very well to the preparation of biological collections, given the preservation of the original morpho-anatomical characteristics of plastinated specimens, depending on the dissection proposal and the carcass conservation status. Despite the high cost, low-cost alternative equipment can be used to produce high-quality specimens that can serve as voucher specimens.

With plastination, the preservation of the coat and the plumage with natural aspect, limitation to other preservation methods, was satisfactory and reproducible, which will allow the application in other laboratories.

This work also showed how advantageous biological collections preserved with the plastination technique can become, since the specimens remain non-toxic, without irritating odors, dry to the touch, resistant to weathering and manipulation, and easy to store in small labs.

This study also showed that the application of plastination can guarantee full accessibility to biological collections, since plastinated specimens can be touched and manipulated unrestrictedly, including by the visually impaired, either in the teaching environment or in exhibitions in museum spaces.

Plastination can give the wild animals victims of roadkill an important significance in biological collections, which can contribute to the population's environmental and scientific education, in addition to scientific research.

Conflicts of interest: The authors have no conflicts of interest.

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REFERENCES

1. Taylor Z, Hoyle B, Knowles R. Modern Transport Geography. *Econ Geogr*, 2001 Jan 1;7.
2. Coffin A. From roadkill to road ecology: A review of the ecological effects of roads. *J Transp Geogr*. 2007 Sept 1, 2007;15:396–406.

3. Bager A, Lucas P, Bourscheit A, Kuczach A, Maia B. Os caminhos da conservação da biodiversidade brasileira frente aos impactos da infraestrutura viária. *Biodiversidade Bras.* 2016 Mar 9; 6:75–86.
4. Auricchio P, Catenacci LS, Santos KR, Britto FB. A protocol for the use of roadkill or stranded animals as material for research and teaching. *SITIENTIBUS Sér Ciênc Biológicas.* 2014;14:1–6.
5. Latorre R, Bainbridge D, Tavernor A, Albors OL. Plastination in anatomy learning: An experience at Cambridge University. *J Vet Med Educ.* 2016;43(3):226–34.
6. Monteiro YF, Silva MVF, Bittencourt APSV, Bittencourt AS. Plastination with low viscosity silicone: strategy for less tissue shrinkage. *Braz J Med Biol Res.* 2022;55:1–10.
7. Sora MC, Latorre R, Baptista C, López-Albors O. Plastination—A scientific method for teaching and research. *J Vet Med Ser C Anat Histol Embryol.* 2019;48(6):526–31.
8. Hagens G, Tiedemann K, Kriz W. The current potential of plastination. *Anat Embryol (Berl).* 1987 Feb 17;175:411–21.
9. Raof A, Henry R, Jr R. Silicone plastination of biological tissue: Room-temperature technique Dow/Corcoran technique and products. *J Int Soc Plast.* 2007 Jan 1;22.
10. Ekim O, Haziroğlu RM, Cingoz Insal B, Bakıcı C, Akgün R, Tunali S. A modified S10B silicone plastination method for preparation and preservation of scaled reptile specimens. *Vet Fakültesi Derg.* 2017 Oct 6;64:155–60.
11. Oliveira ÍM de, Mindêllo MMA, Martins Y de O, Silva Filho AR da. [Analysis of anatomical parts preserved with polyester resin for study in human anatomy]. *Rev Colégio Bras Cir.* 2013 Feb; 40(1):76–80.
12. Barros M, Navarini A, Masuko T. Fungal Contamination of Plastined Specimen. 2015.
13. Saeed M, Rufai A, Elsayed S. Mummification to plastination. Revisited. *Saudi Med J.* 2001 Nov;22(11):956—9.
14. Ottone NE, Baptista CAC, del Sol M, Muñoz Ortega M. Extraction of DNA from plastinated tissues. *Forensic Sci Int.* 2020;309:110199.
15. Ramos M, Paula T, Zerlotini M, Silva V, Carazo L, Paula M, et al. A Comparison of Different De-plastination Methodologies for Preparing Histological Sections of Material Plastinated with Biodur® S10/S3. 2018;30:10–5.
16. O’Sullivan E, Mitchell BS. Plastination for gross anatomy teaching using low-cost equipment. *Surg Radiol Anat.* September 1, 1995;17(3):277–81.
17. Miklosová M, Miklos V. Plastination with silicone method S 10--monitoring and analysis causes of failure. *Biomed Pap Med Fac Univ Palacký Olomouc Czechoslov.* 2004;148(2):237–8.
18. Auricchio P, Salomão MG, Eskinazi-leça e, Macedo SJ, Lima T, Barros, HM. [Vertebrates collection and preparation techniques]. São Paulo: Arujá. Instituto Pau Brasil de História Natural. 2002. 348 p.:il. Ed. Universitária da UFPE, Recife, 2000. 252 p.
19. Bispo de Sousa EP, Barbosa MVM. [Zcollections: the jose hidasi museum of zoology and taxidermy of the state university of tocantins]. October 15, 2020;4(2):7-13.



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