Comparative analysis of wild and domestic animals hair species

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Abstract. The article reveals the search for a fast and reliable techniques for identifying hair samples from wild animals, which occur during an expert evaluation in the Amur branch of WWF Russia. The relevance of the presented data is that the main methods for determining the species of hair are electron microscopy, analysis by spectrogram or chemical composition of the hair, which is a long-term method and requires special equipment. In practice, the above-root parts of hair are often received for examination, and it is unsuitable for genetic research since some have already passed through the digestive tract. The authors considered the search for a technique that allows analyzing the hair of the Amur tiger (Panthera tigris altaica), Domestic cat (Felis catus domesticus) and Domestic dog (Canis lupus familiaris) in comparison, the method of imprints on gelatin, on a photographic plate, and on a colorless varnish was applied. As a result of the research, one method was chosen for analyzing hair samples on a colorless varnish with subsequent microscopy. Measurements of hair samples were compared in Microsoft Excel, with the construction of charts, using the "trend line" and “linear forecast” functions to predict the average measurement result and “error limits” with the "standard error" function to determine the permissible deviations of measurement results. As a result, we found that samples from different parts of the body of wild and domestic animals are well analyzed in the Microsoft Excel program with the expression of a linear trend, which allows us to determine the permissible deviations of the measurement results.

1. Introduction

One of the most important objects of forensic biological examination is the hair of animals, the analysis of which is issued a conclusion about the species. So, in 70% of cases of expert evaluation in the Amur branch of WWF Russia, samples containing animal hair were provided for examination, in this case, it was hair belonging to the red book species Amur tiger (Panthera tigris altaica).

All animals have outer coverings, but only Mammalia mammals have hair. Both macro and micro studies are used to study animal hair. Micro-research involves studying the structure of the hair, dividing it into types according to the topology of the body.

To identify which type of hair belongs to, you can apply the research of Chernova "Hair architectonics and its diagnostic value" [7]. The author considered the fine structure (architectonics) of mammalian hair and the diagnostic significance of its features, proposed the unification of terminology
and classification of the cuticle, cortical and core layers of hair. The structure of the core, according to Chernova, varies greatly and is therefore used to determine taxons, even at the species level. For example, in the tiger Panthera tigris altaica, the core occupies 1/2 of the diameter of the stem, and primates - 1/3 of the stem’s diameter. The classification of hair cores is based on the feature of the content of cavities and the nature of their distribution in the hair stem. The core may not be there at all. If it is present, it may contain cavities (air-bearing) or not (non-air-bearing). In addition, the shape of the cells is taken into account when identifying the hair. Since it contains generic\species characteristics.

Thus, one of the crucial values is the use of the database of hair microstructures to determine whether the objects of research belong to a particular type of animal.

2. Relevance
The main methods for determining the type of hair is considered to be electron microscopy and analysis by spectrogram or chemical composition of the hair, which is sometimes protracted and requires special equipment. Another method used in forensic medical examination is genetic examination, but for its conduct, again, you only need a hair with the root, as only there can you find the DNA of the owner of the hair. In practice, the above-root parts of the hair that get for examination are unsuitable for genetic research. As a result, empirical macroanalysis remains one of the most frequently used methods for determining species membership [3, 4].

3. The purpose of the study
Describe the diagnostic method for examining the hair structure of the Amur tiger (P. tigris altaica).

4. Research Objectives
Conduct a selection of methods for obtaining a print of the hair cuticle.

Conduct microscopy and morphometry of the obtained hair prints and analyze the obtained measurements from hair samples of three animal species.

5. Materials
Samples of the Amur tiger's guard hair (Panthera tigris altaica) collected on 21.04.2017, 7 samples were collected from one tiger, namely: head, neck, back, paw, tail, shoulder blade, and belly. For comparative analysis, we used samples of guard hair: domestic cat (Felis catus domesticus), Domestic dog (Canis lupus familiaris). Morphometric parameters were studied using a LOMO MS-2 microscope with additional lighting and a Topocam video camera with a resolution of x20. Statistical methods of data processing using Microsoft Excel.

6. Methods
The first stage of the study was to determine which method is best to view and diagnose the hair cuticle.

The hair stem usually consists of a cuticle layer on the outside and a medulla in the center and a cortical layer between them. The cuticle is a single layer of cells, the shape of w

Thich may be different, but, as a rule, poorly distinguishable when viewed directly under a light microscope. There are methods for getting a print of the hair cuticle on gelatin, colorless nail varnish or emulsion of a photographic plate.

There are several methods of obtaining an imprint of the cuticle of the hair, namely:

- on gelatin;
- on a colorless nail polish;
- on the emulsion of a photographic plate.
The first method is to obtain a print of the hair cuticle on gelatin, which was made according to the method proposed by Kuznetsov in the determinant of vertebrate animals of the fauna of the USSR (1975) [4]. Gelatin is immersed in cold water at the rate of 1 weight part of gelatin to 5-10 parts of water. After the gelatin swells, the water is heated until the gelatin completely dissolves. A drop of solution is applied to the slide, smeared on it with an even and thin layer (as it is done when receiving a blood smear). The thickness of the layer should not exceed half the thickness of the hair being studied. On a smear of gelatin, a sample of the remaining hair, washed previously in ether, is placed and the composition is allowed to dry. Then the hair is removed, and in its place remains a cuticle imprint, which is studied under a microscope.

The second method is to get a cuticle print on a colorless nail Polish. Method of conducting the experiment: apply a thin layer of colorless varnish on a slide and apply a hair (previously washed in ether) to it, let the varnish dry and after drying remove the hair. Received the imprint of the cuticle will microscopium.

The third method is to get prints of the hair cuticle on photographic plates. Method of conducting a hair print: the photographic plate is cut into pieces according to the size of the slides; then the glasses are fixed in a conventional photographic fixer until the emulsion layer discolors; then the glasses are washed and dried, in this form they can be stored for a long time. Before starting work, a piece of plate is placed in water heated to about 50 C and kept until the emulsion layer swells (5 min). Pre-washed hair is freely lowered to the emulsion surface of the photographic plate. Allow the emulsion layer to dry, and after removing the hair from the glass, you can start studying the cuticle print.

The brain substance of the hair is formed by large, loosely connected cells. The shape of the cells of the brain matter in different animal species is different, in some mammals the hair does not contain brain matter. In some mammals, the hair is highly pigmented, and as a result, it is not possible to examine the core [5, 6]. Core types are described in sufficient detail in the definition table of S. N. Gashev and A. G. Selyukov [2].

The second stage of research was to study the histostructure of the thickness of hair samples under a microscope and search for visible differences. When conducting research, we noted that our data confirm the literature sources about the proportional species-specificity of the difference in the size of the hair core to the cuticle layer [2; 5].

The method of microscopy and measurements was as follows: the width of the hair medulla (core), the width of the hair cuticle, the ratio of the width of the hair medulla to the total thickness of the hair, the ratio of the thickness of the hair medulla to the cuticle layer with measurement on one side.

7. The results of the research
In the course of research on the choice of methods for obtaining the best hair print, the method of printing on a colorless varnish was chosen.

The results of microscopy and morphometry of samples of hair prints obtained on colorless varnish are shown in figure 1 and table 1.

Table 1. Morphometry of hair samples from different body parts of the Amur tiger (Panthera tigris altaica), domestic cat (Felis catus) and domestic dog (Canis lupus family).

| Species | № Hair sample | Hairline sampling area | Brain matter of the hair, nm | Cuticle (from 1 side), nm | The width of a hair, nm | The ratio of the size of the brain matter of the hair to the width of the hair | The ratio of the cuticle to the brain matter of the hair |
|---------|---------------|------------------------|-----------------------------|--------------------------|------------------------|-------------------------------------------------|-------------------------------------------------|
| Amur tiger (Panthera tigris altaica) | 1 | Head | 143.9 | 93.4 | 365.96 | 2.543 | 1.298 |
| | 2 | Neck | 190.89 | 106.54 | 395.28 | 2.070 | 1.116 |
| | 3 | Blade | 244.06 | 109.16 | 463.93 | 1.901 | 0.894 |
| Species                  | Location     | Length    | Width     | Height   | Thickness | Angle |
|-------------------------|--------------|-----------|-----------|----------|-----------|-------|
| Panthera tigris altaica | Back         | 130.13    | 62.7      | 275.02   | 2.113     | 0.963 |
|                         | Stomach      | 139.82    | 69.38     | 316.56   | 2.264     | 0.992 |
|                         | Tail         | 143.84    | 110.3     | 314.29   | 2.184     | 1.534 |
|                         | Paw          | 244.06    | 109.16    | 463.93   | 1.901     | 0.894 |
| Domestic cat (Felis catus) | Head        | 19.07     | 5.55      | 28.48    | 1.493     | 0.582 |
|                         | Neck         | 54.45     | 16.12     | 79.54    | 1.461     | 0.487 |
|                         | Blade        | 39.11     | 12.16     | 63.42    | 1.621     | 0.622 |
|                         | Back         | 53.64     | 13.21     | 78.75    | 1.468     | 0.492 |
|                         | Stomach      | 65.86     | 44.5      | 156.41   | 2.375     | 1.351 |
|                         | Tail         | 32.24     | 6.87      | 46.82    | 1.452     | 0.426 |
|                         | Paw          | 19.80     | 13.5      | 46.12    | 2.329     | 1.364 |
| Domestic dog (Canis lupus familiaris) | Head    | 46.44     | 9.58      | 63.51    | 1.367     | 0.412 |
|                         | Neck         | 49.67     | 8.78      | 63.01    | 1.268     | 0.353 |
|                         | Blade        | 54.26     | 7.26      | 64.76    | 1.193     | 0.268 |
|                         | Back         | 55.53     | 8.19      | 70.33    | 1.266     | 0.295 |
|                         | Stomach      | 95.79     | 9.51      | 112.23   | 1.171     | 0.198 |
|                         | Tail         | 86.67     | 7.4       | 99.64    | 1.150     | 0.171 |
|                         | Paw          | 84.21     | 8.2       | 104.47   | 1.240     | 0.195 |

**Figure 1.** Samples of prints of hair, native sample (SW. X20). a - sample of the guard hairs with the blades of the Amur tiger (Panthera tigris altaica); b - sample of the guard hairs from the shoulder of the Domestic cat (Felis catus); c - sample of the guard hairs from the shoulder of the Domestic dog (Canis lupus familiaris).
For comparison of the results obtained by morphometry samples of guard hairs, we used the program Microsoft Excel charting the results, using the "trend line" and the "linear prediction" functions to predict the average outcome measure and the "error limits" function then the "standard error" to determine the tolerance of the measurement results.

The resulting diagram is shown in figure 2.

![Figure 2. Comparative characteristics of hair with the expression of a linear trend (in the legend of the chart, the ratio of the width of the hair/the width of the core).](image)

Data from table 1 and figure 2 indicate possible deviations of the obtained research results, particularly interesting data are noted in the 5th and 6th samples from a domestic cat (Felis catus), where the deviation from the average trend line is quite large, but when processing possible numerical expressions by determining the trend, we can see that the average trend line is preserved and this deviation is an exception.

Also, when analyzing the ratio of the hair width to the width of the brain substance, the indicators for the Amur tiger (Panthera tigris altaica) remain in the region of 2, for the Domestic cat (Felis catus) this value is close to 1.5, and for the Domestic dog (Canis lupus familiaris) - 1.2.

8. Conclusions
As a result of the tasks set, we determined:

- the most optimal method of hair impression for microscopy and morphometry was determined by the method of hair impression on a colorless varnish;
- the conducted morphometry of hair samples is well analyzed in the Microsoft Excel program with the expression of a linear trend, which allows for determining the permissible deviations of the measurement results.

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