Comparing a mini barcoding methodology to hair-morphology in order to identify fecal samples from Neotropical felids

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Abstract

Molecular approaches have been employed to identify feces found in the field, but this methods require a complete molecular biology laboratory, and usually also require very fresh fecal samples to avoid DNA degradation, what is difficult to access in the field. In this study we tested a molecular identification technique, using the ATP6 region as a marker and compared its efficacy to that of a morphological identification key, built by us, for guard hairs of eight Neotropical felids (*Leopardus pardalis, L. wiedii, L. colocolo, L. geofreoyii, L. tigrinus, Puma concolor, P. yiaguourundi* and *Panthera onca*). For this molecular method, we replicated some field circumstances by postponing sample-conservation procedures, in order to mimic some field conditions. A blind test of the identification key achieved a nearly 70% overall success rate, which we considered equivalent to or better than the results of some molecular methods (probably due to DNA degradation) found in other studies. Results regarding our own molecular approach were excellent, since all blind-tested samples were correctly identified. Part of these identifications were made from samples kept in suboptimal conditions, with some of them lasting outdoors for up to seven days, simulating field conditions

Methods

In this study **[1]**, using hairs collected from museum specimens and from feces of captive individuals of eight species of Neotropical felids, we constructed a morphological identification key using color, banding pattern, size, scale and medullar patterns. Using two different sets of samples of feces from these same captive individuals, we blind-tested the effectiveness of the key and compared it to the effectiveness of a molecular protocol, one that used the ATP6 region as a marker. To perform the blind tests, each sample was coded with a number for each species. The codes were revealed only after the samples were identified with both methods.

Results

Guard hairs from the species analyzed showed great variation in the cuticle scale pattern along the length of each individual hair. Therefore, for practical purposes and for a more precise description, we considered three regions: shield; transitional region between shield and shaft; and shaft.

As in some previous studies, we also found that the shaft is the most important region for differentiating among species based on cuticle scales (**Fig 1**).

Fig 1: Cuticle scale patterns on the shaft of guard hairs of selected Neotropical felids. (a) regular wavy pattern; (b) irregular wavy pattern; (c) irregular wavy pattern with ornamented edges.

On the medulla (**Fig 2**), two regions were observed, shield and shaft. We considered three of their features: 1) Continuity: the medulla may be continuous or not; 2) Width: we compared the width of the medulla with that of the cortex; 3) Shape profile: diagonal uniseriate, with most cells arranged diagonally in relation to the hair axis, creating a morphological configuration similar to some alphabet letters, such as N, M, Y, H; trabecular, with the medulla showing a pattern of rodlike serial structures, parallel and adjacent to each other and transverse to the hair axis; uniseriate ladder, with cells forming regular or irregular squares or rectangles of variable size. In many cases, the medulla is nearly transparent, consisting mainly of air vacuoles, delimited by filaments

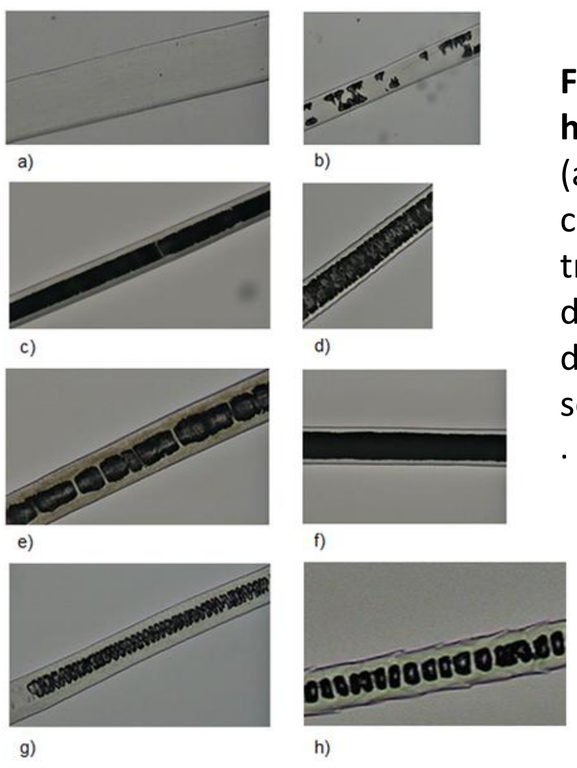

Fig 2: Medullar patterns on guard hairs of selected Neotropical felids. (a) absent; (b) fragmented; (c) continuous with filaments; (d) trabecular; (e) uniseriate ladder; (f) deeply pigmented continuous; (g) diagonal uniseriate; (h) small squares
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Using specific primers to unravel the identity of species from fecal samples, we obtained 100% success for all eight species that were blind-tested (**Table 1**). There was no difference in the results for the test conditions of the samples, *i.e*. the results did not vary if the sample was preserved in ethanol and frozen immediately after it was collected (condition fresh/freeze), or if it was frozen only a week later (week/freeze), or if both preservation in ethanol and freezing were done only seven days after the collection (week/week)

Table 1: Number of samples used for molecular blind-test. All tests were successful.

Species	N of samples fresh/freeze	N of samples week/freeze	N of samples week/week	Total N of samples	% of success	Efficiency
P. onca	3	3	3	9	100	Excellent
P. yagouaroundi	3	3	3	9	100	Excellent
P. concolor	3	3	3	9	100	Excellent
L. colocolo	3	3	3	9	100	Excellent
L. geoffroyi	3	3	3	9	100	Excellent
L. pardalis	3	3	3	9	100	Excellent
L. tigrinus	3	3	3	9	100	Excellent
L.wiedii	3	3	3	9	100	Excellent
TOTAL	**24**	**24**	**24**	**72**	**100**	**Excellent**

The effectiveness of the identification key can be considered excellent for detecting hairs of *P. onca*, *P. yagouaroundi*, *P. concolor* and *L. colocolo*, with success rates of 100%, 100%, 100% and 80% respectively. The results of the key test can be considered good, with 60% success, for *L. geoffroyi*. The results for the ocelot and oncilla can be considered moderate, with a success rate of 40%. Only for *L. wiedii* were the key results poor. The overall success rate of the key, for the group of specimens used here, was 68.35% (**Table 2**).

Table 3 lists the species with more-similar morphological features in their guard hair and which therefore can be confused with each other, leading to false-positive identifications.

Table 2: Results of blind tests of hair identification key.

Species	N of samples	% of success	Key efficacy
P. onca	10	100	Excellent
P. yagouaroundi	10	100	Excellent
P. concolor	9	100	Excellent
L. colocolo	10	80	Excellent
L. geoffroyi	10	60	Good
L. pardalis	10	40	Regular
L. tigrinus	10	40	Regular
L.wiedii	10	30	Poor
TOTAL	**79**	**68,35**	**Good**

Table 3: Species confused with each other in the tests of the key.

Species	N of times confused	% of error
L. tigrinus , L. geoffroyi	6	35
L. pardalis , L. wiedii	4	20
L. colocolo , L. wiedii	2	15
L. wiedii , L. geoffroyi	3	15
L. tigrinus , L. colocolo	2	10
L. tigrinus , L. pardalis	2	10
L. colocolo , L. geoffroyi	1	5
L. colocolo , L. pardalis	1	5
L. pardalis , L. geoffroyi	1	5
L. tigrinus , L. wiedii	1	5

Conclusions

It appears that both methods can be used, depending on the available laboratory facilities and on the expected results.

Reference

1 - Alberts CC, Saranholi BH, Frei F, Galetti PM Jr (2017) Comparing hair-morphology and molecular methods to identify fecal samples from Neotropical felids. PLoS ONE12(9): e0184073. https://doi.org/10.1371/journal.pone.0184073