



New non-invasive photo-identification technique for free-ranging giant anteaters (*Myrmecophaga tridactyla*) facilitates urgently needed field studies

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ABSTRACT

Pelage patterns, colouration and other biometric traits are perceived to be uniform in the Neotropical giant anteater (*Myrmecophaga tridactyla*), a conception precluding the identification of individuals, which is essential for field research on the little known aspects of the species' ecology and behaviour.

Here we present a new, non-invasive technique of matrix photo-identification to identify individual giant anteaters by their natural markings. In a long-term field study in the Brazilian Pantanal, photographs of 475 giant anteater observations (396 = direct sightings, 79 = camera traps) were captured from 2010 to 2015 and considered for our analysis. Photographs were stored in a catalogue and coded in a computerised identification table, with biometric traits being categorised and described for each observed individual in a matrix. In 71% of all photographed giant anteaters, differences in pelage marking patterns, as well as other characteristics such as ear shape and scars, allowed individual recognition. We ensured consistency of the method by conducting a double-blind verification by an experienced researcher and naïve volunteers.

This simple, non-invasive method can push the level of information about life history and population structure of giant anteaters, as it applies to a large array of study designs. It can thus enhance future studies, be integrated in ongoing research projects or supply additional information out of older data sets. It is applicable to expand data collection and raise awareness in local communities, and potentially for participatory citizen science methods. Altogether these are important cornerstones for conservation actions on the species which is listed as 'Vulnerable' on the International Union for Conservation of Nature Red List of Threatened Species.

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Introduction

Individual recognition of animals is of vital importance for a wide range of conservation-relevant ecological and behavioural population studies, such as life histories, social-organisation structures and demographic processes (e.g. Kelly 2001; Oliveira-

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Santos et al. 2009; Gomez-Salazar et al. 2011; Bolger et al. 2012). Unambiguous individualisation can be achieved via invasive methods that rely on marking, tagging or chipping animals (Silvy et al. 2005), or via non-invasive alternatives such as biometric identification techniques utilising characteristic natural markings of animals (Pennycuik 1978; Markowitz et al. 2003; Sherley et al. 2010; Gomez-Salazar et al. 2011). The most obvious morphological patterns are variations in the colouration of hair, feathers, skin or scales (Kelly 2001; Burghardt et al. 2004; Bolger et al. 2012). For decades individuals of mammals with obvious pelage patterns, such as giraffes (Foster 1966), giant pandas (Zheng et al. 2016) or zebras (Petersen 1972; Lahiri et al. 2011), have been identified by these traits. In most of these studies, sightings of animals were recorded via photographic cameras or camera traps, and photographs or videos were later analysed for identification (Karczmarski and Cockcroft 1998; Markowitz et al. 2003; Anderson et al. 2007). This analysis via photo-/video-identification is traditionally executed manually, by setting up photo catalogues (a collection of photographs of observed individuals of a certain animal species, e.g. Würsig and Jefferson 1990) and corresponding identification tables, in which characteristic traits of an individual are categorised and/or described (e.g. Karczmarski and Cockcroft 1998). Available software and personal digital assistant (PDA)/smartphone applications for management of geotagged photographs, particularly developed for participation of citizen science, can nowadays facilitate fieldwork and manual categorisation of photographs (Liebenberg et al. 1999; Ansell & Koenig 2011). For the study of larger wild populations, image-processing software has been developed to automatically analyse characteristic biometric traits (Kelly 2001; Albu et al. 2008; Sherley et al. 2010; Bolger et al. 2012; Óscar et al. 2015). Whatever method is used, the necessary precondition for discriminating individuals of a certain species by analysing photographs is the existence of unique natural markings that are consistent over time (Oliveira-Santos et al. 2009; Bolger et al. 2012).

Ecological and behavioural studies on animals without these biometric traits rely on methods that have been criticised as inaccurate (Sollmann et al. 2013), invasive and/or dependent on expensive marking techniques (summarised by e.g. Silvy et al. 2005). Due to these obstacles, field research on such species is often under-represented (Oliveira-Santos et al. 2009). However, several other external traits such as the shape of the dorsal fins of humpback whales (Katona and Whitehead 1981) or whisker-spot patterns in polar bears (Anderson et al. 2007) were shown to enable the identification of individuals when distinct coat markings are missing. In the Neotropics, individuals of unicoloured pumas and Brazilian tapirs were identified in camera-trap photographs by scars and other unique characteristics (Kelly et al. 2008; Oliveira-Santos et al. 2009). However, accuracy was lower compared to studies on mammal species with more prominent biometric traits (Karanth 1995; Silver et al. 2004).

While working out how to identify individuals of Neotropical species without pelage patterns, surprisingly little attention has been paid to the quite distinct colouring of the endangered and poorly investigated giant anteater (*Myrmecophaga tridactyla*) (Diniz and Brito 2012). It is listed as Vulnerable on the Red List of Threatened Species (IUCN 2015). Threats are the increasing human population density and intensification of land use in vast parts of its distribution (e.g. Silveira et al. 1999; Koster 2008; Di Blanco et al. 2015;

Quiroga et al. 2016). Roads pose a particular threat to this species which is often a victim of collisions with vehicles (de Freitas et al. 2015).

Giant anteaters are deep brown, or sometimes greyish-black, with white forelegs, and a characteristic black-and-white stripe that reaches from the breast up to the flanks and is assumed to play a role in camouflage. Female anteaters carry their cubs for up to 9 months on their back, where the stripes of mother and young merge, thus blurring the contours of the cub (Krumbiegel 1966). Immature giant anteaters that are already independent from their mother can be distinguished from fully grown individuals by smaller body size (Shaw et al. 1987) and a light grey crest of long hair which can be pilo-erected together with the hair of the tail when the animal feels threatened (pers. obs.), probably to camouflage the smaller body size. In adult giant anteaters the crest is darker and appears to be shorter (at least proportionally to the rest of the body, pers. obs.), but can still be pilo-erected with the tail hair during agnostic encounters (Shaw et al. 1987; Kreutz et al. 2009) or when the animal feels threatened (pers. obs.). There is no information regarding at what age young anteaters are no longer discernible from adults, but Shaw et al. proposed in 1987 it might be after 2 or 3 years. Fully grown, the body length of the animal can exceed 2 m including the long and bushy tail; the species lacks any obvious sexual dimorphism (Shaw et al. 1987; Nowak 1991).

The colour pattern of giant anteaters was thought to be uniform and hence excluded the possibility of individual identification. This is probably one reason for the existing lack of behavioural knowledge and long-term studies, especially concerning social organisation or life history, of free-ranging giant anteaters. *In situ* research projects cover only a small number of sites and populations (Diniz and Brito 2012). Most of these were short-term studies carried out more than 20 years ago (Montgomery 1985; Shaw et al. 1987; Medri and Mourão 2005). Others study the behaviour of reintroduced animals which may differ from that of animals that grew up in a natural environment (Di Blanco et al. 2015).

Existing behavioural studies used invasive methods and equipped giant anteaters with very high frequency (VHF) transmitters and global positioning system (GPS) collars to track their movements (Montgomery 1985; Shaw et al. 1987; Medri & Mourão 2005). Radio-tracking is a very effective method to locate and follow animals in the wild and to study activity and rare or cryptic behaviour (Kays et al. 2011). GPS collars can provide an incomparably complete documentation of movement patterns and activity of animals (Kays et al. 2015). At the same time, fitting an animal with a transmitter can be considered intrusive, as it needs to be captured, sedated and equipped with a radio collar of a certain weight. All these procedures can pose a risk to the welfare of the animal. This is especially true for long-term studies, as battery capacity is restricted, and animals need to be recaptured to replace expired collars (Mech and Barber 2002).

Non-invasive studies of giant anteater populations estimated densities using methods that do not require individual identification, such as density calculation by sighting/area during car observations (Kreutz et al. 2012), behavioural observations and measurements on scratching trees (Braga et al. 2010), or strip transect methods (Desbiez and Medri 2010). However, without individual recognition, these studies could not take behavioural aspects or analysis of individual movement patterns into account.

The technique presented here, of identification of giant anteaters by analysing photographs and setting up an identification table, will provide the possibility of

individual recognition in non-invasive field studies and thus facilitate research on the life history and social organisation of the animal, providing information that is urgently needed for effective conservation efforts on its behalf.

We ensured the consistency of the developed technique, by testing the agreement between an experienced researcher and naïve volunteers in identifying individual giant anteaters from photographs in a double-blind trial.

Material and methods

Our study site covers an area of about 100 km² at a sustainably managed cattle ranch in the southern Pantanal in Brazil (*Fazenda Barranco Alto*, 19.576°S, 56.153°W; [Figure 1](#)). Digital photographs of giant anteaters were collected between 2010 and 2015 by tourists, farm inhabitants and the authors, searching for the animals by car and taking pictures with digital photo cameras (the authors used mostly a Sony A100 with a Sigma 70-300 mm 2.8 Zoom Lens) or using camera traps (RECONYX Hyperfire HC500, settings: two frames per second, no delay, trigger time: 1/5, 1080P HD). The camera trap survey design was based on the Terrestrial Vertebrate Monitoring Protocol (TEAM 2008) with some specifications: The spatial layout was 100 positions in a regular grid at a density of one camera trap per km². At each position a camera trap was left in a tree or on a pole at knee height for at least 7 consecutive days, and a maximum interval of 12 months.

Car observations took place mostly in the afternoon, covering about 20 km² per day at an average speed of 15 km/h. On consecutive days different regions of the study area were covered. The location of opportunistic sightings of giant anteaters during these observations was recorded with a handheld GPS device (Garmin 76 CSx) or a smartphone (Honor 5C with the 'Cyber Tracker' application, www.cybertracker.org), or later at the computer by setting a waypoint in Google Earth. The local Google Earth satellite image has a resolution of < 5 m per pixel in the region, enabling us to identify the precise location where an animal was by orientation with respect to landmarks like single bushes and trees. Giant anteaters were photographed, ideally with both lateral sides of the body and the forehead unobscured by obstacles such as grass or branches, to document the complete fur pattern of the animal. If this was not possible, pictures of as many physical characteristics as possible were taken with the aim to document features that varied among individuals. Cubs riding on their mother's back, as well as immature giant anteaters which could be distinguished from adult anteaters by their grey crest and smaller body size, were not considered, as morphological patterns may change until the animal has fully grown.

The photographs were sorted in digital folders that were named with the coordinates, time, and date of each sighting. In the identification matrix, each sighting was described by a consistent set of data including date, time and locality, presence/absence of a cub on the back, and the relative maturity of the individual (adult/juvenile). Photographs of the animal are described according to the photographed sides of the body (l = left, r = right, b = both, f = front) and the observed coat marking patterns, in hierarchical order of obviousness and permanence. Additionally, the presence and absence of scars (presence = 1, absence = 0, no available photographs = X) and their position (head: left, right, front; leg and rump: left, right) was recorded in the matrix.

Two columns for remarks were attached to the table, one to record the quite variable ear shape and possible cuts in the ear, the other for general remarks about unique features of the sighted animal (e.g. pigmentation, lesions, broken-off claws).

When coding of a sighting was completed, the table could be searched for individuals with comparable characteristics (possible re-sightings), sorting column by column in descending order. The photographic evidence for each possible re-sighting was then visually compared with other photographs of the similar individual. If the photographs did not match, the new sighting was considered a new individual and included in the photo database. A photo catalogue of all photographically documented and clearly identifiable individuals was thus created.

To assess the applicability of the methodology, we conducted a double-blind identification test, in which the first author and nine naïve volunteers, recruited via social network (Facebook), classified photographs of 21 independent anteater sightings using the classification matrix. All participants (researcher and volunteers) were unaware of how the others had assessed the photographs, and how many individuals they believed to have identified. A 30-minute online video tutorial and a short guide were provided. We simplified the procedure for volunteers by providing only one photograph per sighting, showing the animals straight from the side, in contrast to a normal data set, which mostly comprises several photographs per sighting, showing various angles of the animal. Inter-rater agreement was compared pairwise among first author and volunteers and among volunteers only. To test whether differences in the quality of photographs, due to varying light conditions and focus as well as distance and angle to camera, influence the ability of an observer to discern individual features, we categorised the photographs according to a quality scale (low, medium, high, excellent quality) and compared agreement and quality of photograph using a one-way analysis of variance (ANOVA). To find out whether relative classification was easier with certain individuals compared to others, we compared with a one-way ANOVA the grade of average agreement by volunteers for the single anteaters identified by the first author.

Results

Photographs of 475 giant anteater sightings were considered in this study, 396 of which were taken during direct sightings and 79 by camera traps (in 3227 trapping nights). In 77% of the photographs of direct sightings the quality was sufficient for individual recognition. At only 38%, the proportion of photographs taken by camera traps that allowed the identification of individual giant anteaters was considerably lower. Moving animals were blurred in these photos, or they passed the trap at an angle that allowed only limited or no analysis of pelage marking pattern.

The analysis of the photographic collection allowed us to identify several obvious and subtle biometric traits that showed variation among individuals (Figure 2 and Table 1). Discrimination of free-ranging giant anteaters by these identified traits is possible. Classification of an individual by consideration of various of these markers offered greater reliability. The body shape was not considered for further analyses, as the animal's silhouette was observed to change substantially when the long and bushy hair of crest and tail was being pilo-erected. Also, the length of hair might have an

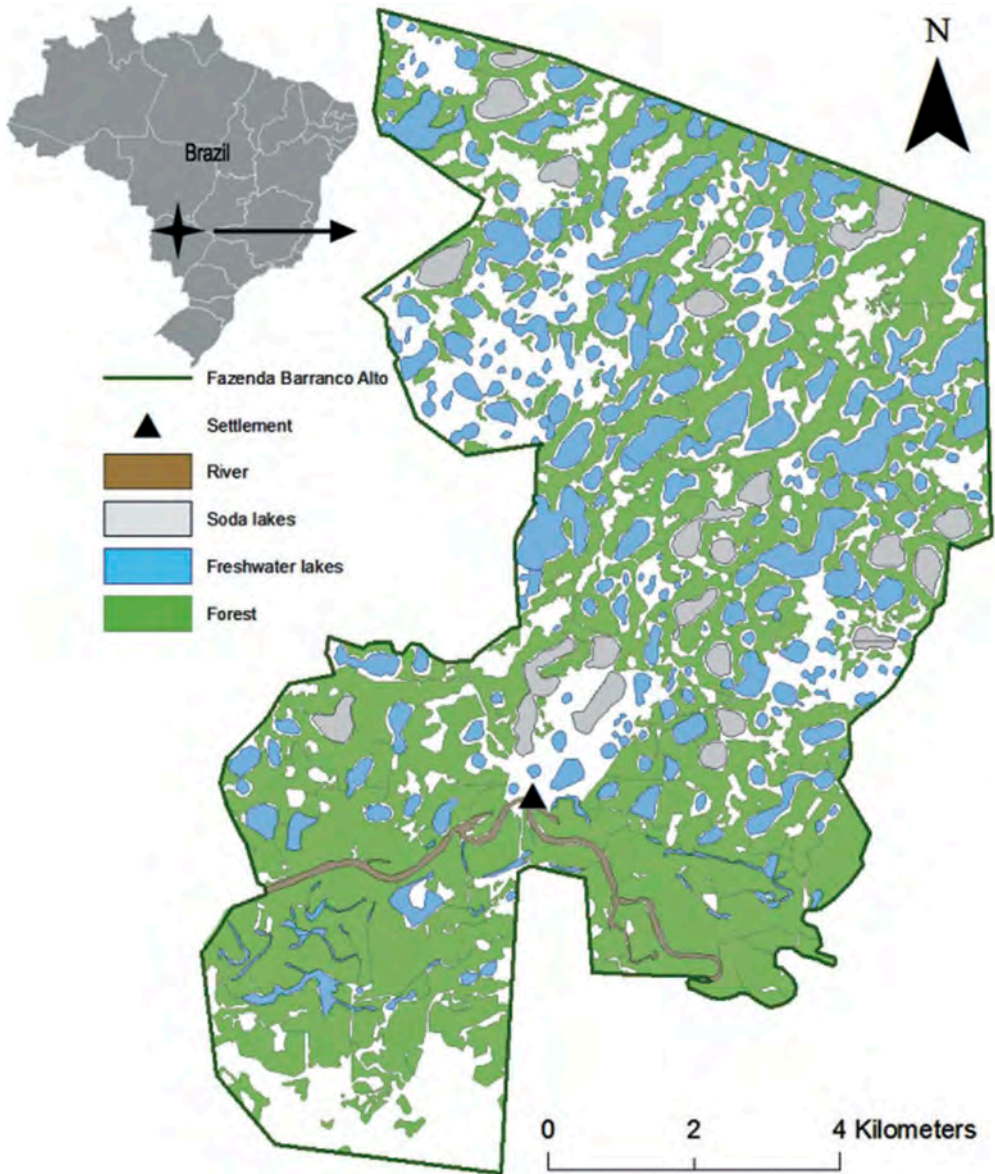


Figure 1. Map of the study area, Fazenda Barranco Alto in the Southern Pantanal. The inset indicates its location in Brazil.

impact on body shape and there is no information on whether hair length of adult giant anteaters changes over a period of years.

Three giant anteater individuals were unmistakably and especially easy to recognise by a large set of unique biometric markers and scars. They served as a control for the methodology and the consistency of individual coat marking patterns over several years (Figure 3).

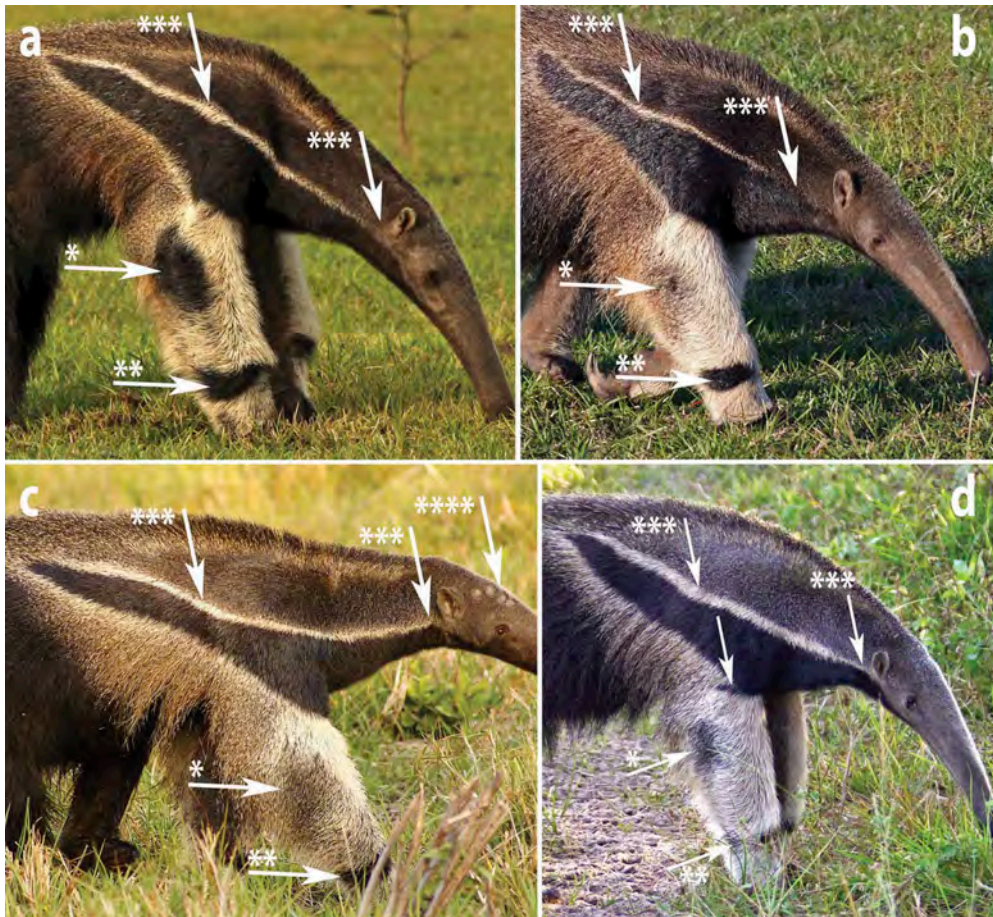


Figure 2. Example of coded photo-ID of four individual giant anteaters (*Myrmecophaga tridactyla*) sighted in the Brazilian Pantanal. For every picture the date, time and locality of the sighting were recorded. The arrows point to biometric traits that enable discrimination of individuals. These have been categorised in Table 1; stars refer to categories in this matrix (*foreleg, **bracelet, ***stripe, ****scars). As an example, the animals are here only shown from one lateral side. In practice, other photographs, preferably of both lateral sides as well as the front, were considered for the coding of the matrix and the catalogue. Photos by Lydia Möcklinghoff.

Poor-quality images could often be subsequently linked to a sighting of the same animal with better photographic documentation, when the visible portion on the picture showed one identifying feature that could be clearly discerned.

The following characteristics were found to apply for individual recognition of giant anteaters.

Coat-marking patterns

Coat-marking patterns were shown to be consistent over years (Figure 3) and laterally symmetrical. Characteristic black patches on the white foreleg were documented as the most obvious trait, followed by the considerable variation in width [< 1 cm up to $>$

Table 1. Refer to Figure 2. Matrix for photo-identification of individual free-ranging giant anteaters in the Brazilian Pantanal.

Ind.	Mat.	Cub	Stripe***					Scars****					Remarks			
			Black bracelet**		Shape	Width	Extend	Head	Legs	Rump						
			Foreleg*	0/1/2/3							s/m/l	p/o	s/m/l	1/2/3	L	R
a	A	0	3	l	p	m	2	X	0	X	X	0	X	0	Cut right	White above shoulder
b	A	0	2	m	p	s	1	X	0	X	X	0	X	0	Oval	-
c	A	0	1	m	p	l	2	X	1	X	X	0	X	0	Small dent/cut right	-
d	A	0	2	s	o	l	3	X	0	X	X	0	X	0	Round	Black tip at flag

*Black markings on the white foreleg (0 = none, 1 = shadow, 2 = black dot, 3 = black spot).

**Width and shape of the black 'bracelet' above the front paw (l = large, m = medium, s = small).

***Shape of the white colouring around the black flag at the shoulder (p = ends pointed, o = ends open); width of the white stripe above the black shoulder marking (l = large, m = medium, s = small); extent of the white stripe above the black shoulder marking (1 = ending distant to ear, 2 = ending in a pale stripe near ear, 3 = ending near ear).

****Scars (L = left, R = right, F = front, X = not photographed).



Figure 3. Example of 4 years of consistent morphologic characteristics of a giant anteater (*Myrmecophaga tridactyla*). Variations in boldness of the drop-shaped black spot are typical for varying light situations. Uncommon are some white hairs within the black flag, the white 'blob' in the stripe above it and the ear shape. Photo by Lydia Möcklinghoff in the Brazilian Pantanal.

10 cm as measured in unpublished studies of the first author in the Dortmund Zoo 2007 (n = 8), and during captures of giant anteaters in the study area in 2017 (n = 5)] and shape of the black bracelets that all giant anteaters have above their forefeet. The black 'flag pattern' on the animal's shoulder is very conspicuous. However, its shape could not be considered for individual discrimination as it was observed to be highly conditional on the animal's present posture. An applicable visual marker of this flag pattern was found to be the intensity of the white stripe above the black shoulder marking. This stripe can be bright white or faint, it can reach the ear or fade above the foreleg, and it either merges with the white area below the black flag to create a point above the

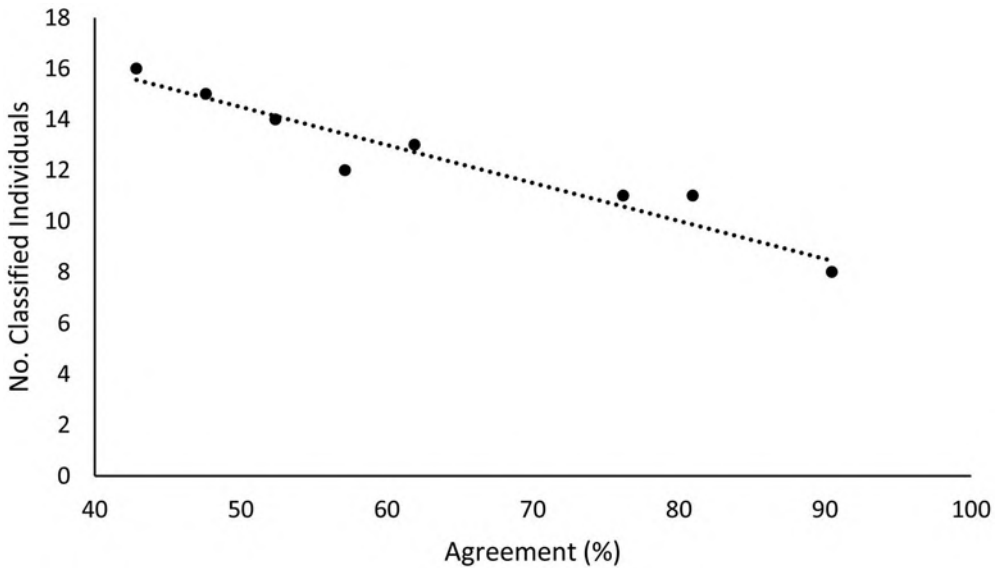


Figure 4. Relation between number of individuals classified by volunteers and grade of inter-observer agreement relative to the identity assigned to individual giant anteaters by the first author.

shoulder or it ends open (Figure 2). The shade of the fur colour was observed to vary strongly depending on light conditions and was therefore not considered for further analyses.

Scars

Scars were often present and mostly located on the forehead of giant anteaters, probably caused by the long and sharp claws of opponents during intraspecific fights.

Some scars were observed to be permanent and an obvious and important marker over many years, while others were covered by hair a certain time after the lesion. Therefore, scars could only be considered for individual recognition in consecutive sightings and/or in combination with other traits. In the double-blind trial that we conducted to verify our methodology and the recognisability of the characteristic traits presented here, the experienced researcher identified seven individuals out of the 21 provided photographs of giant anteaters, each featured in two to four sightings. The average inter-rater agreement among the nine naïve volunteers and the researcher was $68.26\% \pm 5.22\%$ with the highest concordance between researcher and two participants of 90.5% and the lowest agreement with a volunteer of 42.9%. Average inter-rater agreement among participants excluding the researcher's rating was lower, at $49.67\% \pm 4.5\%$. False mismatches made up for 87.5% of disagreement, when volunteers would not detect unique features enabling identification and would classify the specimen as an additional individual. This is reflected by the significant linear negative relationship among number of individuals and grade of agreement, tested with Pearson's correlation ($r = 0.967$, $n = 9$, $p < 0.01$; Figure 4). In conversations following

the test volunteers admitted that they forgot to pay attention to some features even though these were explained in the tutorial. Only 4 times did false matches occur, when volunteers assigned two anteaters as one individual that were associated by the researcher to two different individuals. Some individuals showed more distinct features and were significantly easier to classify, as determined by a one-way ANOVA ($F(6,14) = 13,76, P < 0.01$) that compared average agreement among the seven individuals assigned by the first author. Image quality was shown to have no impact on grade of agreement ($F(3,17) = 0.474, P = 0,71$).

Discussion

Our study shows that identifying individual giant anteaters on the basis of natural pelage marks is possible, even though some experience and routine is required, as characteristic fur patterns are subtler than stripe patterns in tigers (Karanth and Nichols 1998) or spot patterns in giraffes (Foster 1966), for instance. The technique presented here of analysing geotagged photographs by utilising a digitised identification matrix can provide conservation-relevant information about life history, such as individual movement patterns, home-range structure, social organisation, reproduction and behaviour (Moecklinghoff et al. 2014). It is thus an easy and non-invasive alternative or enhancement to invasive capturing methods that cause stress and may influence the life history and natural behaviour of the individual (Mech and Barber 2002; McMahon et al. 2011). The basic methodology applies to a large array of study designs that include photographs of giant anteaters. It can be adopted in future studies as well as in ongoing research or can be used to obtain additional information from older data sets.

Limitations of the technique were the low proportion of camera trap photographs that allowed identification of coat marking patterns, the high expenditure of time the analysis consumed and the significant overrating of population size by naïve volunteers, when experience and intensive training is lacking.

It should be tested whether quality of camera trap footage can be enhanced by using other camera trap types with video capture, incandescent flash at night or higher sensitivity, or by changing the camera-trap setup to a paired camera-trap setting on both sides of trails (Trolliet et al. 2014).

To reduce the time effort of data analysis, we found the free online software and smartphone application Cyber Tracker (www.cybertracker.org) to be very helpful for field data collection, as it allows instant data management and processing during *in situ* sightings of giant anteaters. Cyber Tracker was developed to record data by animal trackers in Africa (Liebenberg et al. 1999; Ansell and Koenig 2011). It is a PDA-based application, enabling the setup of customised data bases. In our case it was used to transform the identification matrix with the biometric traits of giant anteaters, identified here, into an application. This application can be installed to an open number of smartphones and can be directly accessed in the field for a pre-sorting of photographs and sightings. The identified biometric traits in combination with the smartphone application can also serve as the base for future participatory citizen science projects, as Cyber Tracker was initially designed for data collection by indigenous communities and is easy to handle.

Participatory research methods and community-based conservation efforts are often vital factors in field work, as local communities have higher stakes than the state in

preserving their natural resources and better knowledge of local resources (Altrichter 2008). They can therefore add important local knowledge to science and support data collection by extending the capacities of a project (Calheiros et al. 2000; Metha and Heinen 2001). Involving locals can also raise awareness and acceptance of research and conservation efforts, which is of special interest in the Brazilian Pantanal, where our study took place. Ninety-eight percent of the region is privately owned by cattle ranchers, with extensive cattle ranching being the most important source of income. As people, cattle and wildlife are living side by side in this Biosphere Reserve and cattle ranchers are in full control over what happens on their land, close cooperation with locals and community-based conservation and research measures are indispensable. In our last few years of *in situ* research in the Pantanal, our easy, appealing method to learn ‘who is who’ in the anteater community has already piqued the local people’s interest and can be a door opener for communication of nature and wildlife conservation issues. Since the first author explained at the local school (Escola do Rio Negro) in 2016 how to distinguish individuals of giant anteaters, the schoolchildren are running their own catalogue with the fur patterns of anteater individuals living around their school building. This example demonstrates the participatory and educational potential of our method. Combining a PDA- and smartphone-based application for participatory research like Cyber Tracker with our technique presented here enables the coordination and evaluation of the efforts of locals and volunteers to capture geotagged photographs of giant anteater sightings and sort them according to biometric traits, as preparation for a final analysis by an experienced researcher. A test of this community-based research approach is being implemented but can potentially be extended to other areas and projects in Brazil in the future.

It should also be tested in the future whether the identified biometric traits of giant anteaters and the existing photographic database can be used to develop a semi- or fully automated computational recognition procedure. Such photo-matching software has already been developed and implemented in numerous projects and species. A semi-automated approach that helps to analyse the borders of ears, for example, exists for African elephants (Andorvini et al. 2007), while a fully automated system uses the chest patterns of black feathers as an identifier for African penguins in a large colony (Burghardt et al. 2004; Sherley et al. 2010). The (semi-)automatic computer-based identification of giant anteaters could enhance time-consuming human-made photo recognition and thus potentially enable the analysis of larger populations and habitats.

Conclusion

We have shown that there is now a working approach to identify giant anteaters by coat marking patterns and other biometric traits. The identified individual biometric traits can be the basis for a variety of study designs, analyses and application possibilities. We will use our results for population studies, behavioural observations and studies on the life history of giant anteaters in our study area. The work flow with a manual setup of an identification matrix is, however, very time consuming, and thus is only applicable to smaller populations, and dependent on experienced researchers. Therefore, our Computational Bioacoustics Research Unit (CO.BRA, <https://www.cobra.ic.ufmt.br>), an interdisciplinary scientific network based at the Federal University of

Mato Grosso (UFMT), is planning to use our photo databases to develop photo-matching software (automated image recognition). Automatisation would enable the study of larger populations of giant anteaters. Also, it should be tested whether and how coat marking patterns and other biometric traits of young giant anteaters change when they grow up.

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