

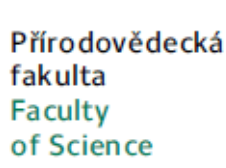
Defining Management Units for European Captive Aardvarks



Ludmila Pohlová,^{1*} Pauline Schepsky,² Thomas Lehmann,³ Axel Hochkirch,² Renata Masopustová,⁴ Jaroslav Šimek,⁵ Wineke Schoo,⁶ Roman Vodička,⁵ and Jan Robovský¹

¹Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
²Department of Biogeography, Trier University, Trier, Germany
³Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt am Main, Germany
⁴Department of Animal Science and Ethology, Faculty of Agrobiological Sciences, University of Life Science Prague, Prague, Czech Republic
⁵Zoological Garden Prague, Prague, Czech Republic
⁶Burgers' Zoo, Arnhem, The Netherlands

*Correspondence to: L. Pohlová, Department of Zoology, Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic.
E-mail: lideila@seznam.cz



Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

INTRODUCTION

The **Aardvark** (*Orycteropus afer*) is a very unique, but relatively widespread African mammal. Although some morphological variation has been observed between forest and savannah populations and among different African regions, they are all considered as **a single species**. However, no modern taxonomic revision is available. All **captive aardvarks in Europe** are believed to stem from wild born animals from **Namibia** [Schoo, 2008, 2009], but recently several new wild-caught aardvarks from **Tanzania** have been integrated into the captive population [Parys et al., 2012]. This raises the question, whether these specimens should be **interbred** with the existing captive population or whether there is a risk of **outbreeding depression**. In order to avoid negative effects of outbreeding, it is important to identify appropriate **management units** [MU] that should be based upon a large number of purebred individuals [Frankham et al., 2002]. We studied the genetic structure of the captive populations by sequencing two mitochondrial genes (**cytochrome *b*** and **16S rRNA**) to assess the degree of genetic differentiation between the two source regions.

MATERIALS AND METHODS

To examine the genetic structure of European captive population of aardvarks, **hair, tissue, and blood samples** were collected from 21 alive and seven dead individuals, **28 in total**, out of the 45 aardvarks kept in Europe [International Species Information System ISIS, September 2013, <http://www.isis.org/>]. Among these, four were wild born individuals from **Tanzania**, whereas the remaining ones were captive-born individuals, which are believed to stem from **Namibian** wild born founders (for details see **Table 1**).

TABLE 1. List of voucher specimens included in the present study, with their origin (Tanzania or Namibia), detected haplotype for cyt <i>b</i> and 16S, studbook number (European studbook; ESB), name or local number, sex and current location (as of December 31, 2012) [Schoo, 2013]						
Voucher number [origin]	Cyt <i>b</i> haplotype	16S haplotype	ESB	Name (local number)	Sex	Location
1_Tanzania	Haplotype 6 ^f	Haplotype 1 ^a	181	In nominate (loc. 209080)	M	Wroclaw Zoo
2_Tanzania	Haplotype 7 ^h	Haplotype 1	187	In nominate (loc. 209081)	M	Wroclaw Zoo
3_Tanzania	Haplotype 8 ^b	Haplotype 2 ^b	194	Ermine	F	Frankfurt n./M. Zoo
4_Tanzania	Haplotype 4	Haplotype 1	195	Ernst	M	Frankfurt n./M. Zoo
5_Namibia	Haplotype 4 ^d	Haplotype 1	72	Tata	M	Prague Zoo [†]
6_Namibia	Haplotype 1 ^a	Haplotype 1	180	Draco	M	Prague Zoo
7_Namibia	Haplotype 1	Haplotype 1	44	Myška	F	Kessingland Zoo
8_Namibia	Haplotype 1	Haplotype 1	74	Dudley	M	Prague Zoo [†]
9_Namibia	Haplotype 1	Haplotype 1	156	Danny	M	Bioparc Valencia Rainforest
10_Namibia	Haplotype 1	Haplotype 1	159	Quote	F	Prague Zoo
11_Namibia	Haplotype 2 ^b	Haplotype 1	161	Snuggy	F	Faunia Zoo Madrid
12_Namibia	Haplotype 1	Haplotype 1	177	Bovenkantje	F	Burgers' Zoo Arnhem
13_Namibia	Haplotype 1	Haplotype 1	103	Oryc	F	Burgers' Zoo Arnhem
14_Namibia	Haplotype 2	Haplotype 1	98	Snuffy	F	Burgers' Zoo Arnhem
15_Namibia	Haplotype 1	Haplotype 3 ^c	145	Irmo	M	Burgers' Zoo Arnhem
16_Namibia	Haplotype 2	Haplotype 1	179	Henkjan	M	Bioparc Valencia Rainforest
17_Namibia	Haplotype 1	Haplotype 1	190	In nominate (loc. 615782)	F	Burgers' Zoo Arnhem [†]
18_Namibia	Haplotype 1	Haplotype 1	170	In nominate (loc. 613151)	F	Burgers' Zoo Arnhem [†]
19_Namibia	Haplotype 2	Haplotype 1	189	In nominate (loc. 615648)	F	Burgers' Zoo Arnhem [†]
20_Namibia	Haplotype 2	Haplotype 1	162	In nominate (loc. 612518)	F	Burgers' Zoo Arnhem [†]
21_Namibia	Haplotype 2	Haplotype 1	172	Misha	F	Zoological Society of London
22_Namibia	Haplotype 3 ^c	Haplotype 1	147	Oq	F	Colchester Zoo
23_Namibia	Haplotype 3	Haplotype 1	151	Plucky	M	Berlin Zoo
24_Namibia	Haplotype 3	Haplotype 1	144	Curly	F	Antwerp Zoo
25_Namibia	Haplotype 1	Haplotype 1	39	Okahandja	F	Burgers' Zoo Arnhem [†]
26_Namibia	Haplotype 1	Haplotype 1	140	Puq	F	Colchester Zoo
27_Namibia	Haplotype 1	Haplotype 1	41	Pieta	F	Prague Zoo
28_Namibia	Haplotype 5 ^e	Haplotype 2	127	Quiggly	M	Kessingland Zoo

GenBank accession number (NCBI; The National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>) for cytochrome *b* haplotypes: KF984292 (a), KF984293 (b), KF984294 (c), KF984295 (d), KF984296 (e), KF984297 (f), KF984298 (g), KF984299 (h), and 16S rRNA haplotypes: KF984300 (a), KF984301 (b), KF984302 (c); (†) deceased animals.

- **DNA extraction**
 - JETQUICK Tissue DNA Spin Kit (Genomed)
- **PCR amplification** (primers)
 - **Cyt *b***: L14724, L14841, and H15915 [Kocher et al., 1989; Irwin et al., 1991]
 - **16S**: 16sAL and 16sBH [Palumbi, 1996]
- **PCR products purification**
 - JETQUICK PCR Purification Spin Kit (Genomed)
- **Sanger Sequencing**
 - The Laboratory of Genomics, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice
- **Editing of sequences** - GenBank, SeqMan, BioEdit, Geneious, Muscle, or Mafft
- **Phylogenetic analyses** - Winclada, PAUP, ModelTest, MrBayes, TCS, MEGA
 - maximum parsimony, Bayesian interference, haplotype network

RESULTS

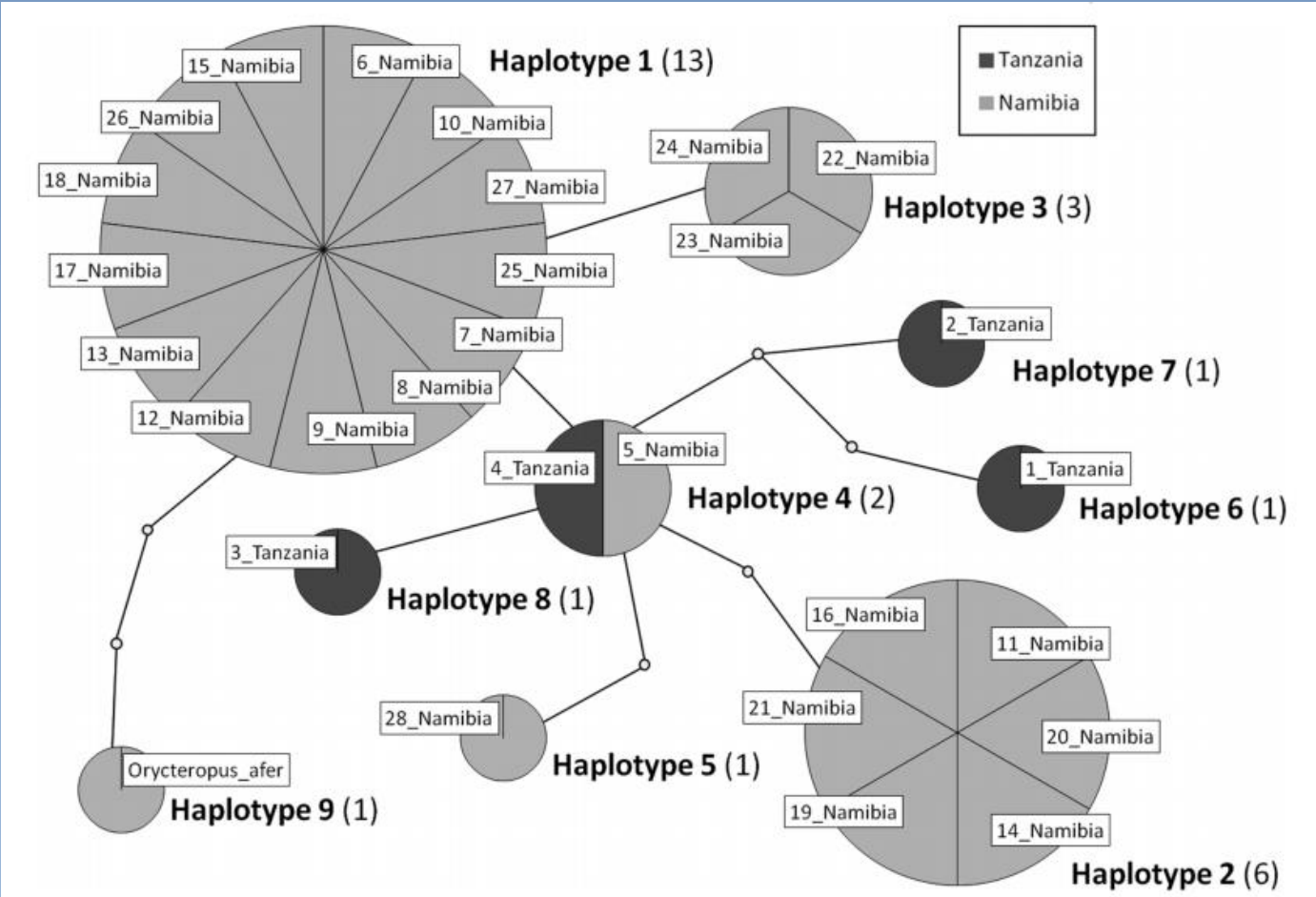


Fig. 1. Haplotype network (TCS) of cyt *b* with frequencies for each haplotype. Each line connecting circle indicates one base pair substitution. Small circles indicate hypothetical internodes.

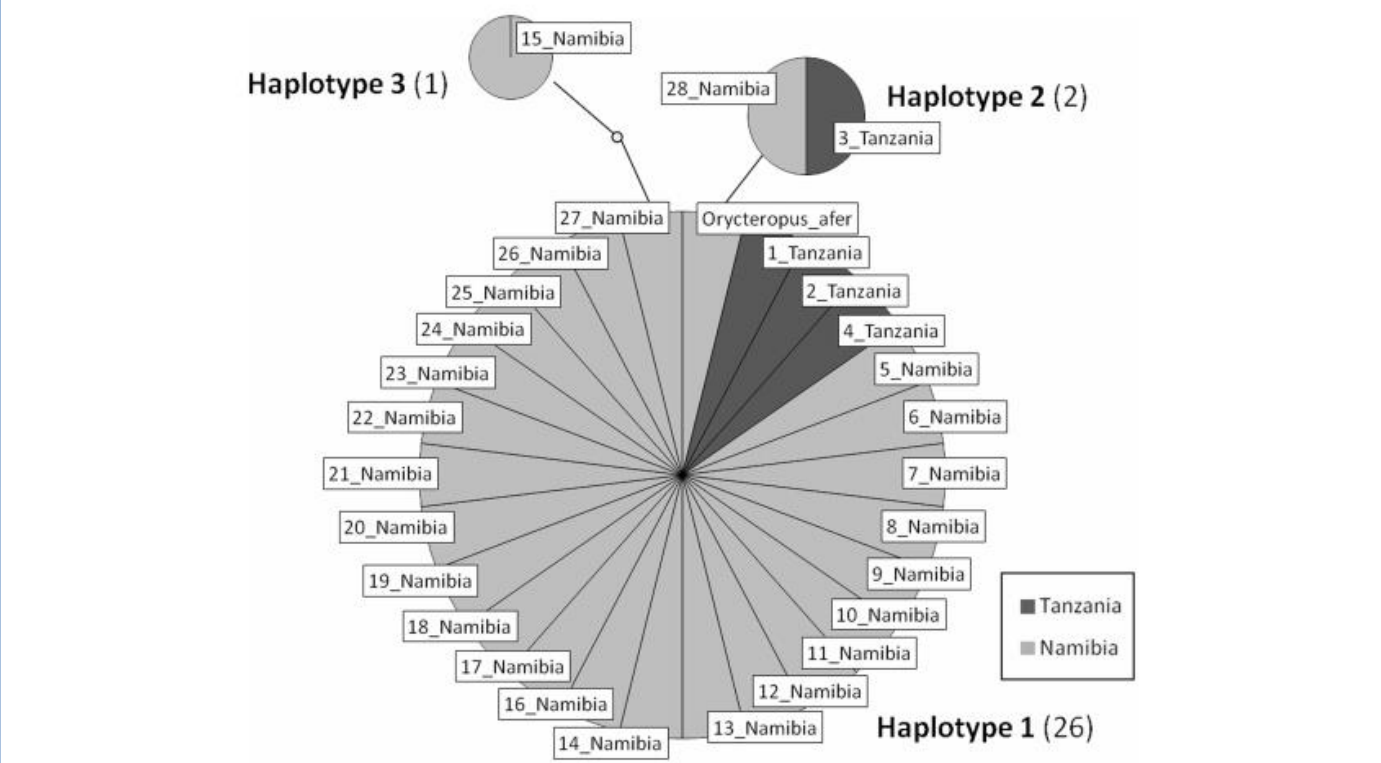


Fig. 2. Haplotype network (TCS) of 16S with frequencies for each haplotype. Each line connecting circle indicates one base pair substitution. Small circles indicate hypothetical internodes.

DISCUSSION

Our data suggests that the aardvarks kept in European zoos belong to the **same phylogenetic (mitochondrial) lineage** as the differentiation of the two studied mitochondrial markers was extremely low. All detected genetic distances of cyt *b* were significantly **lower than 2%**, which is considered by Bradley and Baker [2001], for the same gene and under the same model, to be a criterion marking the boundary between intraspecific and subspecies level. In spite of a geographic distance of approximately 1,200 km, our results suggest that at least some Tanzanian aardvarks could have had rather **recent contacts** with Namibian ones.

PERSPECTIVE

A more comprehensive analysis of a **larger sample with well documented origin** (covering the complete geographic range) and with **more sensitive genetic markers** (for example, the mitochondrial control region, MHC, microsatellites) is needed to infer any final conclusions concerning the aardvark's taxonomy and identification of suitable aardvark management units. Therefore, we **do not recommend mixing** Namibian and Tanzanian individuals until more extensive studies have been conducted.

ACKNOWLEDGEMENTS

We would like to acknowledge the Ministry of Education, Youth and Sports of the Czech Republic (Grant N. 6007665801 to J.R.), Grant Agency of the University of South Bohemia in České Budějovice (Grant N. 04-049/2013/Z to L.P.), as well as the Nature and Biodiversity Conservation Union (NABU-Saarbrücken Naturschutzfonds to P.S.) for funding. We also wish to thank Lubomir Piálek (Department of Zoology, University of South Bohemia) for technical help and advices. Finally, we are indebted to all aardvarks for their stoic collaboration during sample collections.

REFERENCES

- Bradley RD, Baker RJ. 2001. A test of the Genetic Species Concept: cytochrome *b* sequences and mammals. J Mammal 82:960–973.
- Frankham R, Ballou JD, Briscoe DA. 2002. Introduction to conservation genetics. Cambridge: Cambridge University Press. 640p.
- Irwin DM, Kocher TD, Wilson AC. 1991. Evolution of the cytochrome *b* gene of mammals. J Mol Evol 32:128–144.
- Kocher TD, Thomas WK, Meyer A, et al. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86:6196–6200.
- Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, editors. Molecular systematics. 2nd edition. Sunderland, MA: Sinauer and Associates. p 205–247.
- Parys A, Lehmann T, Shoo W, Wilms TM. 2012. Newcomers enrich the European zoo aardvark population. Afrotherian Conser 9:2–5.
- Schoo W. 2008. European studbook for the aardvark (*Orycteropus afer*). Arnhem: Burgers' Zoo. 36p.
- Schoo W. 2009. European studbook for the aardvark (*Orycteropus afer*). Arnhem: Burgers' Zoo. 10p.

• Pohlová L, Schepsky P, Lehmann T, Hochkirch A, Masopustová R, Šimek J, Schoo W, Vodička R and Robovský J. 2014. Defining Management Units for European Captive Aardvarks. Zoo Biol. (DOI: 10.1002/zoo.21164).