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RESOURCES

Isolation and characterization of 30 STRs in Temminck's ground pangolin (*Smutsia temminckii*) and potential for cross amplification in other African species

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Abstract. Temminck's ground pangolin (*Smutsia temminckii*) is one of four species of pangolin, endemic to Africa. Two of the African pangolin species are listed as vulnerable and two are listed as endangered on the International Union for Conservation of Nature Red List of Threatened Species due to their ongoing exploitation for traditional medicine and bushmeat. In this study, we developed 30 species-specific short-tandem repeats (STRs) in Temminck's ground pangolin using next-generation sequencing. The markers were also optimized for cross-amplification in other African species. All the markers amplified successfully in Temminck's ground pangolin with allelic polymorphisms observed in 87% of the markers in giant pangolin (*S. gigantea*) whereas 60% of the markers were amplified polymorphic loci in both white-bellied pangolin (*Phataginus tricuspis*) and black-bellied pangolin (*P. tetradactyla*). Analysis of diversity estimates showed moderate levels of variability in Temminck's ground pangolin (N_a = 5; H_o = 0.559), giant pangolin (N_a = 4.909; H_o = 0.514) and white-bellied pangolin (N_a = 2.686; H_o = 0.541) with lower values being observed in black-bellied pangolin (N_a = 3; H_o = 0.242). This study provides data of the first available STR markers which was amplified in all four African pangolin species that can now be used in conservation genetic and evolutionary aspects of population histories.

Keywords. microsatellites; African pangolin; Temminck's ground pangolin; Smutsiinae; *Smutsia temminckii.*

Introduction

Pangolins, a member of the order Pholidota, currently includes eight extant species that are broadly grouped into two sub-families, Smutsiinae and Maninae (Gaudin *et al.* 2009). The sub-family Smutsiinae was ascribed to the African pangolin species consisting of two genera, *Smutsia* and *Phataginu*s while Maninae was attributed to the Asian pangolin species containing only one genus, *Manis*. The two ground-dwelling species were assigned to the genus *Smutsia*, which comprises Temminck's ground pangolin (*Smutsia temminckii*) and the giant ground pangolin (*S. gigantea*). The two arboreal species were allocated to the genus *Phataginu*s, which comprises the black-bellied pangolin (*Phataginus tetradactyla*) and white-bellied pangolin (*P. tricuspis*) (Gaudin *et al.* 2009; Du Toit *et al.* 2017).

Despite the endangered status of pangolins, as a result of the illegal trade in large numbers, limited molecular genetic

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Zelda Du Toit and Desiré L. Dalton designed and conducted the experiments, coanalysed the data, wrote the paper and reviewed drafts of the paper. Morné Du Plessis coanalysed the data and reviewed drafts of the paper. Antoinette Kotzé conceived the idea of the research, ensured financial support to execute the study and critically revised the paper. Raymond Jansen provided financial support through the African Pangolin Working Group for sample collection by Maxwell Boakye and reviewed drafts of the paper. J. Paul Grobler reviewed drafts of the paper.

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studies have been conducted on the four African species. One of the principal reasons is being secretive and elusive nature, making difficult to obtain samples. Some of the threats include electrocution on game farms in southern Africa, use in traditional medicine and the illegal wildlife trade (Soewu and Adekanola 2011; Pietersen *et al.* 2014a). In 2014, the estimated illicit trade of pangolins was calculated to exceed a million individuals during the last decade, with more than 10,000 of the respective species being traded annually (Challender *et al.* 2016). In light of the increase in illegal trade, the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species agreed to up list all the extant pangolin species. In 2014, the Asian pangolin species were up listed to critically endangered (*Manis pentadactyla* and *M. javanica*) and endangered (*M. crassicaudata* and *M. culionensis*), while the African pangolins were assessed as vulnerable (*S. temminckii*, *S. gigantea*, *P. tetradactyla* and *P. tricuspis*) (Pietersen *et al.* 2014c; Waterman *et al.* 2014a,b,c). In 2019, a review of the Red List status was undertaken and *S. gigantea* and *P. tricuspis* were uplisted to endangered (Nixon *et al.* 2019; Pietersen *et al.* 2019). In addition, the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) elevated all extant pangolin species to appendix I at the CoP17 meeting held in 2016 (IUCN 2016).

Pertaining to the African pangolin species, Temminck's ground pangolin has an extensive recorded distribution range along with being the only species which occur in southern Africa below –20° latitude (Pietersen *et al.* 2014b). The range of countries include Chad, Central African Republic, South Sudan, Uganda, Kenya, Rwanda, Tanzania, Zambia, Malawi, Mozambique, Zimbabwe, Botswana, Namibia and South Africa (Pietersen *et al.* 2014c). Temminck's pangolin is considered to be an adapatable species as it developed the potential to inhabit a variety of niches, however this is dependant on the availability of their preffered food source of selected ants and termite species. These habitats incluce savannah, woodland, floodplain grassland, rocky slopes and sandveld areas (Pietersen *et al.* 2014c, 2016b; Elsner *et al.* 2016). Being a ground-dwelling species, they are mostly absent in habitats such as karroid, highveld grassland, coastal regions, tropical and coastal forests (Pietersen *et al.* 2014c). The mounting pressure of pangolin populations due to illicit trade and poaching (Heinrich *et al.* 2016; Pietersen *et al.* 2016a) prompted us to find suitable genetic techniques to aid forensic

Table 1. Primer sequences of 30 STRs isolated for Temminck's ground pangolin, *S. temminckii*.

Locus	Primer sequence forward (5′–3′)	Primer sequence reverse (5′–3′)	Repeat motif	Annealing temp (°C)
PAN_01	CAGCTGAACACTGAACACAGAA	AGAGCAAGGCAGAGCATTTG	$(GGT)_{12}$	55
PAN_02	TAGAGGCTGTGAGCAGGTTC	GAGGGGATTATGCCAGGAAG	$(TCC)_{9}$	48
PAN_03	ACTTTCCATCCCAAGCCTCT	GGTGGAGAGCGCTAATCAGT	$(GA)_{13}$	48
PAN_05	TTTTCCCCTGTGGAATGTGT	TTTGCGTAAAGTCCCTAGCA	$(GAT)_{11}$	48
PAN_06	CACACAGTAGGGCTCTGCAA	CAACACATCACCTGACAGCA	$(TGAA)_{10}$	48
PAN_07	CTTCTCCCACCTGACCTCCT	CACTTAAGATTTTGGGGCTCA	$(CT)_{15}$	48
PAN_08	CCTCCAGTGTATGTGCGAGT	CCACGGCAAGAACCATTC	$(CA)_{15}$	48
PAN_10	AAAATGGACACAGAGGCATT	ACATTGTGATGTGGACCAGTG	$(TC)_{14}$	48
PAN_11	ACTCGGGTTGTGGGAACAT	CCAGTCCTTGCTGCTCCTAT	$(GT)_{13}$	55
PAN_12	TAGTTCACACACATGGGAAGTT	TGTTATCCTTGCCTCCTGACTT	$(GGA)_{9}$	48
PAN_13	AGCTCCCTGAAGAACCTGCT	CTAGTGCCTGGCCCAACA	$(TCCA)_{9}$	50
PAN_15	GTCCTCAAACTCCTCCGACA	CTGGGGTCCTGTCATCCTG	$(CTC)_{9}$	50
PAN_17	CCCTGTGAAAAATGGAGACC	CTGGTGGCATTATAAGCCTTG	$(GT)_{15}$	45
PAN_18	GCACCTGTGGGTAGTTCCAT	GCATATGGACAAGGCAGGAG	$(CT)_{13}$	55
PAN_19	GAGCATCTATGTTTAGACTGTTCTGC	TGGGCAGAGACCAAGACTGT	$(GT)_{15}$	50
PAN_20	TTTGGGCACAAGTGATCCTA	GAGTCAAGACAGGGCCAGTG	$(AC)_{14}$	50
PAN_21	AGACGGAAGCCCAAGAGG	CCAGAAATGGAAGTTCAGTCC	$(CAC)_{9}$	50
PAN_23	TTCCCATTATGCGACTAGGA	TGCCCCAAATCCAATACTGA	$(GAG)_{9}$	55
PAN_24	AGAAAGGTTCTTGGGGCATT	AGACCATAAGCTCCCTGAAGAA	$(TGGA)_{12}$	55
PAN_26	GGAGATACCAAGGCAAAAAGAA	GGACCATCTGGTCAAATGAGA	$(AG)_{14}$	55
PAN_27	GCTGTTGGCTGGTTACAGGT	CTATGTGTGGGTGTGGGTGT	$(CA)_{13}$	55
PAN_28	CCACCTTTAACCTCAGGGCTA	CACTCCCCTTAACCCATCAA	$(TGAA)_{11}$	50
PAN_29	ACCTTACGCACCTGGAAGAC	AATTCTTGCCTCAGCTCTGC	$(GT)_{17}$	45
PAN_30	TCATATTCATGTGATGTGATGAGG	AAGGCTGCTGCGGTAGAAC	$(GAG)_{8}$	45
PAN_31	CTTCAGTCATCCGTCCGTCT	CACTTGACACAGTGCCTTGC	$(TCCA)_{8}$	45
PAN_32	TCTGAATGATGCCAAGGAGA	TGGCTCTGCAGATTTTCTCA	$(TG)_{14}$	45
PAN_35	GCCTACACTTGCGTGTGAAA	TTCCATCGGTGTGATGATTG	$(CA)_{13}$	55
PAN_36	GGCAGACTTTACCAATGAAGC	AACCATAATGTTGATCATGTAATTG	$(TTG)_{8}$	55
PAN_37	GTCTTCAGCCCACAGTCAGC	TCAAGCTTTCAATAGTCGTCCA	$(TGGA)_{10}$	55
PAN_38	GAAGCCAACAATATCCAATGC	TCGTATTCTCTCAGATAACCCTTTG	$(AC)_{17}$	55

F, forward primer; R, reverse primer.

investigations. Short-tandem repeats (STRs) are considered to be the preferred marker in studies such as assessing divergent lineages and evaluating population genetic structure which can subsequently contribute to conservation management (Ellegren 2004; Luo *et al.* 2007). To date, STR markers have been developed for the Malayan pangolin, *M. javanica* (Luo *et al.* 2007), which displayed cross-species amplification in Chinese pangolin (*M. pentadactyla*) and white-bellied pangolin (Luo *et al.* 2007). However, no STR markers are reported to amplify for all the African species. The aim of this study was to isolate and characterize the polymorphic STRs for Temminck's ground pangolin using next-generation sequencing (NGS) and to determine the cross-amplification potential in the remaining species (*S. gigantean*, *P. tetradactyla* and *P. tricuspis*).

Materials and methods

This study included samples from 32 Temminck's ground pangolins (South Africa), nine black bellied (Ghana) 10 white bellied (Ghana) and 10 giant ground pangolins from a confiscated batch from Hong Kong with an unknown origin. All samples were stored at the NZG Biobank, National Zoological Garden (NZG). DNA was isolated from tissue or blood samples using the QIAamp Micro kit (Qiagen, Novato, USA) along with the respective manufacturers' protocol. The markers were submitted to GenBank with accession numbers MN477255–MN477284. Scale samples were processed according to the protocol of Du Toit *et al.* (2016) using the PrepFiler Forensic DNA Extraction kit (Thermo Scientific, Massachusetts, USA) following the manufacturer's protocol for hair samples. The DNA was quantified on a Qubit v3.0 Fluorometer (Thermo Scientific). One Temminck's ground pangolin blood sample was used for NGS and a rapid run was performed on the Illumina HiSeq 2500 (Illumina Incorporated, San Diego, USA) using the TruSeq DNA LT Sample Prep kit (Illumina Incorporated). The quality of the data was evaluated using the software package FastQC v0.11.7 (Andrews 2010) and Trimmomatic v0.36 (Bolger *et al.* 2014) was implemented to edit and trim the data by removing poor quality sections and adapters. The program tandem repeats finder v4.09 (Benson 1999) was utilized to identify regions comprising dinucleotide, trinucleotide and tetranucleotide repeats of $\geq$8 repeats in length. The unique loci, consisting of dinucleotide,

Table 2. Characterization of 30 STRs in Temminck's ground pangolin, *S. temminckii*.

Locus	GenBank accession number	Size range (bp)	N_a	H_e	H_o	HWE	Null alleles
PAN_01	MN477255	69–118	5	0.813	0.552	***	None
PAN_02	MN477256	85–139	2	0.344	0.285	ns	None
PAN_03	MN477257	89–121	7	0.688	0.713	ns	None
PAN_05	MN477258	98–113	4	0.531	0.546	ns	None
PAN_06	MN477259	97–130	4	0.719	0.655	ns	None
PAN_07	MN477260	91–117	4	0.969	0.558	***	None
PAN_08	MN477261	88–114	5	0.438	0.576	ns	None
PAN_10	MN477262	84–106	3	0.313	0.366	ns	None
PAN_11	MN477263	119–187	5	0.844	0.734	ns	None
PAN_12	MN477264	89–122	5	0.531	0.418	ns	None
PAN_13	MN477265	87–123	5	0.742	0.748	ns	None
PAN_15	MN477266	105–117	3	0.313	0.382	ns	None
PAN_17	MN477267	131–187	12	0.625	0.729	***	None
PAN_18	MN477268	99–121	7	0.844	0.772	***	None
PAN_19	MN477269	105–189	4	0.774	0.674	ns	None
PAN_20	MN477270	96–146	8	0.688	0.688	ns	None
PAN_21	MN477271	97–129	2	0.156	0.144	ns	None
PAN_23	MN477272	111–122	3	0.313	0.389	***	None
PAN_24	MN477273	77–116	5	0.844	0.759	ns	None
PAN_26	MN477274	91–142	4	0.594	0.534	ns	None
PAN_27	MN477275	93–124	9	0.793	0.719	ns	None
PAN_28	MN477276	109–157	3	0.344	0.330	ns	None
PAN_29	MN477277	118–142	5	0.844	0.720	ns	None
PAN_30	MN477278	86–106	2	0.219	0.285	ns	None
PAN_31	MN477279	72–136	2	0.500	0.430	ns	None
PAN_32	MN477280	61–103	2	0.438	0.451	ns	None
PAN_35	MN477281	96–136	7	0.733	0.820	*	None
PAN_36	MN477282	63–111	2	0.000	0.117	***	None
PAN_37	MN477283	86–120	2	0.406	0.359	ns	None
PAN_38	MN477284	80–120	5	0.500	0.599	ns	None

N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; HWE, significant deviation from Hardy–Weinberg proportions; ns, not significant. *P<0.05, ***P<0.001.

Table 3. Cross-species amplification of 30 STRs for African pangolins.

	P. tetradactyla		*P. tricuspis*		*S. gigantea*	
Locus	Amplification	HWE	Amplification	HWE	Amplification	HWE
PAN_01	√	ns	√	ns	√	ns
PAN_02	M		M		√	*
PAN_03	M		√	***	√	ns
PAN_05	M		M		M	
PAN_06	√	ns	√	**	M	
PAN_07	√	***	M		√	**
PAN_08	√	**	√	**	√	ns
PAN_10	√	**	M		√	ns
PAN_11	M		√	ns	√	*
PAN_12	M		M		√	ns
PAN_13	M		√	ns	M	
PAN_15	M		√	*	√	ns
PAN_17	√	ns	√	ns	√	ns
PAN_18	√	**	M		√	ns
PAN_19	√	ns	√	*	√	ns
PAN_20	M		M		√	ns
PAN_21	√	ns	√	*	√	ns
PAN_23	√	ns	√	ns	√	ns
PAN_24	M		√	ns	√	***
PAN_26	√	ns	√	ns	√	ns
PAN_27	√	ns	√	ns	√	*
PAN_28	M		√	ns	NA	
PAN_29	NA		NA		√	ns
PAN_30	√	ns	M		√	*
PAN_31	√	ns	√	ns	√	**
PAN_32	M		M		√	***
PAN_35	√	***	√	*	√	ns
PAN_36	√	ns	NA		√	ns
PAN_37	√	***	√	*	√	ns
PAN_38	√	ns	M		√	ns

HWE, Hardy–Weinberg equilibrium; ns, not significant; M, positive monomorphic amplification; √, positive polymorphic amplification; NA, no amplification. *P<0.05; **P<0.01; ***P<0.001.

trinucleotide and tetranucleotide repeats were identified and primer pair flanking regions was designed using BatchPrimer3 v1.0 (You *et al.* 2008) software. Polymerase chain reaction (PCR) amplification was performed in multiplex reactions with a total reaction volume of 12.5 μL, consisting of *Taq* DNA polymerase master mix red (Ampliqon A/S, Odense, Denmark), forward and reverse primers (0.25 μM each) from multiple loci and 50-ng genomic DNA template. The conditions for the PCR amplification were as follows: denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 45–55°C for 30 s and extension at 72°C for 1 min, followed by final extension at 72°C for 40 min. PCR products were run against a Genescan 500 LIZ internal size standard on an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA). Samples were genotyped using GeneMapper v5.0 software (Applied Biosystems). MICRO-CHECKER v2.2 (Van Oosterhout *et al.* 2004) was used to detect possible genotyping errors, allele dropout and null alleles. The number of alleles per locus (A), observed heterozygosity (H_o), and unbiased heterozygosity (H_z) were calculated with GenAlEx v6.5 (Peakall and Smouse 2012), while Arlequin v3.5 (Excoffier and Lischer 2010) was used to test for deviations from expected Hardy–Weinberg (HW) proportions in genotypes and to evaluate loci for gametic disequilibrium. Associated probability values were corrected for multiple comparisons using Bonferroni adjustment at a significance level of 0.05. Ethical approval was obtained from the Animal Research Ethics Committee, University of the Free State, South Africa (UFS-AED2015/0070) and the NZG Research Ethics and Scientific Committee (NZG/RES/P/001/F/02). All samples were stored in the NZG Biobank. Sample collection in South Africa was approved under a CPC5 permit (02437) from the Department of Agriculture and Rural Development, South Africa and a Biodiversity permit (FAUNA 714/2012) from the Department of Environmental Affairs, South Africa.

Results

The NGS rapid run generated more than 10,000 reads containing STRs of various repeat lengths, from which 40 were identified for further analysis. A total of 30 of the 40 loci

Table 4. Genetic diversity estimates across four African pangolin species based on 11 polymorphic loci.

Population	N_a	N_e	H_o	H_e	H_z
S. temminckii	5.000	2.612	0.559	0.539	0.547
P. tetradactyla	3.000	1.745	0.242	0.349	0.370
P. tricuspis	4.000	2.686	0.541	0.525	0.553
S. gigantea	4.909	3.053	0.514	0.600	0.633
Total	4.227	2.524	0.464	0.503	0.526

N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; H_z, unbiased expected heterozygosity.

tested, amplified consistently (table 1) in Temminck's ground pangolin. Annealing temperatures ranged from 45 to 55°C for all loci. The number of alleles ranged from 2 to 12, with an average of 4.4 alleles per locus. The mean H_o ranged from 0.00 to 0.968 and H_e values ranged from 0.11 to 0.75. No evidence of linkage disequilibrium was observed for the analysed loci. However, six markers deviated from HW equilibrium following Bonferroni correction, which may suggest population substructure or the presence of null alleles (table 2). Cross-species amplification was successful in the three African pangolin species using PCR conditions as described above. However, amplification was not achieved for the following markers: PAN_28 in *S. gigantea*, PAN_29 in *P. tetradactyla* and *P. tricuspis*, and PAN_36 in *P. tricuspis*. In addition, 16 (53%) of the markers were identified as monomorphic, including PAN_2, PAN_3, PAN_5, PAN_6, PAN_7, PAN_10, PAN_11, PAN_12, PAN_13, PAN_15, PAN_18, PAN_20, PAN_24, PAN_28, PAN_30, PAN_32 and PAN_38 (table 3). In total, 26 (87%) markers successfully amplified and were polymorphic for *S. gigantea* and 18 (60%) markers for *P. tetradactyla* and *P. tricuspis*, respectively. Thus in total, 11 markers were polymorphic across all four African pangolin species. Genetic diversity estimates based on these 11 markers (table 4) varied between the four African pangolin species with *S. temminckii* (N_a = 5; H_o = 0.559), *S. gigantea* (N_a = 4.909; H_o = 0.514) and *P. tricuspis* (N_a = 2.686; H_o = 0.541) displaying higher diversity in comparison to *P. tetradactyla* (N_a = 3; H_o = 0.242). These estimates however should be interpreted with caution due to differences in sample sizes of the populations.

Discussion

The STRs presented here will contribute to estimates of genetic diversity, population genetic structure and relatedness studies in African pangolin species. Cross-species amplification was achieved for all three African pangolin species. However, the number of markers in which successful polymorphic amplification was achieved was higher in the two ground-dwelling species (*S. gigantea* and *S. temminckii*) in comparison to the arboreal species, which can be attributed to the relative divergence among the two genera (Du Toit *et al.* 2017). Genetic diversity estimates were found to be low to moderate in African pangolins (0.168–0.544) in comparison to the Malayan pangolin (*Manis javanica*) (0.708; Luo *et al.* 2007).

In conclusion, we developed and optimized 30 STR markers for Temminck's ground pangolin. We also confirm cross-species amplification in the other African pangolin species with a success ranging from 60 to 87%. To our knowledge, this is the first study to describe STR markers of African pangolin species. This marker set may allow for the investigation of evolutionary history, population structure and genetic differentiation in African pangolins. Further, these markers may inform conservation strategies and aid in the forensic investigation of confiscated scale samples from African pangolins.

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References

Andrews S. 2010 FastQC: a quality control tool for high throughput sequence data. Available at http://dnacore.missouri.edu/PDF/FastQC_Manual.pdf, accessed 18 July 2016.

Benson G. 1999 Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res.* **27**, 573–580.

Bolger A. M., Lohse M. and Usadel B. 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120.

Challender D, Baillie J. E. M., Waterman C. and Shepherd C. R. 2016 On scaling up pangolin conservation. *TRAFFIC Bull.* **28**,19–21.

Du Toit Z., Grobler J. P., Kotzé A., Jansen R. and Dalton D. L. 2016 Scale samples from Temminck's ground pangolin (*Smutsia temminckii*): a non-invasive source of DNA. *Conserv. Genet. Resour.* **9**, 1–4.

Du Toit Z., Du Plessis M., Dalton D. L., Jansen R., Grobler P. J. and Kotzé A. 2017 Mitochondrial genomes of African pangolins and insights into evolutionary patterns and phylogeny of the family Manidae. *BMC Genomics* **18**, 746–758.

Ellegren H. 2004 Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.* 435–445.

Elsner J., Deschler-Erb S., Stopp B., Hofreiter M., Schibler J. and Schlumbaum A. 2016 Mitochondrial d-loop variation, coat colour and sex identification of Late Iron Age horses in Switzerland. *J. Archaeol. Sci.* **6**, 386–396.

Excoffier L. and Lischer H. E. L. 2010 Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **10**, 564–567.

Gaudin T. J., Emry R. J. and Wible J. R. 2009 The phylogeny of living and extinct pangolins (mammalia, pholidota) and associated taxa: a morphology based analysis. *J. Mammal. Evol.* **16**, 235–305.

Heinrich S., Wittmann T. A., Prowse T. A. A., Ross J. V., Delean S., Shepherd C. R. *et al.* 2016 Where did all the pangolins go? International CITES trade in pangolin species. *Glob. Ecol. Conserv.* **8**, 241–253.

IUCN 2016 What does the new trade ban mean for pangolin conservation? Available at http://www.iucnredlist.org/news/what-does-the-new-trade-ban-mean-for-pangolin-conservation, accessed 20 October 2016.

Luo S. J., Cai Q-X., David V. A., Li Z., Paolo M., Lim N. T-L. *et al.* 2007 Isolation and characterization of microsatellite markers in pangolins (Mammalia, Pholidota, *Manis* spp.). *Mol. Ecol. Notes* **7**, 269–272.

Nixon S., Pietersen D., Challender D., Hoffmann M., Godwill Ichu I., Bruce T. *et al.* 2019 Giant ground pangolin (https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T12762A123584478.en).

Peakall R. and Smouse P. E. 2012 GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**, 2537–2539.

Pietersen D., Moumbolou C., Ingram D. J., Soewu D., Jansen R., Sodeinde O. *et al.* 2019 White-bellied pangolin (https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T12767A123586469.en).

Pietersen D. W., McKechnie A. E. and Jansen R. 2014a A review of the anthropogenic threats faced by Temminck's ground pangolin, *Smutsia temminckii*, in southern Africa. *South African J. Wildl. Res.* **44**, 167–178.

Pietersen D. W., McKechnie A. E. and Jansen R. 2014b Home range, habitat selection and activity patterns of an arid-zone population of Temminck's ground pangolins, *Smutsia temminckii*. *Afr. Zool.* **49**, 265–276.

Pietersen D. W., Waterman C., Hywood L., Rankin P. and Soewu D. 2014c *Smutsia temminckii*. Available at http://dx.doi.org/10.2305/IUCN.UK.2014-2.RLTS.T12765A45222717.en, accessed 1 August 2014.

Pietersen D. W., Jansen R., Swart J. and Kotzé A. 2016a A conservation assessment of *Smutsia temminckii*. In *The Red List of Mammals of South Africa, Swaziland and Lesotho* (ed. M. F. Child, L. Roxburgh, E. Do Linh San, D. Raimondo and H. T. Davies-Mostert), pp. 1–11. South African National Biodiversity Institute and Endangered Wildlife Trust, South Africa.

Pietersen D. W., Symes C. T., Woodborne S., McKechnie A. E. and Jansen R. 2016b Diet and prey selectivity of the specialist myrmecophage, Temminck's ground pangolin. *J. Zool.* **298**, 198–208.

Soewu D. A. and Adekanola T. A. 2011 Traditional-medical knowledge and perception of pangolins (*Manis* sps) among the Awori people, Southwestern Nigeria. *J. Ethnobiol. Ethnomed.* **7**, 25–35.

Van Oosterhout C., Hutchinson W. F., Wills D. P. M and Shipley P. 2004 MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**, 535–538.

Waterman C., Pietersen D. W., Hywood L., Rankin P. and Soewu D. 2014a *Smutsia gigantea.* Available at http://dx.doi.org/10.2305/IUCN.UK.2014-2.RLTS.T12762A45222061.en, accessed 12 July 2016.

Waterman C., Pietersen D. W., Soewu D., Hywood L. and Rankin P. 2014b *Phataginus tetradactyla*. Available at http://dx.doi.org/10.2305/IUCN.UK.2014-2.RLTS.T12766A45222929.en, accessed 12 July 2016.

Waterman C., Pietersen D. W., Soewu D., Hywood L. and Rankin P. 2014c *Phataginus tricuspis*. The IUCN Red List of Threatened Species 2014:e.T12767A45223135. Available from http://dx.doi.org/10.2305/IUCN.UK.2014-2.RLTS.T12767A45223135.en, accessed 12 July 2016.

You F. M., Huo N., Gu Y. Q., Luo M., Ma Y., Hane D. *et al.* 2008 BatchPrimer3: A high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics* **9**, 253–265.

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