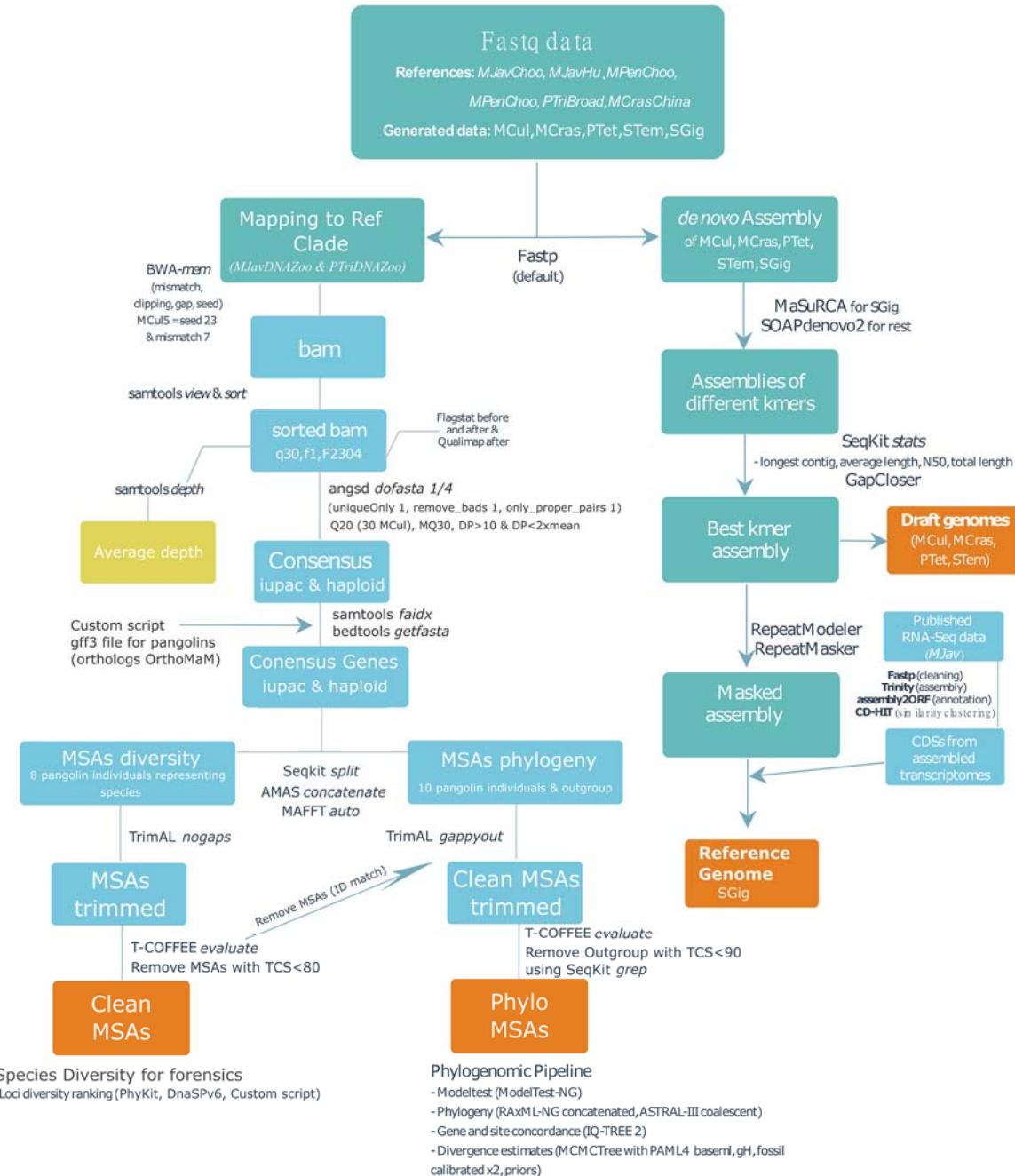
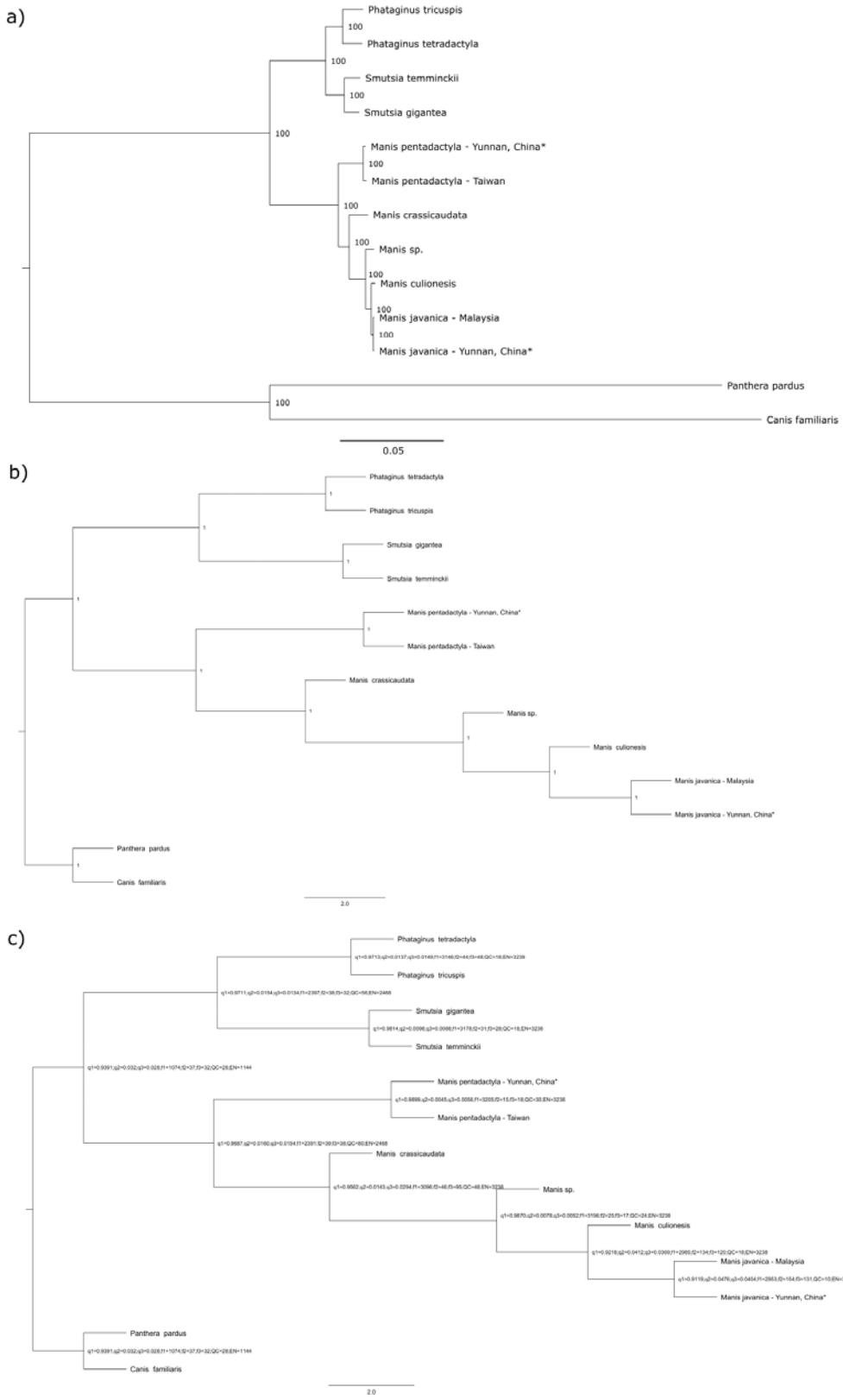


## Whole-Gene Pipeline



**Figure S1:** Orthologous whole-gene marker pipeline and subsequent analyses. This pipeline was formulated and used to create the whole-gene markers (introns and exons) required for further analyses. During this process, the reference genome (*Smutsia gigantea*) and draft genomes for four other pangolin species were created. First, the *de novo* assembly side of the pipeline (right side) was conducted before the whole genome and subsequent whole-gene extraction side of the pipeline could be conducted (left side). Custom scripts can be found at Zenodo (<https://doi.org/10.5281/zenodo.7517409>). We used shorthand notations to indicate the genomes used for each species

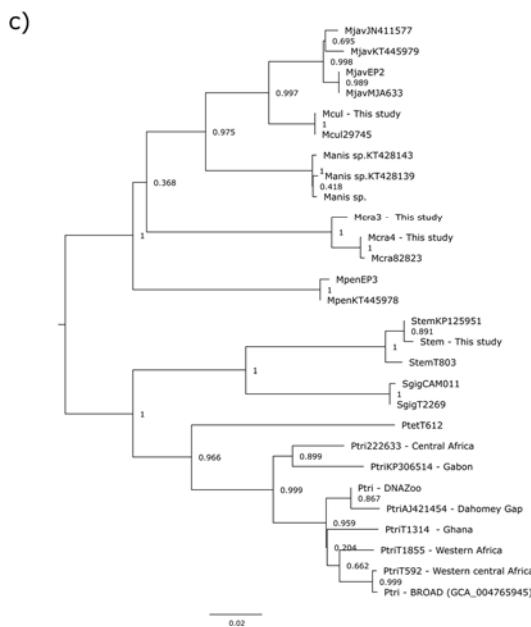
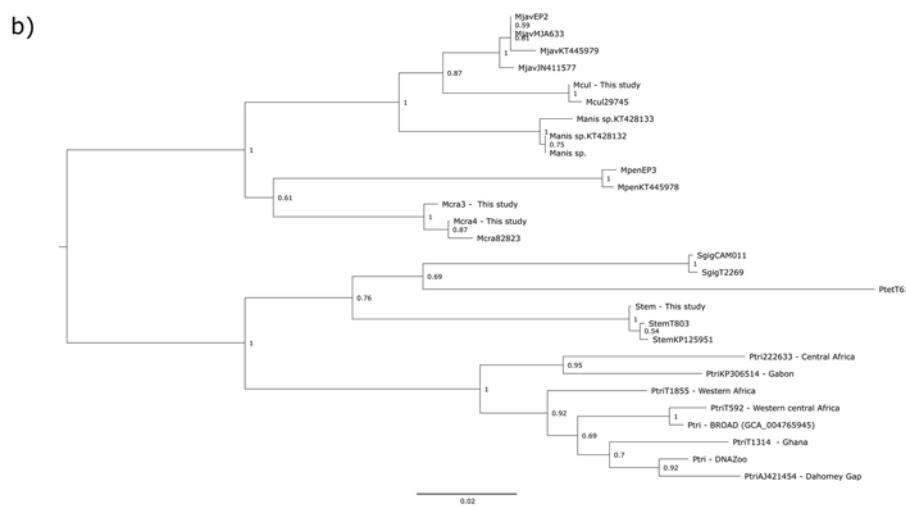
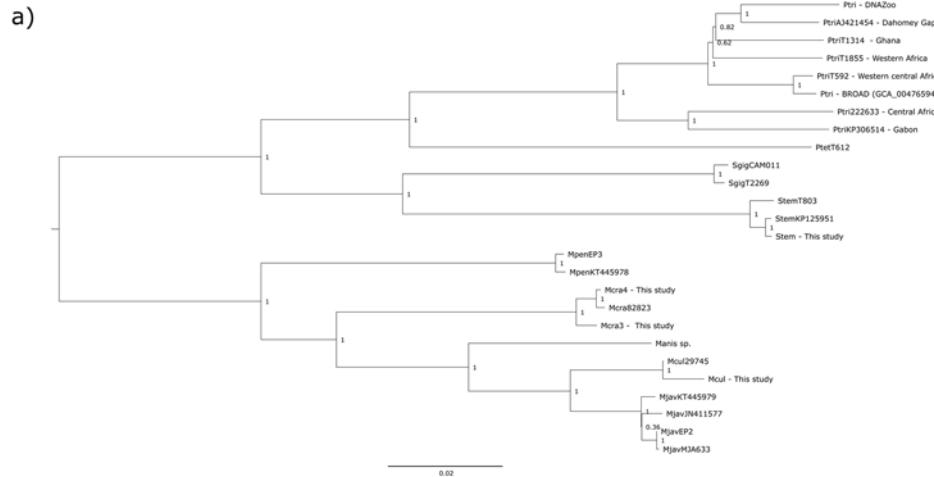
as follows: MJavChoo - *Manis javanica* from Malaysia (Choo, et al. 2016), MJavHu – *M. javanica* confiscated in China (Hu, Hao, et al. 2020), MPenChoo – *Manis pentadactyla* from Taiwan (Choo, et al. 2016), MPenHu – *M. pentadactyla* confiscated in China (Hu, Hao, et al. 2020), MCrasChina – *Manis sp.* confiscated in China (Cao, et al. 2021), PTriBROAD – *Phataginus tricuspis* (unpublished BROAD institute; GCA\_004765945.1), PTriDNAZoo – *P. tricuspis* (unpublished DNA Zoo; [https://www.dnazoo.org/assemblies/Phataginus\\_tricuspis](https://www.dnazoo.org/assemblies/Phataginus_tricuspis)), MCul – *M. culionensis* (this study), MCras – *M. crassicaudata* (this study), PTet – *P. tetradactyla* (this study), SGig – *Smutsia gigantea* (this study), STem – *S. temminckii* (this study). Further information on these genomes and samples can be found in Table S1.



**Figure S2:** Phylogenomic relationships of pangolins inferred from concatenated and coalescent trees.

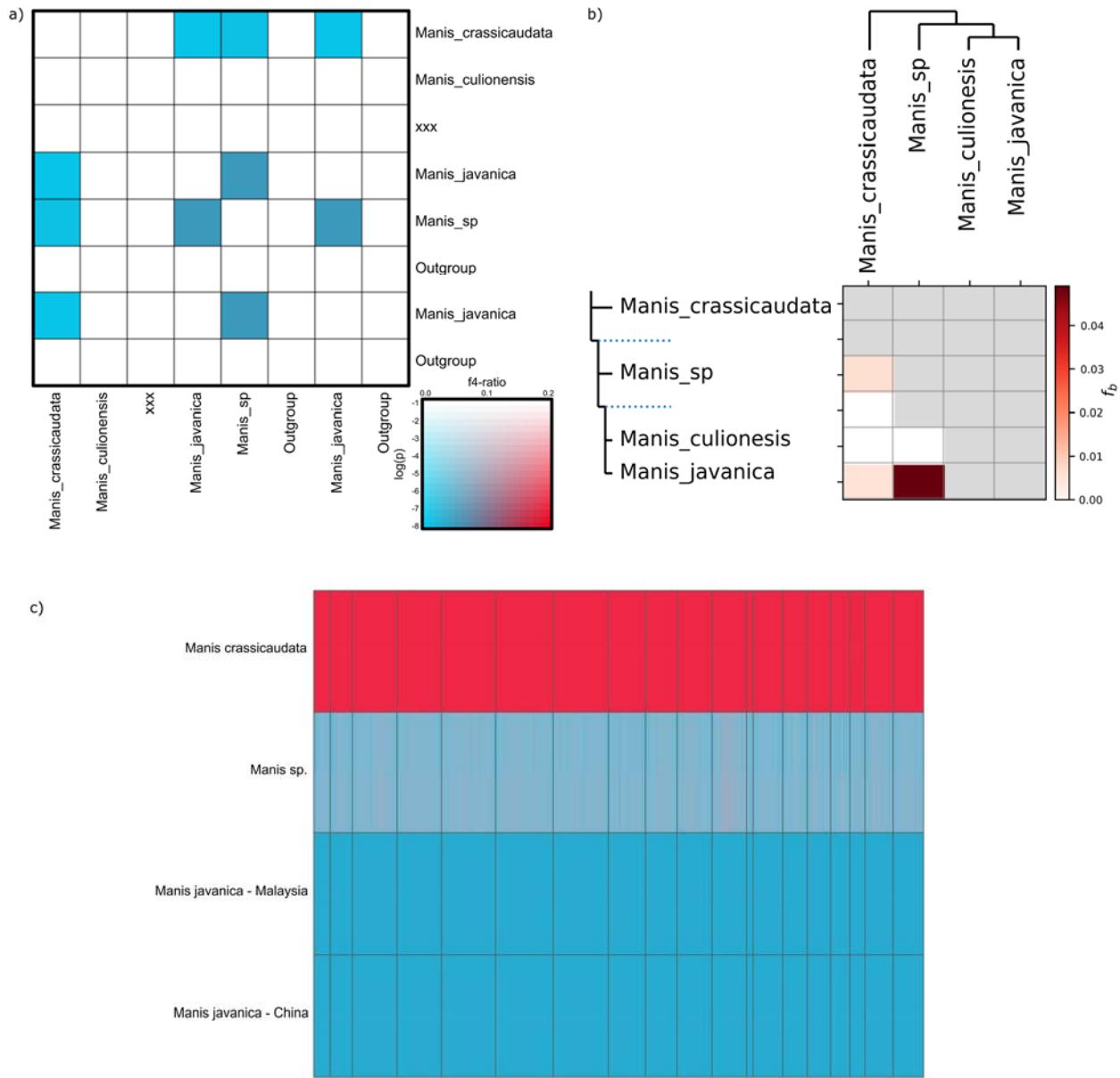
- a) Non-partitioned concatenated phylogeny based on 58 724 014 bp from 2 238 IUPAC consensus whole-gene markers. The pangolin phylogeny consists of 13 individuals from all eight species and is rooted with two representatives of the sister Order Carnivora (*Canis familiaris* and *Panthera pardus*). Nodal values indicate bootstrap support from 1 000 Felsenstein replicates. The model used for the maximum likelihood tree search was the TVM+FO+G4m based on a best-fit DNA model test.
- b) Multiple species coalescent phylogeny based on 2 238 gene trees. Branch lengths are in coalescent units while nodal values indicate local posterior probability support (1=complete support).
- c) Multiple species coalescent phylogeny based on 2 238 gene trees. Branch lengths are in coalescent units. Nodal values indicate the level of gene tree conflict, which is calculated through the number of alternative gene tree quartets that agree with the main species tree quartet topology (this figure) at each internal branch. The normalized quartet score for the entire phylogeny is 0.981. Using a polytomy test we did not identify any polytomies in the tree (which could influence these results). q1, q2, and q3 refer to the proportion of quartets in the gene trees that agree with a branch (quartet support) for the main topology (LR|SO), first alternative (RS|LO) and second alternative (RO|LS), respectively. f1, f2, and f3 refer to the same as above but are the raw number of quartet trees instead of the proportion. QC is the total number of quartets possible around each branch and EN is the number of effective genes for each branch.

Asterisks (\*) indicate confiscated individuals whose origins could not be verified.

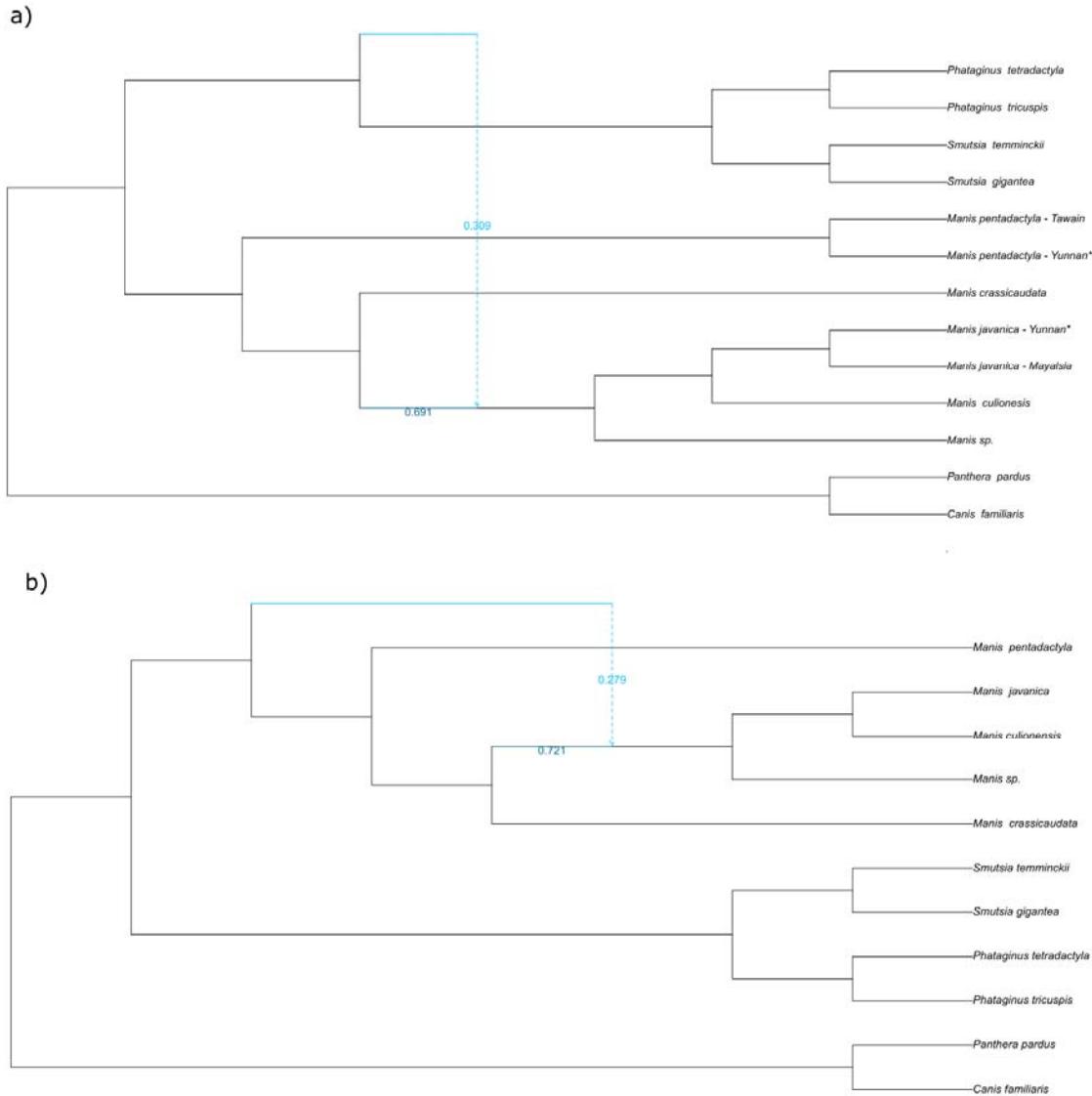


**Figure S3:** Phylogenetic relationships of pangolins inferred from mitochondrial gene and genome trees.

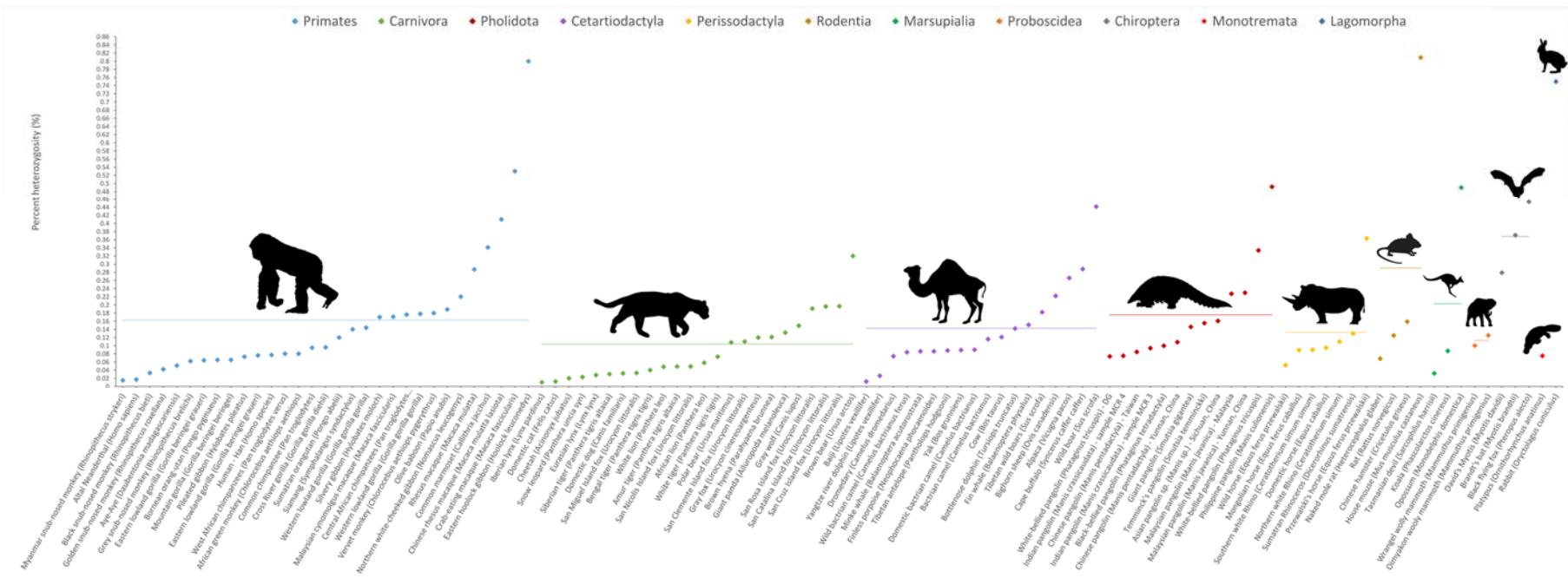
- a) Full mitochondrial genome phylogeny based on 16 437 bp using the Neighbor-Joining method in MEGA X (Kumar, et al. 2018). The pangolin phylogeny consists of 26 individuals from all eight species and six cryptic lineages of *Phataginus tricuspidis*. Nodal values relate to bootstrap support (1 000 replicates). The DNA model of evolution was Kimura 2-parameter with all positions with <90% site coverage eliminated (partial deletion option).
- b) Full cytochrome b (*Cytb*) gene phylogeny based on 399 bp using the Neighbor-Joining method in MEGA X (Kumar, et al. 2018). The pangolin phylogeny consists of 28 individuals, including that from all eight species, the six cryptic lineages of *Phataginus tricuspidis*, and the two samples suggested as a possibly new *Manis* species (Hu, Roos, et al. 2020). Nodal values relate to bootstrap support (1000 replicates). The DNA model of evolution was Kimura 2-parameter with all positions with <90% site coverage eliminated (partial deletion option).
- c) Full cytochrome oxidase subunit I (*COI*) gene phylogeny based on 600 bp using the Neighbor-Joining method in MEGA X (Kumar, et al. 2018). The pangolin phylogeny consists of 28 individuals, including that from all eight species, the six cryptic lineages of *Phataginus tricuspidis*, and the two samples suggested as a possibly new *Manis* species (Hu, Roos, et al. 2020). Nodal values relate to bootstrap support (1000 replicates). The DNA model of evolution was Kimura 2-parameter with all positions with <90% site coverage eliminated (partial deletion option).



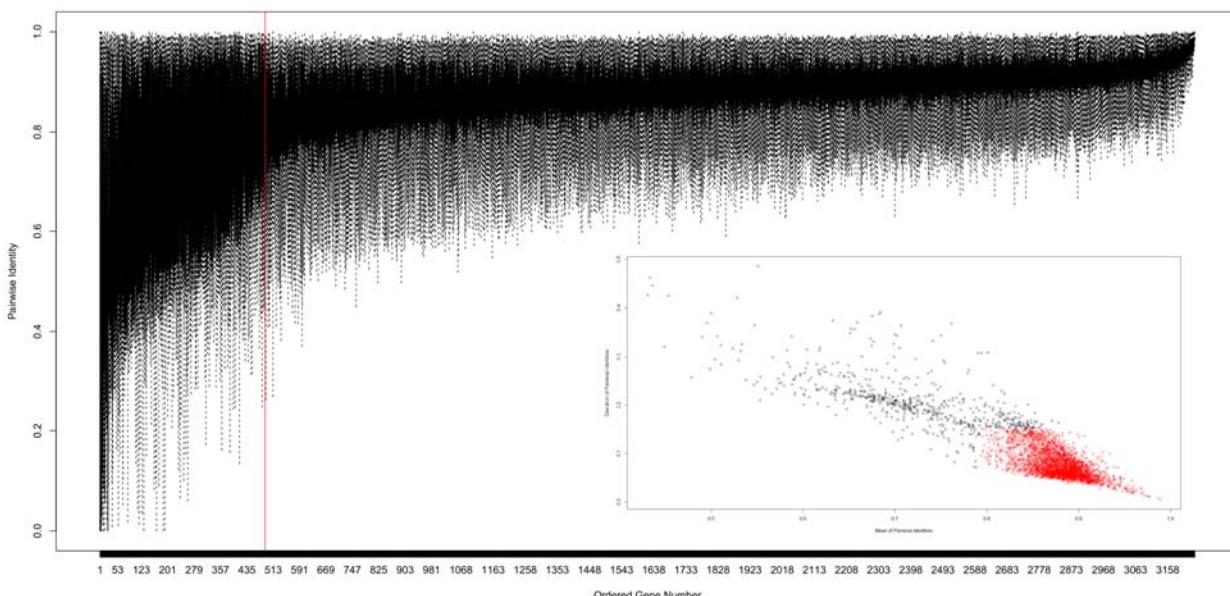
**Figure S4:** Tests of admixture and hybridization between Asian pangolin species. (a) Heatmap of  $f_4$ -ratio statistics whereby warmer colours indicate higher admixture proportions. The ‘Outgroup’ refers to *Manis pentadactyla* individuals whilst ‘XXX’ refers to samples left out of the analysis. (b) Heatmap of  $f$ -branch statistics (uncorrelated  $f_4$ -ratios) whereby warmer colours indicate higher the admixture proportions. Grey shaded areas represent non-testable combinations. The tree on each side represents the phylogenetic relationships between species whereby *Manis pentadactyla* is not included since it was set as the outgroup species. (c) Ancestry painting plot to test for hybrid species whereby the undescribed *Manis* sp. was set as the putative hybrid species of the two putative parental species: *Manis crassicaudata* (red) and *Manis javanica* (blue). The bottom row of the *Manis* sp. sample column is coloured in red and the top row is coloured in blue when the genotype is heterozygous. The proportion of heterozygous genotypes is 0.02 whilst an F1 hybrid would be close to 1.0 (see Table S2 for other parental-hybrid combinations).



**Figure S5:** Phylogenetic networks using maximum pseudolikelihood estimates, depicted as rooted phylogenies to infer reticulation (introgression/hybridization) events within pangolins. Dotted blue lines indicate connection and direction of gene-flow between the donor and recipient taxa. Solid blue lines indicate ancestry of donor and recipient taxa. Numbers correspond to the proportion of genes shared between recipient and donor (light blue) and recipient and ancestor (dark blue). Analyses were run twice, (a) first by using each individual as a spate evolutionary unit and then (b) by indicating that multiple individuals of the same species were the same species. Both analyses indicated one reticulation event as the most likely outcome. Networks were drawn using the PhyloPlot function in Julia. Asterisks (\*) indicate confiscated individuals whose origins could not be verified.



**Figure S6:** Proportion of genome-wide heterozygosity of pangolins (Pholidota) and other mammals. Estimates are grouped and color-coded by mammalian order with the horizontal line indicating the average proportion of heterozygosity for each order. Estimates are updated from the summary by Hu, Hao, et al. (2020), and displayed from smallest (least diverse) to largest (most diverse) in Table S6. Illustrations indicate the various taxonomic orders and are credited as follows (<https://creativecommons.org/licenses/by/3.0/>): Pholidota/Certartiodactyla (Steven Traver), Carnivora (Gabriela Palomo-Munoz), Chiroptera (Margot Michaud), Lagomorpha/Marsupialia/Monotremata (Sarah Werning), Perissodactyla (Oscar Sanisidro), Primates (T. Michael Keesey), Proboscidea (Margot Michaud), Rodentia (Jiro Wada) sourced from PhyloPic (<http://phylopic.org/>).



8

9 **Figure S7:** Diversity of orthologous whole-gene markers. Boxplot of whole-genes ranked from lowest (most diverse)  
10 to highest (least diverse) mean pairwise identity and the deviation of these markers (inner scatter plot). The average  
11 pairwise identity was calculated from the multiple sequence alignments of the eight pangolin species (using a single  
12 representative of each species only), by obtaining the pairwise identity for each combination of pairs and averaging  
13 it. This indicates the level of similarity ( $1=100\%$  mean pairwise identity/similarity) which can be interpreted as  
14 pairwise diversity on the inverse ( $1=0\%$  diverse). The vertical red line on the boxplot indicates the point (marker 566)  
15 at which genes to the left are likely outliers (values  $>2$  Qn deviations from the median of the mean pairwise identities)  
16 as they are too diverse to be reliable (possible influence of parology, repetitive regions, bad alignment, bad gene  
17 annotation, etc.). This cutoff, along with another (values  $>2$  Qn deviations from the median of the standard deviations  
18 of mean pairwise identities) can be viewed in the scatter plot whereby black points are the outliers (first 610 markers  
19 with the lowest mean pairwise identity or highest standard deviation of mean pairwise identity) and red points are  
20 likely reliable markers. These outlier markers have been highlighted in red in the sheet “removed genes” in the  
21 diversity database (Database S1: available at <https://doi.org/10.5281/zenodo.7517409>). The remaining points make up  
22 3 410 610 polymorphisms from 2 623 orthologous whole-genes.

23   **Table S1:** Samples sequenced and references used in this study. BUSCO v5 scores were obtained from mammalian orthologues by uploading the assemblies to  
 24 gVolante (<https://gvolante.riken.jp/>). Unpublished genome assemblies and sequencing data for DNA Zoo versions of *Manis pentadactyla*, *M. javanica*, and  
 25 *Phataginus tricuspis* are used with permission from the DNA Zoo Consortium ([dnazoo.org](http://dnazoo.org)). These DNA Zoo draft assemblies were created and reviewed following  
 26 the Hi-C method (Dudchenko, et al. 2017; Dudchenko, et al. 2018).

Individual	Genbank accession number	Isolate; origin	Study	Collection year; sample type; collector	Sequencing platform	BUSCO scores (completed_S; completed_D; Fragmented; Missing)	Estimated coverage. For samples in this study: (1) using Lander/Waterman equation with 2.45 Gb genome length as standard / (2) mapped to <i>P. tricuspis</i> DNA Zoo reference.	Notes
Chinese pangolin ( <i>Manis pentadactyla</i> )	GCA_000738955.1	MPE899; Taiwan	(Choo, et al. 2016)	N/A	Illumina HiSeq	70.5; 0.7; 10; 18.8	59x	Unpublished DNA Zoo version with additional Hi-C and NovaSeq data can be found here: <a href="https://www.dnazon.org/assemblies/Manis_pentadactyla">https://www.dnazon.org/assemblies/Manis_pentadactyla</a>
Chinese pangolin ( <i>Manis pentadactyla</i> )	GCA_014570555.1	MP20; Confiscated in Yunnan, China	(Hu, Hao, et al. 2020)	N/A	Illumina HiSeq and 10X genomic	93.1; 1.3; 1.2; 4.4	281.6x	Used 77.19 Gb of the 180.66 Gb available data for mapping
Sunda pangolin ( <i>Manis javanica</i> )	GCA_001685135.1	MP_PG03-UM; Malaysia	(Choo, et al. 2016)	N/A	Illumina HiSeq	74.6; 0.6; 6; 8.8	145.7x	Used 71.33 Gb of the 169.4 Gb available data for mapping Unpublished DNA Zoo version with additional Hi-C and HiSeq

								data can be found here: <a href="https://www.dnazon.org/assemblies/Manis_javanica">https://www.dnazon.org/assemblies/Manis_javanica</a>
Sunda pangolin ( <i>Manis javanica</i> )	GCA_014 570535.1	MJ74; Confiscated in Yunnan, China	(Hu, Hao, et al. 2020)	N/A	Illumina HiSeq and 10X genomic	92.3; 0.9; 2; 4.8	411.8x	Used 112.07 Gb of the 316.2 Gb available data for mapping
Philippine pangolin ( <i>Manis culionensis</i> )		MCUP0005; Casuyan, Palawan Isl., Philippines	This study	1945; museum skin; H.H. Hoogstraal / Field Museum of Natural History, Chicago (FMNH 62919)	Illumina HiSeq X Ten	6.2; 0; 12.5; 81.3	(1) 76.7x / (2) 19.9x	
Indian pangolin (3) ( <i>Manis crassicaudata</i> )		MCR3; Confiscated but died in captivity, Sri Lanka	This study	2008; tissue from dead individual; Jayanthi Alahakoon / Colombo Zoo	Illumina HiSeq X Ten	40.9; 0.2, 16.9; 42	(1) 53.1x / (2) 33.2x	
Indian pangolin (4) ( <i>Manis crassicaudata</i> )		MCR4; Confiscated but died in captivity, Sri Lanka	This study	2008; tissue from dead individual; Jayanthi Alahakoon / Colombo Zoo	Illumina HiSeq X Ten	N/A	(1) 16.2x / (2) 9.1x	Only used in Heterozygosity and PSMC analyses due to limited coverage
<i>Manis</i> sp.	GCA_016 801295.1	Confiscated in Sichuan, China	(Cao, et al. 2021)	N/A	Illumina HiSeq	54.5; 0.3; 13.9; 31.3	44x	Indicated at <i>M. crassicaudata</i> on NCBI
Black-bellied pangolin ( <i>Phataginus tetradactyla</i> )		CAM085; Yaoundé bushmeat market, Cameroon	This study	2007; tissue from dead individual; F. Njiokou & P. Gaubert	Illumina HiSeq 3000	55.9; 0.4; 15.3; 28.4	(1) 43.4x / (2) 30.1x	

White-bellied pangolin ( <i>Phataginus tricuspis</i> )	GCA_004765945.1	BS60	Unpublished - BROAD institute	O. Ryder / San Diego Zoo Institute for Conservation Research	Illumina HiSeq	65.5; 0.8; 12.4; 21.3	30.2x	
White-bellied pangolin ( <i>Phataginus tricuspis</i> )	Jaziri		Unpublished - DNA Zoo	Pittsburgh Zoo & PPG Aquarium	Illumina NovaSeq and Hi-C	87.4; 1.5; 4.2; 6.9	Unknown	The assembly can be found here courtesy of DNA Zoo: <a href="https://www.dnazon.org/assemblies/Phataginus_tricuspis">https://www.dnazon.org/assemblies/Phataginus_tricuspis</a>
Temminck's pangolin ( <i>Smutsia temminckii</i> )	STEM 81; Kalahari Oryx Game Farm, South Africa	This study	2012; tissue from dead individual; D.W. Pietersen	Illumina HiSeq X Ten	46.4; 0.4; 17.2; 36	(1) 43.9x / (2) 21.9x		
Giant pangolin ( <i>Smutsia gigantea</i> )	CAM011; Yaoundé bushmeat market, Cameroon	This study	2007; tissue from dead individual; F. Njiokou & P. Gaubert	Illumina HiSeq 3000 and Oxford Nanopore	79.8; 1.3; 7; 11.9	(1) 100.4x / (2) 57.4x	Mapping coverage estimate is from short read data only	

28 **Table S2:** Ancestral painting output statistics related to the test for hybrid individuals between various sets (trios) of putative parental species. P1 and P2 refer to  
 29 putative parental species one and two.

Hybrid test trio	Species	Homozygous genotypes P1	Heterozygous genotypes (P1-P2)	Homozygous genotypes P2	Proportion of heterozygous genotypes (%)
Trio 1 - P1	<i>M. crassicaudata</i>	224566	0	0	0
Trio 1 - Hybrid	<i>Manis species</i>	36814	4492	181552	2
Trio 1 - P2	<i>M. javanica</i>	0	0	224566	0
Trio 2 - P1	<i>M. culionensis</i>	210997	0	0	0
Trio 2 - Hybrid	<i>Manis species</i>	56583	3748	148943	1.8
Trio 2 - P2	<i>M. javanica</i>	0	0	210997	0
Trio 3 - P1	<i>Manis species</i>	222505	0	0	0
Trio 3 - Hybrid	<i>M. culionensis</i>	22313	9281	190001	4.2
Trio 3 - P2	<i>M. javanica</i>	0	0	222505	0
Trio 4 - P1	<i>M. culionensis</i>	1294988	0	0	0
Trio 4 - Hybrid	<i>M. crassicaudata</i>	381209	11247	878312	0.9
Trio 4 - P2	<i>M. javanica</i>	0	0	1294988	0

30

**Table S3:** Output of the concordance analysis implemented in IQ-TREE along with the significance of the Chi-squared test of independence between the two discordant gene counts (gDF1\_N and gDF2\_N). This was used to test whether incomplete lineage sorting (ILS) may be the sole cause of discordance for a branch whereby a significant p-value (\*) indicates the contrary. Branch Clade refers to the clade onto which the branch opens in the phylogenetic tree in Figure 1. The rest of the column ID's follow that of IQ-TREE; ID: Branch ID, gCF: Gene concordance factor (=gCF\_N/gN %), gCF\_N: Number of trees concordant with the branch, gDF1: Gene discordance factor for NNI-1 branch (=gDF1\_N/gN %), gDF1\_N: Number of trees concordant with NNI-1 branch, gDF2: Gene discordance factor for NNI-2 branch (=gDF2\_N/gN %), gDF2\_N: Number of trees concordant with NNI-2 branch, gDFP: Gene discordance factor due to polyphyly (=gDFP\_N/gN %), gDFP\_N: Number of trees decisive but discordant due to polyphyly, gN: Number of trees decisive for the branch, sCF: Site concordance factor averaged over 100 quartets (=sCF\_N/sN %), sCF\_N: sCF in absolute number of sites, sDF1: Site discordance factor for alternative quartet 1 (=sDF1\_N/sN %), sDF1\_N: sDF1 in absolute number of sites, sDF2: Site discordance factor for alternative quartet 2 (=sDF2\_N/sN %), sDF2\_N: sDF2 in absolute number of sites, sN: Number of informative sites averaged over 100 quartet, gEF\_p: p-value of the Chi-squared test of independence for genes.

Branch ID	Branch Clade	gCF	gCF_N	gD_F1	gDF_1_N	gDF_2	gDF_2_N	gDF_P	gDFP_N	gN	sCF	sCF_N	sDF_1	sDF1_N	sDF_2	sDF_2_N	sN	Branch -length	gEF_p
16	<i>M. culionensis/M. javanica</i> Malaysia/ <i>M. javanica</i> China	91.3 2	295 7	3.1 8	103	3.68	119	1.82	59	323 8	86.62 .4	77921 11	7.18	5998. 8.77	6.21	531 8	89238.2	2.13912	0.27735352 1
18	<i>Manis</i> sp./ <i>M. culionensis/M. javanica</i> Malaysia/ <i>M. javanica</i> China	97.5 9	316 0	0.1 2	4	0.06	2	2.22	72	323 8	89.69 1.1	24793 .84	5.21	12487 121 77.4 3	5.1	272596. 4	3.91262	0.41931657 8	
19	<i>M. crassicaudata/M. nis</i> sp./ <i>M. culionensis/M. javanica</i> Malaysia/ <i>M. javanica</i> China	94.6 3	306	2.5	81	1.14	37	1.76	57	323 8	67.99 0	16695 1	16.9 .42	39310 15.1	15.1	356 05.5 3	241866	2.71664	4.98E-05*
20	<i>M. pentadactyla</i> Taiwan/ <i>M. pentadactyla</i> China	98.6 7	319 5	0.3 7	12	0.37	12	0.59	19	323 8	96.5 6.3	31019 39	1.59	4394. 1.91	1.91	519 4.61 3	319785.	4.16025	1
21	<i>Manis</i> (Asian pangolins)	96.1 5	237 3	1.2 2	30	1.22	30	1.42	35	246 8	81.27 9.6	39130 .17	8.93	42936 9.79	9.79	469 76.1 8	481221. 9	3.04534	1
22	Pholidota & Carnivora	93.7 1	107 2	2.8	32	3.15	36	0.35	4	114 4	91.01 25	12351 .86	4.27	57919 4.72	4.72	640 50.9 6 9	135709	2.38006	0.62694318 2

23	African pangolins	96.6 4	238 5	1.0 1	25	1.26	31	1.09	27	246 8	76.93 0.9	34552 2	11.8 .68	53083 5	11.2 5	505 36.6	449141. 2	3.12795 2	0.40978038
24	<i>Phataginus</i>	96.5 4	312 7	1.2 7	41	1.08	35	1.11	36	323 9	79.05 9.4	24855 8	10.2 .15	29904 7	10.6 7	311 91.6	309655. 2	3.13571 4	0.48016122
25	<i>Smutsia</i>	97.4 4	315 5	0.6 5	21	0.8	26	1.11	36	323 8	84.25 7	28046 .21	8.01	24451 7.74	7.74	237 95.5	328713. 7	3.56152 1	0.47905592
17	<i>M. javanica</i> Malaysia/ <i>M.</i> <i>javanica</i> China	90.1 5	291 9	3.7 4	121	4.39	142	1.73	56	323 8	88.39 .07	23207 69	5.95	1502. 69	5.66	144 4.31	26154.0 7	2.02097	0.19449974

**Table S4:** Dates used for soft bound fossil calibrations on specific nodes to be used as priors for the MCMCTree analysis of divergence estimates of pangolins. These calibrations are based on both dated fossils and molecular phylogeny estimates with reasoning provided for each. The calibration of Pholidota is the most recent calibration node possible due to the scarcity of fossils for genus/species-based estimates of the group.

Node	Date	Fossil	Reference	Notes
Ferae	66–87 Ma	Min UALVP 50993 and 50994 (Oldest stem- carnivores - miacids, viverravids)	(Fox, et al. 2010)	Due to no upper estimates of Ferae, we used a molecular dated calibration which has been used in previous studies
		Max Molecular estimate	(Zhou, et al. 2011; Gaubert, et al. 2018)	
Carnivora	37.3–66 Ma	Min <i>Daphoenus</i> & <i>Hesperocyon</i>	(Benton, et al. 2015)	<i>Tapocyon</i> may be an even older caniform; (46– 43 Ma). However, it is placed outside Carnivora (Wesley- Hunt and Flynn 2005). The oldest feliforms may be the nimravids, but this too is uncertain (Hunt 2004).
		Max UALVP 50993 and 50994 (Oldest stem- carnivores - miacids, viverravids)	(Fox, et al. 2010)	
Pholidota	31–45 Ma	Min <i>Manidae</i> (oldest manidae fossil)	(Gebo and Rasmussen 1985)	Messel deposits (Germany). Found with <i>Eurotamandua</i> <i>joresi</i> which has been debated as to whether it should be included in the Pholidota or whether it predates this order but Gaudin, et al. (2009) places it under Pholidota as sister to <i>Eomanis</i> and <i>Euromanis</i>
		Max <i>Euromanis krebsi</i> (oldest) <i>Eurotamandua joresi</i> <i>Eomanis waldi</i>	(Gaudin, et al. 2009; Rose 2012)	

**Table S5:** Estimated posterior mean or median divergence estimates and 95% Highest Posterior Density (HPD) interval of each node for this study and the one conducted by Gaubert, et al. (2018). The latter study involved mitogenomes and nine nuclear genes, included more individuals and fossil calibrations within the order Carnivora, and used the program BEAST (Bouckaert, et al. 2014) to obtain time to most recent common ancestor (TMRCA) estimates. \$ refers to nodes with fossil priors for this study. \* refers to nodes where the 95% HPD of divergence estimates do not overlap in the two studies.

Node	This study		Gaubert et al. (2018)	
	Mean	95% HPD	Median	95% HPD
Ferae\$	79.47	67.66 – 87.24	78.9	69.6 – 87.0
Carnivora\$	49.95	36.49 – 65.44	50.8	44.9 – 57.4
Caniformia			41.6	38.0 – 46.0
<i>Mustela</i> – <i>Ailuropoda</i>			35.4	30.5 – 40.9
Felidae			11.3	10.0 – 13.3
<i>Acinonyx</i> – <i>Felis</i>			7.2	3.3 – 10.2
Pholidota\$	41.34	33.54 – 45.45	37.9	31.4 – 44.6
<i>Smutsia</i> - <i>Phataginus</i>	20.35	12.79 – 28.29	22.9	18.7 – 27.2
<i>Manis</i>	22.62	17.07 – 28.23*	12.9	10.3 – 15.6
<i>M. crassicaudata</i> – ( <i>Manis</i> sp, <i>M. javanica</i> , <i>M. culionensis</i> )	16.84	12.21 – 22.00*	9.1	6.6 – 11.4
<i>Manis</i> sp. – ( <i>M. javanica</i> – <i>M. culionensis</i> )	7.16	4.73 – 10.42		
<i>M. javanica</i> – <i>M. culionensis</i>	2.71	1.70 – 4.21	1.7	0.4 – 2.5
<i>M. javanica</i> China – <i>M. javanica</i> Malaysia	0.77	0.46 – 1.24		
<i>M. pentadactyla</i> China – <i>M. pentadactyla</i> Taiwan	2.82	1.48 – 4.95		
<i>Smutsia</i>	9.78	5.55 – 15.74	9.8	5.6 – 13.2
<i>Phataginus</i>	11.34	6.55 – 17.45	13.3	9.3 – 16.5
<i>P. tricuspis</i>			2.7	0.8 – 4.6
<i>P. tricuspis</i> West Africa – Western Central Africa			1.1	0.0 – 2.4

**Table S6:** Proportion of genome-wide heterozygosity for mammalian species ranked from least to most diverse. As depicted in Figure S6, each mammalian order is colour-coded (Pholidota = dark red), and citations are given for the studies in which the value was originally provided for each species. Heterozygosity values for pangolins (Pholidota) include those calculated in our study as well as Hu, Hao, et al. (2020), who used a different method of calculation (using VCFtools on autosomal SNPs with 50 kb non-overlapping sliding windows). This is an edited and updated version of the table created by Hu, Hao, et al. (2020).

Species	Heterozygosity (%)	Mammalian order	Sources
Iberian lynx ( <i>Lynx pardinus</i> )	0.010	Carnivora	(Abascal, et al. 2016)
Domestic cat ( <i>Felis catus</i> )	0.012	Carnivora	(Cho, et al. 2013)
Baiji ( <i>Lipotes vexillifer</i> )	0.012	Cetartiodactyla	(Zhou, et al. 2013)
Myanmar snub-nosed monkey ( <i>Rhinopithecus strykeri</i> )	0.015	Primates	(Zhang, et al. 2016)
Altai Neanderthal ( <i>Homo sapiens</i> )	0.017	Primates	(Prüfer, et al. 2014)
Cheetah ( <i>Acinonyx jubatus</i> )	0.020	Carnivora	(Dobrynin, et al. 2015)
Snow leopard ( <i>Panthera uncia</i> syn)	0.023	Carnivora	(Cho, et al. 2013)
Yangtze river dolphin ( <i>Lipotes vexillifer</i> )	0.026	Cetartiodactyla	(Zhou, et al. 2013)
Eurasian lynx ( <i>Lynx lynx</i> )	0.028	Carnivora	(Abascal, et al. 2016)
Siberian tiger ( <i>Panthera tigris altaica</i> )	0.030	Carnivora	(Dobrynin, et al. 2015)
Domestic dog ( <i>Canis familiaris</i> )	0.032	Carnivora	(Lindblad-Toh, et al. 2005)
Tasmanian devil ( <i>Sarcophilus harrisii</i> )	0.032	Marsupialia	(Cho, et al. 2013)
Black snub-nosed monkey ( <i>Rhinopithecus bieti</i> )	0.033	Primates	(Zhou, et al. 2016)
Island fox ( <i>Urocyon littoralis</i> ) - San Miguel	0.033	Carnivora	(Robinson, et al. 2016)
Bengal tiger ( <i>Panthera tigris tigris</i> )	0.040	Carnivora	(Dobrynin, et al. 2015)
Golden snub-nosed monkey ( <i>Rhinopithecus roxellana</i> )	0.042	Primates	(Zhou, et al. 2016)
White lion ( <i>Panthera leo</i> )	0.048	Carnivora	(Cho, et al. 2013)
Amur tiger ( <i>Panthera tigris altaica</i> )	0.049	Carnivora	(Cho, et al. 2013)
Island fox ( <i>Urocyon littoralis</i> ) - San Nicolis	0.049	Carnivora	(Robinson, et al. 2016)
Aye-Aye ( <i>Daubentonia madagascariensis</i> )	0.051	Primates	(Perry, et al. 2011)
Wild horse ( <i>Equus ferus przewalskii</i> )	0.052	Perissodactyla	(Huang, et al. 2014)
African lion ( <i>Panthera leo</i> )	0.058	Carnivora	(Cho, et al. 2013)
Grey snub-nosed monkey ( <i>Rhinopithecus brelichi</i> )	0.062	Primates	(Zhou, et al. 2016)
Eastern lowland gorilla ( <i>Gorilla beringei graueri</i> )	0.064	Primates	(Xue, et al. 2015)
Bornean orangutan ( <i>Pongo pygmaeus</i> )	0.065	Primates	(Locke, et al. 2011)
Mountain gorilla ( <i>Gorilla beringei beringei</i> )	0.065	Primates	(Xue, et al. 2015)

Naked mole rat ( <i>Heterocephalus glaber</i> )	0.068	Rodentia	(Kim, et al. 2011)
Pileated gibbon ( <i>Hylobates pileatus</i> )	0.073	Primates	(Carbone, et al. 2014)
White tiger ( <i>Panthera tigris tigris</i> )	0.073	Carnivora	(Cho, et al. 2013)
White-bellied pangolin ( <i>Phataginus tricuspis</i> ) - DG	0.074	Pholidota	Sequencing data from DNAZoo (Wu, et al. 2014)
Dromedary ( <i>Camelus dromedarius</i> )	0.074	Certartiodactyla	
Platypus ( <i>Ornithorhynchus anatinus</i> )	0.075	Monotremata	(Warren, et al. 2008)
Indian pangolin ( <i>Manis crassicaudata</i> ) – MCR4	0.075	Pholidota	This study
Eastern lowland gorilla ( <i>Gorilla beringei graueri</i> )	0.076	Primates	(Scally, et al. 2012)
Human Han ( <i>Homo</i> species)	0.077	Primates	(Meyer, et al. 2012)
West African chimpanzees ( <i>Pan troglodytes verus</i> )	0.080	Primates	(Mikkelsen, et al. 2005)
African green monkey ( <i>Chlorocebus aethiops aethiops</i> )	0.080	Primates	(Warren, et al. 2015)
Wild bactrian camel ( <i>Camelus bactrianus ferus</i> )	0.084	Certartiodactyla	(Wang, et al. 2012)
Chinese pangolin ( <i>Manis pentadactyla</i> ) - Taiwan	0.085	Pholidota	Sequencing data from Choo, et al. (2016). Analysis from this study
Sunda pangolin ( <i>Manis javanica</i> ) - Average over 74 individuals	0.085	Pholidota	Samples in Hu, Hao, et al. (2020). Analysis in their study
Minke whale ( <i>Balaenoptera acutorostrata</i> )	0.086	Certartiodactyla	(Yim, et al. 2014)
Finless porpoise ( <i>Neophocaena phocaenoides</i> )	0.086	Certartiodactyla	(Yim, et al. 2014)
Koala ( <i>Phascolarctos cinereus</i> )	0.087	Marsupalia	(Johnson, et al. 2018)
Tibetan antelope ( <i>Pantholops hodgsonii</i> )	0.088	Certartiodactyla	(Ge, et al. 2013)
Yak ( <i>Bos grunniens</i> )	0.089	Certartiodactyla	(Qiu, et al. 2012)
Mongolian horse ( <i>Equus ferus caballus</i> )	0.089	Perissodactyla	(Huang, et al. 2014)
Domestic bactrian camel ( <i>Camelus bactrianus</i> )	0.090	Certartiodactyla	(Wang, et al. 2012)
Southern white rhinoceros ( <i>Ceratotherium simum simum</i> )	0.090	Perissodactyla	(Tunstall, et al. 2018)
Indian pangolin ( <i>Manis crassicaudata</i> ) – MCR3	0.094	Pholidota	This study
Common chimpanzee ( <i>Pan troglodytes</i> )	0.095	Primates	(Mikkelsen, et al. 2005)
Domestic horse ( <i>Equus ferus caballus</i> )	0.095	Perissodactyla	(Wade, et al. 2009)
Cross River gorilla ( <i>Gorilla gorilla diehli</i> )	0.096	Primates	(Xue, et al. 2015)
Black-bellied pangolin ( <i>Phataginus tetradactyla</i> )	0.100	Pholidota	This study
Wrangel woolly mammoth ( <i>Mammuthus primigenius</i> )	0.100	Proboscidea	(Palkopoulou, et al. 2015)
Polar bear ( <i>Ursus maritimus</i> )	0.108	Carnivora	(Liu, et al. 2014)
Chinese pangolin ( <i>Manis pentadactyla</i> ) – Yunnan, China confiscation	0.109	Pholidota	Sequencing data from Hu, Hao, et al. (2020).

				Analysis from this study
Island fox ( <i>Urocyon littoralis</i> ) - San Clemente	0.110	Carnivora	(Robinson, et al. 2016)	(Robinson, et al. 2016)
Northern white rhinoceros ( <i>Ceratotherium simum cottoni</i> )	0.110	Perissodactyla	(Tunstall, et al. 2018)	(Tunstall, et al. 2018)
Chinese pangolin ( <i>Manis pentadactyla</i> )	0.114	Pholidota	Sample MP20 in Hu, Hao, et al. (2020). Analysis in their study	Sample MP20 in Hu, Hao, et al. (2020). Analysis in their study
Bactrian camel ( <i>Camelus bactrianus</i> )	0.116	Certartiodactyla	(Wu, et al. 2014)	(Wu, et al. 2014)
Sumatran orangutan ( <i>Pongo abelii</i> )	0.120	Primates	(Xue, et al. 2015)	(Xue, et al. 2015)
Gray fox ( <i>Urocyon cinereoargenteus</i> )	0.120	Carnivora	(Robinson, et al. 2016)	(Robinson, et al. 2016)
Brown hyena ( <i>Parahyaena brunnea</i> )	0.121	Carnivora	(Westbury, et al. 2018)	(Westbury, et al. 2018)
Cow ( <i>Bos taurus</i> )	0.121	Certartiodactyla	(Corbett-Detig, et al. 2015)	(Corbett-Detig, et al. 2015)
Rat ( <i>Rattus norvegicus</i> )	0.125	Rodentia	(Leffler, et al. 2012)	(Leffler, et al. 2012)
Oimyakon woolly mammoth ( <i>Mammuthus primigenius</i> )	0.125	Proboscidea	(Palkopoulou, et al. 2015)	(Palkopoulou, et al. 2015)
Chinese pangolin ( <i>Manis pentadactyla</i> ) - Average over 23 individuals	0.127	Pholidota	Samples in Hu, Hao, et al. (2020). Analysis in their study	Samples in Hu, Hao, et al. (2020). Analysis in their study
Sumatran rhinoceros ( <i>Dicerorhinus sumatrensis</i> )	0.130	Perissodactyla	(Mays, et al. 2018)	(Mays, et al. 2018)
Giant panda ( <i>Ailuropoda melanoleuca</i> )	0.132	Carnivora	(Li, et al. 2010)	(Li, et al. 2010)
Siamang ( <i>Sympthalangus syndactylus</i> )	0.140	Primates	(Carbone, et al. 2014)	(Carbone, et al. 2014)
Bottlenose dolphin ( <i>Tursiops truncatus</i> )	0.142	Certartiodactyla	(Yim, et al. 2014)	(Yim, et al. 2014)
Western lowland gorilla ( <i>Gorilla gorilla gorilla</i> )	0.144	Primates	(Xue, et al. 2015)	(Xue, et al. 2015)
Giant pangolin ( <i>Smutsia gigantea</i> )	0.146	Pholidota	This study	This study
Gray wolf ( <i>Canis lupus</i> )	0.149	Carnivora	(Corbett-Detig, et al. 2015)	(Corbett-Detig, et al. 2015)
Fin whale ( <i>Balaenoptera physalus</i> )	0.151	Certartiodactyla	(Yim, et al. 2014)	(Yim, et al. 2014)
Sunda pangolin ( <i>Manis javanica</i> )	0.152	Pholidota	Sample MJ74 in Hu, Hao, et al. (2020). Analysis in their study	Sample MJ74 in Hu, Hao, et al. (2020). Analysis in their study
Temminck's pangolin ( <i>Smutsia temminckii</i> )	0.155	Pholidota	This study	This study
Chinese hamster ( <i>Cricetus griseus</i> )	0.159	Rodentia	(Lewis, et al. 2013)	(Lewis, et al. 2013)
Asian pangolin sp. ( <i>Manis</i> sp.) - Sichuan, China confiscated	0.161	Pholidota	Sequencing data from Cao, et al. (2021). Analysis in this study.	Sequencing data from Cao, et al. (2021). Analysis in this study.
Silvery gibbon ( <i>Hylobates moloch</i> )	0.17	Primates	(Carbone, et al. 2014)	(Carbone, et al. 2014)
Malaysian cynomolgus macaque ( <i>Macaca fascicularis</i> )	0.171	Primates	(Higashino, et al. 2012)	(Higashino, et al. 2012)
Central African chimpanzees ( <i>Pan troglodytes troglodytes</i> )	0.176	Primates	(Mikkelsen, et al. 2005)	(Mikkelsen, et al. 2005)

Western lowland gorilla ( <i>Gorilla gorilla gorilla</i> )	0.178	Primates	(Scally, et al. 2012)
Vervet monkey ( <i>Chlorocebus aethiops pygerythrus</i> )	0.180	Primates	(Warren, et al. 2015)
Wild boar ( <i>Sus scrofa</i> ) - Tibetan	0.182	Certartiodactyla	(Li, et al. 2013)
Olive baboon ( <i>Papio anubis</i> )	0.189	Primates	(Corbett-Detig, et al. 2015)
Island fox ( <i>Urocyon littoralis</i> ) - San Rosa	0.191	Carnivora	(Robinson, et al. 2016)
Island fox ( <i>Urocyon littoralis</i> ) - San Catalina	0.196	Carnivora	(Robinson, et al. 2016)
Island fox ( <i>Urocyon littoralis</i> ) - San Cruz	0.197	Carnivora	(Robinson, et al. 2016)
Northern white-cheeked gibbon ( <i>Nomascus leucogenys</i> )	0.220	Primates	(Carbone, et al. 2014)
Bighorn sheep ( <i>Ovis canadensis</i> )	0.222	Certartiodactyla	(Corbett-Detig, et al. 2015)
Sunda pangolin ( <i>Manis javanica</i> ) - Malaysia	0.224	Pholidota	Sequencing data from Choo, et al. (2016). Analysis from this study
Sunda pangolin ( <i>Manis javanica</i> ) - Yunnan, China confiscation	0.230	Pholidota	Sequencing data from sample MJ74 in Hu, Hao, et al. (2020). Analysis from this study
Alpaca ( <i>Vicugna pacos</i> )	0.266	Certartiodactyla	(Wu, et al. 2014)
David's myotis ( <i>Myotis davidii</i> )	0.279	Chiroptera	(Zhang, et al. 2013)
Rhesus macaque ( <i>Macaca mulatta</i> )	0.287	Primates	(Corbett-Detig, et al. 2015)
Cape buffalo ( <i>Syncerus caffer caffer</i> )	0.288	Certartiodactyla	(de Jager, et al. 2021)
Brown bear ( <i>Ursus arctos</i> )	0.320	Carnivora	(Liu, et al. 2014)
White-bellied pangolin ( <i>Phataginus tricuspis</i> ) - CWA	0.334	Pholidota	Sequencing data from Genbank (GCA_00476594 5.1). Analysis from this study
Common marmoset ( <i>Callithrix jacchus</i> )	0.341	Primates	(Consortium 2014)
Przewalski's horse ( <i>Equus ferus przewalskii</i> )	0.363	Perissodactyla	(Corbett-Detig, et al. 2015)
Brandt's bat ( <i>Myotis brandtii</i> )	0.371	Chiroptera	(Seim, et al. 2013)
Chinese rhesus macaque ( <i>Macaca mulatta lasiota</i> )	0.410	Primates	(Yan, et al. 2011)
Wild boar ( <i>Sus scrofa</i> )	0.441	Certartiodactyla	(Corbett-Detig, et al. 2015)
Black flying fox ( <i>Pteropus alecto</i> )	0.453	Chiroptera	(Zhang, et al. 2013)
Opossum ( <i>Monodelphis domestica</i> )	0.490	Marsupialia	(Mikkelsen, et al. 2007)
Philippine pangolin ( <i>Manis culionensis</i> )	0.492	Pholidota	This study

Crab-eating macaque ( <i>Macaca fascicularis</i> )	0.530	Primates	(Yan, et al. 2011)
Rabbit ( <i>Oryctolagus cuniculus</i> )	0.750	Lagomorpha	(Carneiro, et al. 2014)
Eastern hoolock gibbon ( <i>Hoolock leuconedys</i> )	0.800	Primates	(Carbone, et al. 2014)
House mouse ( <i>Mus musculus castaneus</i> )	0.809	Rodentia	(Corbett-Detig, et al. 2015)

**Table S7:** Estimated generation times of each species of pangolins based on the available literature. These times include a sum of the gestation period (passing on genomic information to the next generation at conception) and time until sexual maturity (when genomic information can be passed on to the next generation again). These estimates were used in the PSMC (pairwise sequentially Markovian coalescent) model analysis on pangolins in order to get the timing of changes in IICR (inverse instantaneous coalescence rate) as accurate as possible. We defined generation time as from the conception of an individual to the time until the first born of that individual is conceived.

Species	Estimated generation time	Evidence	Citations
Chinese pangolin ( <i>Manis pentadactyla</i> )	2 years	Reaches sexual maturity at 1–1.5 years old, but may be as low as 6–7 months; gestation is 6–7 months (captive data)	(Chin, et al. 2012; Zhang, et al. 2016)
Sunda pangolin ( <i>Manis javanica</i> )	1.5 years	1 year (supposed sexual maturity of males is 1.5 years based off sperm analysis – A. Kurniawan, unpublished) until sexual maturity but may be as low as 6–7 months; gestation is 6 months (captive and seizure data)	(Zhang, et al. 2015; Zhang, et al. 2017)
Philippine pangolin ( <i>Manis culionensis</i> )	1.5 years	No data, but likely similar to the Sunda pangolin.	
Indian pangolin ( <i>Manis crassicaudata</i> )	3 years	Up to 3 years until sexual maturity (unpublished data), gestation is around 6–8 months (135–251 days; captive data).	(Mohapatra, et al. 2018; Mahmood, et al. 2020)
Black-bellied pangolin ( <i>Phataginus tetradactyla</i> )	2.5 years	2 years (unpublished suggestion – book chapter), gestation is 5 months (150 days; wild data).	(Pagès 1972a; Gudehus, et al. 2020)
White-bellied pangolin ( <i>Phataginus tricuspis</i> )	2.5 years	No data for maturity (probably similar to Black-bellied pangolin), gestation is 5 months (140–150 days; wild data).	(Pagès 1972b)
Temminck's pangolin ( <i>Smutsia temminckii</i> )	2.5 years	Sexual maturity is likely around 2 years but no home range is established for a few more years (assuming home range is important, then 3–7 years), gestation is 3.5–4.5 months (105–140 days; both captive and wild data).	(van Ee 1966; Pietersen, et al. 2020)
Giant pangolin ( <i>Smutsia gigantea</i> )	3 years	No data available, but likely similar, if not longer than, Temminck's pangolin due to larger size.	

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