1   **Manuscript for review in *PLOS One***

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3   **Evolutionary history of Carnivora (Mammalia, Laurasiatheria) inferred**

4   **from mitochondrial genomes**

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Abstract

The order Carnivora, which currently includes 296 species classified into 16 families, is distributed across all continents. The phylogeny and the timing of diversifications are still a matter of debate.

Here, complete mitochondrial genomes were analysed to reconstruct the phylogenetic relationships and to estimate divergence times among species of Carnivora. We assembled 51 new mitogenomes from 13 families, and aligned them with available mitogenomes by selecting only those showing more than 1% of nucleotide divergence and excluding those suspected to be of low-quality or from misidentified taxa. Our final alignment included 220 taxa representing 2,442 mitogenomes. Our analyses led to a robust resolution of suprafamilial and intrafamilial relationships. We identified 22 fossil calibration points to estimate a molecular timescale for carnivorans. According to our divergence time estimates, crown carnivorans appeared during or just after the Early Eocene Climatic Optimum; all major groups of Caniformia (Cynoidea/Arctoidea; Ursidae; Musteloidea/Pinnipedia) diverged from each other during the Eocene, while all major groups of Feliformia (Nandiniidae; Feloidea; Viverroidea) diversified more recently during the Oligocene, with a basal divergence of *Nandinia* at the Eocene/Oligocene transition; intrafamilial divergences occurred during the Miocene, except for the Procyonidae, as *Potos* separated from other genera during the Oligocene.

Key-words: Carnivora, Caniformia, Feliformia, mtDNA, molecular dating, base composition

Introduction

52

53   The order Carnivora is composed of 296 extant species (IUCN, 2020) currently ranged

54 into two suborders: the Caniformia which includes nine families, namely the Canidae (dog-

55 like species), Ailuridae (red panda), Mephitidae (skunks and stink badgers), Mustelidae

56 (weasels, badgers, martens, otters, etc.), Odobenidae (walrus), Otariidae (eared seals),

57 Phocidae (earless seal), Procyonidae (raccoons, coatis, kinkajous, etc.), and Ursidae (bears);

58 and the Feliformia which is represented by seven families, namely the Felidae (cat-like

59 species), Eupleridae (Malagasy carnivorans), Herpestidae (mongooses), Hyaenidae (hyenas),

60 Nandiniidae (African palm civet), Prionodontidae (Asiatic linsangs), and Viverridae (civets

61 and genets). The oldest known fossils of Carnivora have been found in the late Paleocene;

62 they belong to the extinct families Miacidae and Viverravidae, and have small body-size,

63 comparable to extant weasels and martens (Spaulding & Flynn, 2012; Solé et al., 2014; 2016).

64 The timing of the emergence of the crown carnivorans and their relationships to Paleocene

65 and Eocene fossils are still unresolved. However, there is consensus for supporting two higher

66 fossil taxa: the Carnivoraformes, which are composed of the crown group plus the stem

67 family Miacidae, which is probably paraphyletic; and the Carnivoramorpha, which groups the

68 Carnivoraformes and the Viverravidae (Flynn et al., 2010; Solé et al., 2016).

69   The phylogeny of several carnivoran families has been extensively studied based on

70 mitochondrial and nuclear data, including the Felidae, the Mustelidae, and the Ursidae

71 (Kutschera et al., 2014; Li et al., 2016; Law et al., 2018), while other families remain poorly

72 studied, specifically the Eupleridae, Herpestidae, Mephitidae, Procyonidae, and Viverridae.

73 The phylogeny and timescale of diversification of the Carnivora have been studied by Eizirik

74 et al. (2010) using a molecular supermatrix of 7,765 base pairs (bp) containing 14 nuclear

75 genes for 50 species, which represents less than 17% of the species diversity of the order

76 Carnivora. The evolutionary history of carnivorans has also been inferred using a supertree

77 approach by Nyakatura & Bininda-Emonds (2012). These two studies were based on different

78   methods and data, and different fossils were used as calibration points. Although several

79   nodes showed similar ages in the two studies, such as the most recent common ancestor

80   (MRCA) of the Carnivora (59.2 versus 64.9 Mya), Arctoidea (42.6 vs 47.8 Mya) and

81   Pinnipedia (24.5 vs 22.4 Mya), some nodes were highly discordant, including the MRCA of

82   the Caniformia (48.2 vs 61.2 Mya), Feliformia (44.5 vs 53.2 Mya), Feloidea (Felidae +

83   Prionodontidae) (33.3 vs 52.9 Mya) and Canidae (7.8 vs 16.3 Mya). Most other studies to

84   date have focused on either the Caniformia (e.g. Law et al., 2018) or the Feliformia (e.g. Zhou

85   et al., 2017) and the timing of diversification in some families appears highly elusive or

86   uncertain in the absence of a molecular timescale based on a high diversity of species from all

87   carnivoran families.

88   With the development of next-generation sequencing (NGS) technologies, the number

89   of mitochondrial genomes available in the international nucleotide databases has considerably

90   increased during the last decades (Coissac et al., 2016). For the order Carnivora, there are

91   currently more than 2,400 complete mitogenomes, and some of these were sequenced from

92   Pleistocene fossils, such as polar bear (Lindqvist et al., 2010), giant short-faced bears

93   (Mitchell et al., 2016), cave lion (Barnett et al., 2016) or saber-toothed cats (Paijmans et al.,

94   2017). This huge and diversified dataset offers an excellent opportunity to better understand

95   the evolutionary history of the order Carnivora, as problematic sequences and taxonomic

96   issues can be more easily detected, and more importantly, as many fossils can be included as

97   calibration points for estimating divergence times. The latter aspect is particularly relevant

98   considering the paleontological record of Carnivora has been significantly improved over the

99   last ten years, with the discovery of several key fossils (e.g. Abella et al., 2012; Grohé et al.,

100   2013; Tseng et al., 2014; Berta et al., 2018; Valenciano et al., 2020).

101   Here we analysed complete mitochondrial genomes to reconstruct phylogenetic

102   relationships among carnivorans and to estimate divergence times. We sequenced 43

103   mitogenomes using various methods (Sanger sequencing of PCR products, or next-generation

104   sequencing of long PCR products or Illumina shotgun sequencing). We also assembled eight

105   mitogenomes from Sequence Read Archive (SRA) data. The 51 new mitogenomes belong to

106   several families, namely Canidae (2), Eupleridae (6), Felidae (4), Herpestidae (12), Hyaenidae

107   (1), Mustelidae (10), Otariidae (2), Phocidae (1), Prionodontidae (1), Procyonidae (3),

108   Ursidae (1), Viverridae (7) for Carnivora, as well as Tapiridae (1) for the order

109   Perissodactyla, which was included as an outgroup. These new mitogenomes were compared

110   to all mitogenomes available for Carnivora in public repositories. At the intraspecific level,

111   we selected only mitogenomes that were separated by more than 1% of nucleotide divergence

112   and excluded those suspected to be of low-quality or from misidentified taxa. Our final

113   alignment includes 220 taxa, which represent 2,442 mitogenomes. Our main objective was to

114   build one of the largest time-trees of Carnivora estimated using a selection of fossil

115   calibration points in order to provide further insights into the evolution of this broadly

116   distributed and morphologically diverse taxonomic group.

117

Material and Methods

DNA extraction, amplification, sequencing and mitogenome assembly

120   Total DNA was extracted from cells, muscle, or skin samples using the DNeasy Blood

121   and Tissue Kit (Qiagen, Hilden, Germany). Details on the 43 samples extracted for this study

122   are given in S1 Appendix. The mitochondrial genomes were sequenced using one of the three

123   following approaches: Sanger sequencing of $\approx$ 20 overlapping PCR products (length between

124   700 and 2000 bp); next-generation sequencing (NGS) of five overlapping long PCR products

125   of around 4-5 kb; and shotgun Illumina sequencing.

126   In the first approach, PCR amplifications were carried out as described in Hassanin et

127   al. (2012) using the primers listed in S2 Appendix. The amplicons were then sequenced in

128   both directions by Eurofins MWG Operon (Ebersberg, Germany). Genomes were assembled

129   with electropherograms of overlapping amplicons using Sequencher 5.1 (Gene Codes

130   Corporation, Ann Arbor, MI, USA).

131   In the second approach, five overlapping PCR products of around 4-5 kb were

132   amplified as described in Hassanin et al. (2020), and they were sequenced at the "Service de

133   Systématique Moléculaire" (UMS CNRS 2700, MNHN, Paris, France) using the Ion Torrent

134   Personal Genome Machine (Thermo Fisher Scientific, Waltham, MA, USA).

135   The third approach was based on shotgun Illumina sequencing. DNA samples were

136   quantified with a Qubit® 2.0 Fluorometer using the Qubit dsDNA HS Assay Kit (Thermo

137   Fisher Scientific, Waltham, MA, USA). Libraries were prepared using the TruSeq® Nano

138   DNA Library Prep kit (Illumina, San Diego, CA, USA) after pooling 150 ng of total DNA of

139   10 species belonging to distant taxonomic groups (i.e. different phyla, classes, orders or

140   families). Libraries were sequenced at the "Institut du Cerveau et de la Moelle épinière"

141   (Paris, France) using a NextSeq® 500 system and the NextSeq 500 High Output Kit v2 (300

142   cycles) (Illumina).

143   The NGS reads generated with either Ion Torrent or Illumina sequencers were

144   assembled by baiting and iterative mapping approach on Geneious® 10.2.2 (Biomatters Ltd.,

145   Auckland, New Zealand) using available mitochondrial references, including cytochrome b,

146   cytochrome c oxidase subunit I, 12S and 16S rRNA genes. The 43 new mitochondrial

147   genomes generated for this study were annotated on Geneious and deposited in GenBank

148   under accession numbers XXXXXX-XXXXXX.

149   Eight mitochondrial genomes were assembled from SRA downloaded from NCBI for

150   the following species: *Bassaricyon neblina* (SRX1097850), *Bassariscus sumichrasti*

151   (SRX1099089), *Helogale parvula* (SRR7637809), *Lontra canadensis* (SRR10409165),

152   *Mirounga angustirostris* (SRR10331586), *Mungos mungo* (SRR7704821), *Prionodon linsang*

153   (ERR2391707), and *Zalophus wollebaeki* (SRR4431565).

154

Mitochondrial alignments

156   The 51 mitochondrial genomes assembled in this study were compared with other

157   genomes available in the NCBI nucleotide database (see accession numbers in S1 Appendix)

158   for the different families. At the intraspecific level, we selected only mitochondrial haplotypes

159   separated by more than 1% of divergence. Our final alignment includes 218 mitogenomes of

160   Carnivora representing a large taxonomic diversity in the 16 following families (in

161   parentheses number of mitogenomes): Ailuridae (2), Canidae (21), Eupleridae (7), Felidae

162   (46), Herpestidae (15), Hyaenidae (4), Mephitidae (3), Mustelidae (39), Nandiniidae (1),

163   Odobenidae (1), Otariidae (12), Phocidae (30), Prionodontidae (2), Procyonidae (6), Ursidae

164   (21), and Viverridae (19). A tapir (*Tapirus terrestris*) and a pangolin (*Phataginus tricuspis*)

165   were used to root the carnivoran tree because they represent two Laurasiatherian orders,

166   Perissodactyla and Pholidota, respectively, which were shown to be closely related to

167   Carnivora in previous molecular studies (e.g. Murphy et al., 2001; Meredith et al., 2011).

168   The 220 mitochondrial genomes were aligned using AliView 1.22 (Larsson, 2014).

169   Ambiguous regions for primary homology were excluded from the alignment. To limit the

170   impact of missing data, we also removed from the alignment all indels (insertions or

171   deletions) detected in only one genome. The final alignment, named *mtDNA*, contains 220

172   taxa and 14,892 nucleotide sites. Two other datasets were used for the analyses: (1) the

173   *mtDNA-Tv* dataset (transversions only), which corresponds to the *mtDNA* dataset in which the

174   nucleotide G was replaced by A and the nucleotide T by C; and (2) the *PCG-DNA* dataset

175   (10,809 nt) in which all regions other than protein-coding genes were removed (i.e. control

176   region, 12S and 16S rRNA genes and tRNA genes), as well as the *ND6* gene (because it is

177   located on the opposite strand of other protein-coding genes). The three datasets used in this

178   study are available at https://osf.io/XXX.

179

Analysis of base composition

181   The alignment of the protein-coding genes of 220 mitochondrial genomes (*PCG-DNA*

182   dataset) was used to calculate the frequency of the four bases (A, C, G and T) at each of the

183   three codon-positions (S3 Appendix). The twelve variables measured were then summarized

184   by a principal component analysis (PCA) using the FactoMineR package (Lê et al., 2008) in

185   R version 3.5.3 (from http://www.R-project.org/). The strand bias in nucleotide composition

186   was studied at third codon-positions of the *PCG-DNA* dataset by calculating the relative

187   frequencies of A and T nucleotides (AT3 skew = [A - T] / [A + T]) and the relative

188   frequencies of C and G nucleotides (CG3 skew = [C - G] / [C + G]) (Lobry, 1995; Hassanin et

189   al., 2005; Arabi et al., 2010).

190

Phylogenetic analyses

192   Two datasets (*mtDNA* and *mtDNA-Tv*) were analysed with probabilistic methods for

193   tree reconstruction using the resources available from the CIPRES Science Gateway (Miller et

194   al., 2010). The Bayesian analyses were done with MrBayes 3.2.7 (Ronquist et al., 2012) and

195   the two following models: GTR+I+G for the *mtDNA* dataset, and JC69+I+G for the *mtDNA-*

196   *Tv* dataset. The posterior probabilities (PP) were calculated using 10,000,000 Metropolis-

197   coupled MCMC generations, tree sampling every 1000 generations, and a burn-in of 25%.

198   To examine the phylogenetic signal along the *mtDNA* dataset, we also performed

199   Bayesian analyses (with the same parameters) on 10 half-overlapping sub-datasets (i–x) of the

200   about the same length (i.e., 2978 or 2980 nt), corresponding to the following positions: (i) 1-

201   2978; (ii) 1489-4466; (iii) 2979-5956; (iv) 4467-7444; (v) 5957-8934; (vi) 7445-10422; (vii)

202   8935-11912; (viii) 10423-13400; (ix) 11913-14892; and (x) 13401-14892 + 1-1488. The use

203   of half-overlapping sub-datasets (sliding window of ≈ 2978 nt) implies that all nucleotide

204   sites of the total *mtDNA* alignment are represented twice in these Bayesian analyses. The lists

205   of bipartitions obtained from Bayesian analyses of the 10 sub-datasets were transformed into

206   a weighted binary matrix for supertree construction using SuperTRI v57 (Ropiquet et al.,

207   2009). Each binary character corresponds to a node, which was weighted according to its

208   frequency of occurrence in one of the 10 lists of bipartitions. In this manner, the SuperTRI

209   method takes into account both principal and secondary signals, because all phylogenetic

210   hypotheses found during the 10 Bayesian analyses were used for the calculation of the

211   following two reliability indices for each node of interest: (1) the supertree bootstrap

212   percentages (SBP) were obtained from PAUP 4* version 4b10 (Swofford, 2003) after 1000

213   bootstrap replicates of the MRP (Matrix Representation with Parsimony) matrix of 3,398

214   binary characters (reconstructed under SuperTRI v57); and (2) the mean posterior

215   probabilities percentages (MPP) were directly calculated on SuperTRI v57. The SBP and

216   MPP values were reported on the Bayesian tree found with the total alignment of 14,892 nt.

217   Here, the SuperTRI analyses were conducted to test for phylogenetic signal along the mtDNA

218   genome. If a robust node in the Bayesian tree (PP ≥ 0.95) is recovered with high SBP (≥ 95%)

219   and MPP values (≥ 0.80), it means that the signal is present all along the mtDNA genome. If a

220   node in the Bayesian tree is recovered with low MPP values (< 0.50), it means that the signal

221   is weak or confined to a few fragments of the mtDNA genome. If there is a robust topological

222   conflict between Bayesian and SuperTRI results, it suggests that at least one of the studied

223   genomes was partially contaminated by a mitochondrial DNA sequence from another species

224   or by a nuclear DNA sequence of mitochondrial origin (Numt). An example was published in

225   Hassanin et al. (2010) for the mitochondrial genomes of domestic goat.

226

227 **Molecular dating**

228   Divergence times were estimated on the CIPRES Science Gateway (Miller et al.,

229 2010) using the *mtDNA* dataset and the Bayesian approach implemented in BEAST v.2.4.7

230 (Bouckaert et al., 2014). Twenty-two calibration points were selected for molecular dating

231 (**Table 1**). Most of these were interpreted from the fossil record using maximum (Max) and

232 minimum (Min) ages. We applied two strategies for fossil calibration: (1) a uniform

233 distribution between Max and Min on the calibrated node ages; or (2) a log-normal

234 distribution on the calibrated node ages using Min as offset, M = Max - Min / 4, and S =

235 0.926 (to match the 97.5% quantile to Max) (see details in Table 1). The second strategy

236 relies on the fact that minimum ages are generally more accurate and reliable than maximum

237 ages because younger fossils are more abundant and more accurately dated than older fossils

238 as a consequence of taphonomic processes and dating methods (Crees et al., 2019). We

239 applied a GTR + I + G model for the *mtDNA* alignment (with a proportion of invariants of

240 0.425) and a relaxed-clock model with uncorrelated lognormal distribution for substitution

241 rates. Node ages were estimated using a calibrated Yule speciation prior and 2.10^8

242 generations, with tree sampling every 2,000 generations, and a burn-in of 25% generations.

243 MCMC mixing efficiency and convergence were assessed using the ESS values (>200) in

244 Tracer v.1.7.1. The chronogram was reconstructed with TreeAnnotator, which is included in

245 the BEAST package (Bouckaert et al., 2014).

246

247 **Results**

248 **Variation in base composition**

249   The base composition (frequency of the nucleotides A, C, G and T) was analysed at

250 the three codon-positions of the *PCG-DNA* dataset (S3 Appendix). The 12 variables measured

251 for 220 taxa were summarized by a principal component analysis (PCA) based on the first

252 two principal components (PC), which contribute 45.80% and 24.48% of the total variance,

253   respectively (**Figure 1**). The variables factor map shows that the variance can be explained by

254   similar differences in base composition at the three codon-positions. Some taxa have a

255   mtDNA genome characterized by a divergent base composition.

256   Most members of the Mustelidae are found near the middle of the graph, except

257   *Mellivora* because its mtDNA genome contains a higher percentage of guanine (15.65%

258   *versus* "mean in other Mustelidae" [MoM] = 13.18%) and a lower percentage of adenine

259   (28.35% *versus* MoM = 30.95%). This trend is observed at each of the three codon positions,

260   and is more marked at third positions (S3 Appendix): G1 = 22.19% *versus* MoM = 20.87%,

261   G2 = 12.19% *versus* MoM = 11.84%, G3 = 12.58% *versus* MoM = 6.82%; A1 = 30.55%

262   *versus* MoM = 31.95%, A2 = 19.13% *versus* MoM = 19.41%, A3 = 35.38% *versus* MoM =

263   41.51%.

264   Within Viverridae, the mitogenome of the otter civet (*Cynogale bennettii*) is

265   characterized by a higher percentage of thymines (32.90% *versus* "mean in other Viverridae"

266   [MoV] = 30.73%) (**Figure 1**). This is observed at each of the three codon positions and is

267   more marked at third positions: T1 = 25.47% *versus* MoV = 23.79%, T2 = 42.71% *versus*

268   MoV = 42.17%, T3 = 30.52% *versus* MoV = 26.21%.

269   Within Ursidae, the giant panda (*Ailuropoda melanoleuca*) shows a different base

270   composition, with more adenines (30.63% *versus* "mean in other Ursidae" [MoU] = 29.55%)

271   and thymines (31.02% *versus* MoU = 28.98%) than other species of the family. This trend is

272   mainly explained by differences at third codon-positions, in which the giant panda shows

273   more adenines (40.91% *versus* MoU = 38.46%) and thymines (28.52% *versus* MoU =

274   23.38%).

275   Most members of the Felidae, including *Smilodon populator* (Machairodontinae), are

276   found near the middle of the graph, except *Homotherium latidens* (Machairodontinae) for

277   which the mtDNA genome shows a very atypical base composition. At third codon-positions,

278   *Homotherium* is characterized by a lower percentage of guanine (2.80% *versus* "mean in other

279   Felidae" [MoF] = 6.25%) and a higher percentage of thymine (25.41% *versus* MoF =

280   20.79%). At second codon-positions, its mitogenome contains a lower percentage of thymine

281   (40.80% *versus* "mean in other Felidae" [MoF] = 41.89%) and a higher percentage of guanine

282   (12.58% *versus* MoF = 11.86%). At first codon-positions, the mtDNA of *Homotherium* shows

283   a lower percentage of adenine (30.08% *versus* "mean in other Felidae" [MoF] = 32.07%), a

284   higher percentage of guanine (22.65% *versus* MoF = 20.92%), and a higher percentage of

285   guanine (23.07% *versus* MoF = 22.01%).

286   Within Canidae, the two extant tribes can be distinguished based on their nucleotide

287   composition, as the mitogenomes of the Vulpini (fox-like canines) show more guanines ($\geq$

288   13.89% *versus* $\leq$ 13.28%) and less adenines ($\leq$ 29.53% *versus* $\geq$ 29.83%) than those of the

289   Canini (dog-like canines). This trend is mainly explained by a strong bias observed at third

290   codon-positions, in which the Vulpini taxa have more guanines (G3 $\geq$ 8.39% *versus* $\leq$ 7.30%)

291   and less adenines (A3 $\leq$ 37.18% *versus* $\geq$ 37.80%) than the Canini.

292   At third codon-positions, the Eupleridae and Hyaenidae have higher percentages of

293   cytosines than other Feliformia (mean C3: Eupleridae = 36.78%; Hyaenidae = 35.55%; other

294   Feliformia = 30.65%).

295

296   **Pairwise mtDNA distances**

297   Uncorrected pairwise distances were calculated on PAUP4* (Swofford, 2003) using

298   the *mtDNA* dataset (S4 Appendix).

299   Intraspecific similarity distances are generally lower than 2%. However, there are

300   several exceptions involving individuals from different geographic regions, which are or

301   could be assigned to different subspecies or even species (see discussion): the extinct

302   Japanese river otter (*Lutra lutra*, LC050126) *versus* extant representatives of *Lutra lutra*

303   (2.1%); *Ursus arctos isabellinus versus* other brown bear subspecies (2.3-2.4%); *Ursus*

304   *thibetanus laniger versus Ursus thibetanus mupinensis* (2.3%); the three samples of *Melogale*

305   *moschata* (2.2-2.9%); the three samples of *Viverricula indica* (2.5-3.1%); *Canis lupus*

306   *familiaris versus Canis lupus chanco* (2.6%); the two samples of *Paradoxurus*

307   *hermaphroditus* (3.0%); the two samples of *Leopardus pardalis* (2.9%); the two mtDNA

308   lineages identified in *Prionailurus bengalensis* (3.6%); the two samples of *Herpestes*

309   *javanicus* (4.5%), although BLAST searches in NCBI suggest that the sequence NC_006835

310   belongs in fact to *Herpestes auropunctatus* (see discussion); the two samples of *Mungos*

311   *mungo* (6.3%), but we suggest that the sample SRR7704821 may belong in fact to *Mungos*

312   *gambianus* (see discussion).

313   Interspecific distances are generally higher than 2% of similarity. However, several

314   species show more similar mitogenomes: *Martes martes* and *Martes zibellina* (1.9%); *Phoca*

315   *largha* and *Phoca vitulina* (1.9%); *Mustela putorius versus Mustela nigripes* (1.6%) and

316   *Mustela eversmannii* (1.2%); *Felis catus* and *Felis silvestris* (0.7%); *Zalophus californianus*

317   and *Zalophus wollebaeki* (0.5%); *Urocyon cinereoargenteus* and *Urocyon littoralis* (0.4%).

318   Within *Arctocephalus forsteri*, there are two mtDNA lineages: 17 mitogenomes are similar to

319   the mitogenome of *Arctocephalus australis* (MG023139) (1.3%), whereas the 28 other

320   mitogenomes are more divergent (2.1%). Within *Ursus arctos*, we found six mtDNA lineages

321   showing between 1.1% and 2.4% of nucleotide divergence, but brown bears sampled on the

322   Alaskan ABC islands (northern portion of the Alexander Archipelago) have mitogenomes

323   which are highly similar to those of *Ursus maritimus* (0.4-0.5%).

324

325   **Phylogeny of Carnivora**

326   The Bayesian tree of **Figure 2** was reconstructed from the *mtDNA* alignment. The

327   results show that most nodes of the tree were highly supported (PP ≥ 0.95) and were similarly

328   recovered as monophyletic entities using two other methods (nodes highlighted with a filled

329   back circle in **Figure 2**), i.e. in the Bayesian tree reconstructed using the *mtDNA-Tv*

330   alignment and JC69+I+G model (S5 Appendix) and in the Bootstrap 50% majority-rule

331   consensus tree reconstructed from the MRP matrix of the SuperTRI analysis (S6 Appendix).

332   Many of these nodes show maximal support in the SuperTRI analysis, as they were found

333   monophyletic in all 10 Bayesian trees reconstructed from the 10 half-overlapping sub-datasets

334   of the *mtDNA* alignment. For all these nodes, the phylogenetic signal is therefore robust

335   across all parts of the *mtDNA* alignment.

336   Among the nodes supported by all three methods of tree reconstruction and at least 7

337   of the 10 Bayesian SuperTRI analyses are the following taxa: order Carnivora; suborders

338   Caniformia and Feliformia; infraorders Arctoidea, Cynoidea, Feloidea and Viverroidea; all

339   the 14 families represented by at least two members in our analyses (i.e. all families except

340   Odobenidae and Nandiniidae); the subfamilies Euplerinae, Felinae, Galidiinae, Genettinae,

341   Guloninae, Helictidinae, Hemigalinae, Herpestinae, Ictonychinae, Lutrinae,

342   Machairodontinae, Melinae, Monachinae, Mungotinae, Mustelinae, Paradoxurinae, Phocinae,

343   Tremarctinae, Ursinae and Viverrinae; the genera *Catopuma*, *Felis*, *Genetta*, *Herpestes*,

344   *Leopardus*, *Lutra*, *Lynx*, *Martes*, *Meles*, *Mirounga*, *Monachus*, *Mungos*, *Panthera*,

345   *Paradoxurus*, *Phoca*, *Prionailurus*, *Prionodon*, *Puma*, *Urocyon*, *Vulpes* and *Zalophus*; and

346   the species *Ailurus fulgens*, *Canis lupus*, *Civettictis civetta*, *Leopardus pardalis*, *Lutra lutra*,

347   *Melogale moschata*, *Mustela sibirica*, *Panthera onca*, *Panthera pardus*, *Panthera tigris*,

348   *Panthera uncia*, *Paradoxurus hermaphroditus*, *Prionailurus planiceps*, *Procyon lotor*, *Ursus*

349   *maritimus, Ursus spelaeus*, *Ursus thibetanus* and *Viverricula indica*. The results support the

350   non-monophyly of four genera: (1) *Arctocephalus*, because *Arctocephalus pusillus* is

351   divergent from *Neophoca*, *Phocarctos* and other species of *Arctocephalus*; (2) *Canis*, because

352   *Canis adustus* is more distantly related to other *Canis* species than to *Cuon alpinus*; (3)

353   *Mustela*, because *Mustela frenata* is the sister-species of *Neovison vison*, whereas all other

354   species of *Mustela* are enclosed into a robust clade; and (4) *Ursus*, because *Helarctos*

355   *malayanus* is closely related to *Ursus americanus* and *Ursus thibetanus*, whereas *Ursus*

356   *arctos*, *Ursus maritimus*, and *Ursus spelaeus* are grouped together. In addition, three species

357   are not found monophyletic: (1) *Arctocephalus forsteri* is paraphyletic with respect to

358   *Arctocephalus australis*; (2) *Prionailurus bengalensis* is paraphyletic with respect to

359   *Prionailurus viverrinus*; and (3) *Ursus arctos* is paraphyletic with respect to *Ursus maritimus*.

360

361   **BEAST chronogram inferred from the *mtDNA* alignment**

362   Molecular estimates of divergence times are shown in the chronogram provided in

363   **Figure 3**. The ages were inferred with BEAST using the *mtDNA* alignment and the 22 fossil

364   calibration points detailed in **Table 1**. Two analyses were performed using either a uniform or

365   log-normal prior distribution on the calibrated node ages (named "U approach" and "L

366   approach", respectively). The two BEAST chronograms show the same tree topology (**Figure**

367   **3**), which is similar to the one reconstructed under MrBayes (**Figure 2**). As expected, the

368   chronogram inferred with the "L approach" show more recent estimates of divergence times

369   (values highlighted in blue in **Figure 3**) than the chronogram inferred with the "U approach".

370   Using the Geologic Time Scale v. 5.0 (Walker et al., 2018) for the correspondence

371   between estimated divergence times and geologic epochs, the results suggest that the crown

372   Carnivora divided into Caniformia and Feliformia during the early Eocene at around 52.7-

373   46.7 Mya. Subsequently, the Canidae separated from other families in the early/middle

374   Eocene at around 48.0-42.5 Mya, followed by the Ursidae and Pinnipedia in the middle/late

375   Eocene at around 43.4-38.3 Mya and 41.4-36.5 Mya, respectively. The diversification of the

376   Musteloidea began near the Eocene/Oligocene boundary at around 37.4-33.0 Mya, but the

377   Mustelidae diverged from the Procyonidae in the early Oligocene at around 34.4-30 Mya. The

378   family Phocidae separated from the Otarioidea in the Oligocene at around 27.2-22.9 Mya,

379   whereas the separation between the Otariidae and Odobenidae occurred in the early Miocene

380   at around 20.0-16.7 Mya. Within the Feliformia, the Nandiniidae diverged from other families

381   at the Eocene-Oligocene transition at around 34.4-31.1 Mya. Then, the Feloidea and

382   Viverroidea split in the Oligocene at around 29.7-27.0 Mya. The separation between the

383   Felidae and Prionodontidae occurred in the Oligocene at around 27.4-24.7 Mya. Within the

384   Viverroidea, the Viverridae diverged from other families in the Oligocene at around 28.2-25.8

385   Mya, followed by the Hyaenidae in the Late Oligocene at around 24.5-22.9 Mya, whereas the

386   split between the Eupleridae and Herpestidae took place in the Miocene at around 22.3-21.2

387   Mya. Within carnivoran families, the generic diversification occurred during the Miocene (all

388   genera of Canidae, Herpestidae, Mephitidae, Procyonidae, and Viverridae; most genera of

389   Eupleridae, Felidae, Hyaenidae, Mustelidae, Phocidae, and Ursidae) or more rarely during the

390   Pliocene (most genera of Otariidae, excepting *Callorhinus*; *Aonyx* / *Lutrogale*; *Caracal* /

391   *Profelis*; *Histriophoca* / *Pagophilus*; *Hyaena* / *Parahyaena*; *Hydrurga* / *Leptonychotes*;

392   divergence of *Galidictis*; divergence of *Helarctos*) or Pleistocene (*Halichoerus* / *Phoca* /

393   *Pusa*; *Mungotictis* / *Salanoia*).

394

Discussion

Mitogenomic variations at the species level

397   In 88% of the species for which at least two mitogenomes were available (65 out of

398   74), intraspecific distances were found to be less than 2%. In nine species, we detected

399   mtDNA distances greater than 2% (S4 Appendix). Such a high mitogenomic divergence

400   suggests that the samples may belong to two or more distinct species, because of either

401   imperfect taxonomy or species misidentification (human error). Other explanations are

402   however possible, such as a mtDNA introgression from another species or high levels of

403   mtDNA divergence due to strong female philopatry.

404   In mammals, females are generally philopatric, spending their lives close to their

405   birthplace, whereas males typically undertake longer-distance dispersals during their lives

406   (Greenwood, 1980; Dobson, 2013; Li & Kokko, 2019). Indeed, because they have to take care

407   of their young, females tend to stay in areas where they can predict the risks as well as the

408   resources. With time, this behaviour can result in a high spatial genetic structure of mtDNA

409 variation since the mitogenome is transmitted maternally. The impact of female philopatry on

410 the mtDNA evolution is expected to be more important if the preferred habitat-type is

411 delimited by strong geographic barriers representing a high risk for the dispersal of females

412 (and their young), for example, open areas for small forest fruit bats (Hassanin et al., 2015) or

413 large rivers and mountains for giraffes (Petzold & Hassanin, 2020). Female philopatry may

414 therefore result in the selection of highly divergent mtDNA haplotypes in geographically

415 isolated maternal lineages, whereas gene flow can be still maintained through long-distance

416 male dispersals. Female philopatry can be advanced to explain the high intraspecific mtDNA

417 distances found between populations of bears of the species *Ursus arctos* (6 lineages, 1.1-

418 2.4%) and *Ursus thibetanus* (4 linages, 1.0- 2.3%). This hypothesis is supported by previous

419 phylogeographic studies showing strong geographic structure of mtDNA variation (Hirata et

420 al., 2013; Wu et al., 2015) and by field data on brown bears indicating that natal dispersal

421 distances are five times smaller for females (Steyaert et al., 2012).

422 In several other species, we found two or three mitogenomic haplotypes or

423 haplogroups which are separated by more than 2%. In the Eurasian otter (*Lutra lutra*), Waku

424 et al. (2016) sequenced the mtDNA genome of three extinct river otters of Japan: the two

425 mtDNA genomes of otters from Honshu are similar to those found in extant Eurasian otters (<

426 1%), whereas the genome of the otter from Shikoku Island differs by 2.1%, suggesting that it

427 belongs to a distinct species (*Lutra nippon*) or subspecies (*Lutra lutra nippon*). Since female

428 philopatry *versus* male biased dispersal was well attested by both radiotracking and genetic

429 data in European otters (Quaglietta et al., 2013), we consider that nuclear data are needed to

430 solve this taxonomic issue.

431 The three mtDNA haplotypes available for the small-toothed ferret badger (*Melogale*

432 *moschata*) show comparatively high nucleotide distances, i.e., between 2.2 % and 2.9 %. The

433 genus *Melogale* includes five species (IUCN, 2020): *Melogale cucphuongensis* known from a

434 few animals collected in northern Vietnam (Nadler et al., 2011; Li et al., 2018), *Melogale*

435   *everetti* on Borneo, *Melogale moschata* which is widely distributed from North-east India to

436   Taiwan through China and Indochina, *Melogale orientalis* on Java, and *Melogale personata*,

437   which is found in mainland Southeast Asia. In agreement with the recent study of Rozhnov et

438   al. (2019), our phylogenetic analysis based on cytochrome b (*CYB*) sequences (S7 Appendix)

439   shows that *Melogale cucphuongensis* is the sister-group of a clade composed of three

440   divergent mtDNA lineages: (i) *Melogale personata*, as represented by 16 samples from

441   Vietnam; (ii) a first mtDNA haplogroup of *Melogale moschata*, which includes five samples

442   from Vietnam (including our V0735A sample) and the reference mitogenome (NC_020644,

443   unknown origin); and (iii) a second mtDNA haplogroup of *Melogale moschata*, which

444   contains a sample from Taiwan (subspecies *subaurantiaca*) and a sample from China

445   (MN400429, from Fei Zhou). Since the holotype of *Melogale moschata* was collected in the

446   Guangdong Province of South China (Storz & Wozencraft, 1999), our *CYB* tree suggests that

447   the two samples from China and Taiwan represent the species *Melogale moschata*, whereas

448   the samples from Vietnam currently assigned to *Melogale moschata* may belong to a new

449   species, confirming that Vietnam is a key hot spot for the genus *Melogale*.

450   Three mitogenomic haplotypes were available for the small Indian civet (*Viverricula*

451   *indica*), representing the three main mtDNA lineages previously identified in Gaubert et al.

452   (2017), i.e. the subspecies *V. i. indica* from India, Madagascar (where it was introduced), Sri

453   Lanka, West China and northern Indochina; *V. i. rasse* from southern Indochina to Java; and

454   *V. i. pallida*, from East China to Taiwan. The mitogenomic divergence are between 2.5% and

455   3.1%, suggesting that they may be treated as different species, but nuclear markers are needed

456   to further investigate this taxonomic issue. Gaubert et al. (2017) concluded that these three

457   mitogenomic lineages diverged from each other in the Pliocene, between 3.2 Mya and 2.6

458   Mya, but our dating estimates rather support that the divergences occurred in the Pleistocene,

459   between 2.2-1.9 Mya and 1.6-1.4 Mya.

460   In the common palm civet (*Paradoxurus hermaphroditus*), the two mtDNA haplotypes

461   differ by 2.9%, suggesting that they may come from two separate species. The

462   phylogeographic studies of Patou et al. (2010) and Veron et al. (2015) based on both

463   mitochondrial and nuclear sequences (*CYB*, control region, intron 7 of *FGB*) supported the

464   existence of three distinct species of *Paradoxurus*: *P. hermaphroditus* (Indian and

465   Indochinese regions), *P. musangus* (mainland Southeast Asia, Sumatra, Java and other small

466   Indonesian islands) and *P. philippinensis* (Mentawai Islands, Borneo and the Philippines).

467   Only the first two of them were sampled in our mitogenomic study. Since they have

468   overlapping range in mainland Southeast Asia, where it can be assumed that *P.*

469   *hermaphroditus* dispersed from the north of India while *P. musangus* arrived from the south

470   (see Figure 2 in Patou et al., 2010), the possibility of interspecific hybridization should be

471   investigated in this region.

472   The two mitogenomes sequenced for the ocelot (*Leopardus pardalis*) differ by 3.0%,

473   suggesting that this taxon may be split into two separate species, as proposed by Nascimento

474   (2010) based on a morphological analysis of 591 specimens of *Leopardus* spp. Using an

475   alignment of the 5'-part of the mitochondrial control region, Eizirik et al. (1998) have

476   suggested a further division into four geographic groups. However, there is a 100-bp stretch

477   of missing data in all their sequences, which is located in a region where we detected three

478   motifs of 80 bp repeated in tandem in the mitogenome of *Leopardus pardalis mitis* and only

479   two motifs in that of *Leopardus pardalis pardalis*. These repetitive sequences, named RS2 in

480   Hoelzel et al. (1994), were found in variable number in all families of Feliformia, from two in

481   *Prionodon pardicolor* (Hassanin, 2016) to five in *Nandinia binotata* (Hassanin & Veron,

482   2016). These variations in RS2 tandem repeats may pose serious problems of homology for

483   DNA alignment. If RS2 repeats are removed from the alignment of Eizirik et al. (1998), there

484   is no robust signal for phylogenetic relationships within *Leopardus pardalis* (data not shown).

485   We recommend therefore to study the phylogeography of ocelot lineages with mitochondrial

486   protein-coding genes, such as *CYB* and *CO1* genes, or full mitogenomes. For taxonomy

487   purpose, the analyses should be completed with nuclear markers, as Wultsch et al. (2016)

488   have shown evidence for female philopatry *versus* male-biased dispersal in ocelots sampled in

489   Belize (Central America).

490   Two divergent mtDNA haplogroups were found for the leopard cat *Prionailurus*

491   *bengalensis* (3.6%), corresponding to the Asian mainland leopard cat and Sunda leopard cat

492   (Luo et al., 2014; Patel et al., 2017). A species-level separation was confirmed by Y

493   chromosome and whole-genome SNP studies (Luo et al., 2014; Li et al., 2016). Therefore, we

494   follow Kitchener et al. (2017) in recognizing two species of leopard cats: the mainland

495   leopard cat *Prionailurus bengalensis* for Asian mainland leopard cats, and the Sunda leopard

496   cat *Prionailurus javanensis* for leopard cats from Java, Sumatra and Borneo. Previous

497   molecular estimates have provided different ages for their divergence, between 2.67 Mya and

498   0.92 Mya (Luo et al., 2014; Li et al., 2016; Patel et al., 2017). Although based on different

499   calibration points, our estimate of 2.7-2.4 Mya corroborates that of Luo et al. (2014). This

500   suggests that maternal linages were first isolated at the Pliocene/Pleistocene boundary, when

501   the glacial/interglacial oscillations started. However, the mitogenomic tree does not support a

502   sister-group relationship between the two species of leopard cats, because *Prionailurus*

503   *bengalensis* is closer to *Prionailurus viverrinus*, the fishing cat, than to *Prionailurus*

504   *javanensis*. This result contrasts with the nuDNA trees of Luo et al. (2014) and Li et al.

505   (2016) showing a sister relationship of *Prionailurus bengalensis* and *Prionailurus javanensis*.

506   In agreement with the scenario proposed by Li et al. (2016), such a discordance suggests that

507   a mtDNA introgression occurred from *Prionailurus bengalensis* to *Prionailurus viverrinus* in

508   the Pleistocene epoch, here dated at 2.1-1.8 Mya.

509   The two mitogenomes of the Javan mongoose (*Urva javanica*, previously named

510   *Herpestes javanicus*, see Patou et al., 2009 for details) differ by 4.5% in our alignment.

511   BLAST searches in NCBI show that the reference genome sequenced for *Urva javanica*

512 (NC_006835) belongs in fact to its sister-species, *Urva auropunctata* (Small Indian

513 mongoose, previously named *Herpestes javanicus* or *Herpestes auropunctatus* (Patou et al.,

514 2009). In agreement with this, the sample was collected in Fiji where the Small Indian

515 mongoose is known to have been introduced (Veron & Jennings, 2017).

516 Two highly divergent mtDNA haplotypes (6.3%) were detected in banded mongooses

517 *Mungos mungo* from Halle Zoological Garden (Germany) and from the San Diego Zoo

518 Institute for Conservation Research (USA). The geographic origins of these animals are

519 unknown, but such a high pairwise distance suggests that the two mitogenomes may represent

520 the two species currently described in the genus *Mungos*: the banded mongoose (*Mungos*

521 *mungo*), which is widely distributed in sub-Saharan Africa, and the Gambian mongoose

522 (*Mungos gambianus*), which is endemic to West Africa. BLAST searches in NCBI show that

523 our MMC78 sample share 99.9% of *CYB* identity with a mongoose from Kenya (AY928674;

524 Koepfli et al., 2006), confirming that it belongs to the species *Mungos mungo*. By contrast,

525 the sample SRR7704821 shows only 93% of identity with *Mungos mungo* AY928674,

526 suggesting that alternatively it may belong to *Mungos gambianus*. Morphologically, the two

527 species are similar but *Mungos mungo* is characterized by 10 to 15 dark dorsal stripes that are

528 absent in *Mungos gambianus*. These marked differences render the hypothesis of

529 misidentification unlikely. As an alternative, a mitochondrial introgression from *Mungos*

530 *gambianus* to *Mungos mungo* may have occurred either in captivity or in the wild, as the two

531 species can be found in sympatry in West Africa (IUCN, 2020).

532

Shallow mitochondrial phylogeny

534 The mtDNA distances calculated between closely-related species are generally higher

535 than 2% (73% of the comparisons detailed in S4 Appendix). However, there are several

536 exceptions in the genera *Arctocephalus*, *Felis*, *Martes*, *Mustela*, *Phoca*, *Urocyon*, *Ursus* and

537 *Zalophus*. Four main hypotheses can be advanced to explain the existence of similar mtDNA

538   genomes in two putative species: species misidentification (human error), imperfect

539   taxonomy (species synonymy), mtDNA introgression, or recent speciation event during the

540   Pleistocene (Hassanin et al., 2012).

541   Within *Arctocephalus*, two divergent mtDNA lineages (2.1%) were sequenced for the

542   New Zealand fur seal (*Arctocephalus forsteri*) (Emami-Khoyi et al., 2017). One of them was

543   found to be more similar to the single mitogenome available for the South American fur seal,

544   *Arctocephalus australis* (1.3%). The mitochondrial paraphyly of *Arctocephalus forsteri*

545   suggests a mtDNA introgression from *Arctocephalus australis* at 0.8-0.7 Mya. Nuclear data

546   should be sequenced to confirm this hypothesis and to further explore a possible taxonomic

547   issue between South American and New Zealand fur seals.

548   Within *Felis*, the mitogenome of the domestic cat (*Felis catus*) is very similar to those

549   of the wild cat, *Felis silvestris* (0.7%), including the African wild cat (*Felis silvestris lybica*),

550   European wild cat (*Felis silvestris silvestris*) and Chinese desert cat (*Felis silvestris bieti*).

551   These three subspecies and the domestic cat are either considered separate species (Kitchener

552   et al., 2017) or subspecies of *Felis silvestris* (e.g. Driscoll et al., 2007; Mattucci et al., 2015).

553   Such a genome similarity between the domestic cat and wild cat was expected because the cat

554   was domesticated from the near Eastern wild cat *Felis silvestris lybica* (Driscoll et al., 2007)

555   and can hybridize with wild forms (Randi, 2008). Our results confirm that these taxa should

556   be considered subspecies rather than species.

557   Within *Martes*, the Pine marten (*Martes martes*) from western Europe and the Sable

558   (*Martes zibellina*) from Siberia and adjacent areas have quite similar mitogenomes (1.9%

559   divergence), and our estimation of divergence time is 1.1-1.0 Mya, which is in perfect

560   agreement with Law et al. (2018). However, Rozhnov et al. (2010) have detected high levels

561   of reciprocal mtDNA introgression in northern Urals, where populations of the two species

562   are found in sympatry. In this hybrid zone, levels of gene flow should be further studied using

563   nuclear markers to determine if introgression between the two taxa is asymmetric or not.

564   Within *Mustela*, several species show low levels of mitogenomic divergence (1.2-1-

565   6%), including the western polecat (*M. putorius*) distributed in western Europe, the steppe

566   polecat (*M. eversmanii*) found in eastern Europe and Asia, and the black-footed ferret (*M.*

567   *nigripes*) endemic to North America. In addition, the mitogenome of the European mink, *M.*

568   *lutreola* (MT304869, not included in our phylogenetic analyses) shares between 99.1% and

569   99.2 % of identity with the four mitogenomes available for *M. putorius*. Our estimations

570   indicate that these four closely related species of *Mustela* have diverged from each other after

571   1.1-1.0 Mya. However, *M. putorius*, *M. eversmanii*, and *M. lutreola* can be found in sympatry

572   in Europe (IUCN, 2020), suggesting possible mtDNA introgression during the Pleistocene

573   epoch. Cabria et al. (2011) have shown evidence for mtDNA introgression from *M. lutreola*

574   to *M. putorius*, resulting apparently from asymmetric interspecific hybridization, i.e.

575   involving only females of European mink and males of polecat. Since the western and steppe

576   polecats are known to occasionally hybridize in Eastern Europe (Abramov et al., 2016), gene

577   flow should be also studied between the two taxa.

578   Within *Phoca*, harbor seals (*P. vitulina*) and spotted seals (*P. largha*) are parapatric

579   sibling species, showing 1.9% of differences in our mitogenomic alignment. Although the two

580   species are known to hybridize in captivity (IUCN, 2020), they were found reciprocally

581   monophyletic in previous mtDNA studies (Nakagawa et al., 2010; Cordes et al., 2017) and

582   there is no evidence of mixed ancestry in wild populations found in sympatry (e.g. in Alaska;

583   Cordes et al., 2017). The hypothesis of a recent Pleistocene speciation, at 1.1-1.0 Mya

584   according to our estimation, is therefore the most likely.

585   Within *Urocyon*, the island fox (*U. littoralis*), restricted to six California Channel

586   Islands located off the coast of southern California, USA, and the mainland grey fox (*U.*

587   *cinereoargenteus*) share very similar mtDNA genomes (0.4%). In addition, the species *U.*

588   *cinereoargenteus* was found to be paraphyletic in the mitochondrial tree of Hofman et al.

589   (2015) due to the inclusive placement of *U. littoralis*. Even if the island fox is approximately

590   25% smaller than the mainland grey fox (Schutz et al., 2009), we suggest that the island fox

591   should be rather treated as a subspecies of *U. cinereoargenteus*. Nuclear genome comparisons

592   are however needed to definitively clarify this taxonomic issue.

593   Within *Ursus*, bears living on the Alaskan ABC islands have mitogenomes which are

594   very similar (0.4-0.5%) to those sequenced for extant polar bears and the fossil of Svalbard

595   dated between 130 ka and 110 kya (Lindqvist et al., 2010). Two possible scenarios of

596   mitochondrial introgression have been previously proposed: Hailer et al. (2012) suggested

597   that the ancestor of polar bears was introgressed by brown bears between 166 and 111 kya;

598   whereas Hassanin (2015) suggested that different populations of brown bears were

599   introgressed by polar bears at two glacial periods of the Pleistocene, at 340 ± 10 ka in western

600   Europe, and at 155 ± 5 ka on the ABC islands, and probably also in Beringia and Ireland

601   based on ancient DNA sequences.

602   Within *Zalophus*, two species are currently recognized: the Californian Sea Lion (*Z.*

603   *californianus*), which is found on the Pacific coasts of North America; and the Galápagos sea

604   lion (*Z. wollebaeki*), which has been considered as a subspecies of *Z. californianus* by some

605   authors (Aurioles-Gamboa & Hernández-Camacho, 2015). Despite the low mitogenomic

606   distance measured here between the two species (0.5%), Wolf et al. (2007) have previously

607   concluded that they are separate species because they were found reciprocally monophyletic

608   with D-loop and cytochrome b sequences and numerous private alleles were detected for both

609   taxa at most of the 25 investigated microsatellite loci. Our molecular estimate of divergence

610   time between *Z. californianus* and *Z. wollebaeki* is 0.3-0.2 Mya, which is much more recent

611   than previous estimations based on D-loop sequences, i.e. 0.8 Mya (Schramm et al., 2009)

612   and 2.3 ± 0.5 Ma (Wolf et al., 2007).

613

Mitogenomic variations at the genus level

614

615 In Mammals, most events of interspecific diversification at the genus level are more

616 recent than 10 Mya and the great majority of them occurred during the Pliocene and

617 Pleistocene epochs. This trend, previously reported in various genera of Cetartiodactyla

618 (Hassanin et al., 2012) and Primates (Pozzi et al., 2014), is here confirmed in the following 25

619 carnivoran genera: *Arctocephalus*, *Catopuma*, *Felis*, *Genetta*, *Leopardus*, *Lutra*, *Lynx*,

620 *Martes*, *Meles*, *Melogale*, *Monachus*, *Mirounga*, *Mungos*, *Mustela*, *Panthera*, *Paradoxurus*,

621 *Phoca*, *Prionailurus*, *Prionodon*, *Puma*, *Pusa*, *Ursus*, *Urva* and *Vulpes*. Our analyses suggest

622 however that two mustelid species separated from their congeneric representatives in the

623 Middle Miocene: *Martes pennanti* at 14.2-12.4 Mya; and *Mustela frenata* at 13.4-11.8 Mya.

624 In addition, the phylogenetic positions of these two species result in the polyphyly of the two

625 genera *Martes* and *Mustela*, as previously found using both mtDNA and nuDNA markers

626 (Law et al., 2018). Taken together, these results clearly indicate that *Martes pennanti* should

627 be placed in its own genus *Pekania*, whereas *Mustela frenata* should be placed in the genus

628 *Grammogale*. Indeed, the genus *Grammogale* Cabrera, 1940 was used by Cabrera & Yepes

629 (1960) to unite *Mustela africana* and *Mustela felipei* into a separate genus. In addition, several

630 molecular studies have shown that these two species fall into a robust clade with *Mustela*

631 *frenata* and that they are the sister-group of *Neovison vison* (Harding & Smith, 2009; Law et

632 al., 2018). We recommend therefore to include the four species *M. africana*, *M. felipei*, *M.*

633 *frenata*, and *N. vison* into the genus *Grammogale*.

634 Within the tribe Canini, our mitogenomic tree shows that *Cuon alpinus* (dhole) is the

635 sister-group of all *Canis* species, excepting *Canis adustus* (side-striped jackal) which diverged

636 from them at 8.7-7.4 Mya. The African wild dog (*Lycaon pictus*) occupies a more basal

637 position, but this placement is only supported by 6/10 overlapping datasets in our SuperTRI

638 analyses, indicating that an alternative position cannot be excluded. The mitochondrial

639 phylogeny differs from the nuclear phylogeny of Lindblad-Toh et al. (2005) and Koepfli et al.

640   (2015) in which *C. adustus* was found to be related to *Canis mesomelas* (black-backed

641   jackal), but more divergent from the clade composed of other *Canis* species, *Cuon* and

642   *Lycaon*. Following these results, in order to keep the genus *Canis* monophyletic, the species

643   *C. adustus* and *C. mesomelas* should be placed in a different genus, which is *Lupulella*

644   according to Viranta et al. (2017). The mito-nuclear discordance for the monophyly of the

645   genus *Lupulella* was not discussed in previous studies. Our interpretation is that a

646   mitochondrial introgression occurred at 6.2-5.2 Mya from an ancestor of *Canis* species to the

647   lineage leading to *Lupulella mesomelas*. In agreement with the genomic study of

648   Gopalakrishnan et al. (2019), which concluded to pervasive gene flow among *Canis* species,

649   our results suggest that interspecies hybridization has been also frequent in the early

650   evolutionary history of canid genera in Africa.

651

652   Our chronogram in **Figure 3** shows that no intergeneric divergence occurred during

653   the Pleistocene epoch, except the split between the Malagasy genera *Mungotictis* and

654   *Salanoia*, and the separation between the seal genera *Halichoerus*, *Phoca* and *Pusa*. A

655   Pleistocene diversification was also found in previous molecular dating analyses on the

656   Eupleridae and Phocidae (Higdon et al., 2007; Nyakatura & Bininda-Emonds, 2012). In the

657   absence of striking morphological feature for diagnosing these genera, we recommend

658   synonymizing *Halichoerus* and *Pusa* with *Phoca*, as proposed in previous studies (e.g.

659   Arnason et al., 2006), and *Mungotictis* with *Salanoia*.

660

661   **Changes in base composition and their impact on evolutionary rates**

662   Mitogenomic rearrangements did not occur during the evolutionary history of the

663   Carnivora. This deeply contrasts with other animal groups for which inversions of

664   mitochondrial protein-coding genes and control region have resulted in many local or full

665   reversals of base composition (see Hassanin et al., 2005; Arabi et al., 2010 for more details).

666 Despite this, five carnivoran species in our Bayesian tree (**Figure 2**) have a longer terminal

667 branch than other representatives of the same family or superfamily: *Cynogale bennetti* within

668 the Viverridae, *Homotherium latidens* within the Felidae, *Mellivora capensis* within the

669 Mustelidae, *Nasua nasua* within the Procyonidae, and *Odobenus rosmarus* within the

670 Otarioidea. In addition, two higher taxa of Caniformia show longer branches: Otarioidea and

671 Musteloidea. Since branch length is proportional to the number of substitutions, these long

672 branches indicate either an acceleration in the rates of substitution or alternatively an

673 important change in the pattern of substitution (Tamura & Kumar, 2002; Hassanin, 2006). As

674 shown in **Figure 1**, *Cynogale*, *Homotherium*, and *Mellivora* show a divergent base

675 composition. For *Cynogale* and *Mellivora*, we can therefore assume that a change in the

676 pattern of substitution has taken place during their recent evolution. The case of *Homotherium*

677 is more problematic. The mitogenome of this extinct genus was sequenced from a bone (YG

678 439.38) dated at >47,500 years (Paijmans et al., 2017). Since ancient DNA molecules are

679 known to exhibit a high rate of cytosine deamination, the sequences can contain artefactual

680 C=>T and G=>A substitutions (Briggs et al., 2010). By comparing the mitogenomes of all

681 felid species, this is exactly the pattern observed for third-codon positions, as *Homotherium* is

682 characterized by less cytosines (28.94% *versus* 28.96-35.21% in other felid species), more

683 thymines (25.41% *versus* 17.44-23.51%), less guanines (2.80% *versus* 4.69-8.41%) and more

684 adenines (42.85% *versus* 38.41-42.79%). As a consequence, we conclude that sequencing

685 errors introduced by DNA damage are the cause of the long branch of *Homotherium* in the

686 tree of **Figure 2**.

687 The mitogenomes of *Nasua* and *Odobenus* have a base composition that is not so

688 divergent from their closest relatives, suggesting that an acceleration of rates of substitution is

689 the cause of their long branch in the tree of **Figure 2**. As pointed out by Bromham (2011),

690 mammals evolving with faster evolutionary rates have generally a small body-size, but the

691 cause of this body-size effect continues to be debated, because there are many possible

692   mechanisms including shorter generation times, shorter lifespan, higher fecundity, larger litter

693   size, and higher metabolic rates. From this point, the walrus (*O. rosmarus*) is intriguing

694   because it is one of largest pinnipeds. Its field metabolic rate is 381.2 MJ/day, which is 6 to

695   32 times more than in other species of Pinnipedia (Acquarone et al., 2006). Whereas most

696   other pinnipeds hunt pelagic organisms, such as fish and cephalopod species, in the water

697   column, the walrus feeds on benthic species and prefers molluscs, especially clams (Lowry,

698   2016). This special diet and associated behaviour, with long exposure in the cold coastal

699   waters of the Arctic Ocean, may therefore explain the increased rate of mtDNA evolution

700   detected in the walrus. Repetitive bottlenecks generated by climatic oscillations during the

701   Pleistocene may be proposed as an alternative hypothesis. Nuclear genome comparisons

702   between pinniped species will help to decipher between these two main hypotheses, as we can

703   expect accelerated rates of substitution in both mtDNA and nuDNA genomes in case of

704   repetitive bottlenecks.

705

706   **Robust and reliable mitogenomic phylogeny for deep relationships within**

707   **Carnivora**

708
709   Previous molecular studies have shown that inferring deep phylogenetic relationships

710   using mitogenomes can be problematic. An important issue concerns the reversals of strand

711   mutational bias: detected in many animal phyla, such as Arthropoda, Chordata, Mollusca, etc,

712   it may be particularly misleading for phylogenetic reconstruction (Hassanin et al., 2005; Arabi

713   et al., 2010). The mitogenome of mammals is not affected by this kind of bias, as its structure

714   is highly conserved among the 27 mammalian orders. However, mutational saturation is a

715   different issue: since the mtDNA evolves with higher mutation rates than the nuclear genome

716   (Brown et al., 1979), it is more prone to multiple substitutions at the same site which leads,

717   with time, to the disappearance of the phylogenetic signal (Philippe & Forterre, 1999; Xia et

718   al., 2003). Although mutational saturation may be particularly problematic for reconstructing

719   Precambrian, Paleozoic, and Mesozoic divergences, it is expected to have less impact for

720   inferring more recent divergences, such as those during the Cenozoic. This explains why

721   mitogenomic sequences have been largely used for inferring interfamilial and intrafamilial

722   relationships in most mammalian groups, including Cetartiodactyla (Hassanin et al., 2012),

723   Feliformia (Zhou et al., 2017), Primates (Finstermeier et al., 2013; Pozzi et al., 2014),

724   Pholidota (Hassanin et al., 2015), Xenarthra (Gibb et al., 2016), etc. In agreement with this

725   view, all families and inter-familial levels shown in our mtDNA tree of **Figure 2** received

726   high support with all the three phylogenetic approaches (Bayesian trees of *mtDNA* and

727   *mtDNA-Tv* data sets, and SuperTRI analysis), indicating considerable stability of the

728   topology. Interfamilial relationships are conformed to the nuclear tree published by Eizirik et

729   al. (2010), except the position of the Mephitidae as the sister-group of the Ailuridae.

730   However, this relationship was also found by Law et al. (2018) based on 46 genes (4

731   mitochondrial and 42 nuclear genes) and 75 species of Musteloidea.

732

733

734   **Diversification of the Carnivora during the Cenozoic**

735   The mtDNA phylogeny reconstructed here presents a good opportunity to study the

736   evolution of the order Carnivora. Indeed, our dense taxonomic sampling allow us to include

737   many fossils as calibration points for estimating divergence times. This point is particularly

738   relevant as the fossil record of Carnivora has been significantly improved over the last ten

739   years, with the discovery of several key fossils (e.g., Abella et al., 2012; Grohé et al., 2013;

740   Tseng et al., 2014; Berta et al., 2018; Valenciano et al., 2020). As pointed out in Warnock et

741   al. (2015), the most effective means of establishing the quality of fossil-based calibrations is

742   through a priori evaluation of the intrinsic palaeontological, stratigraphic, geochronological

743   and phylogenetic data. We identified therefore 22 calibration points (**Table 1**), including one

744   tip calibration (the mitogenome of a late Pleistocene fossil of *Ursus maritimus* published in

745   Lindqvist et al., 2010) and 20 well-constrained calibration points having both minimum and

746   maximum age boundaries interpreted from fossils with known position with respect to extant

747   taxa included in our study. Molecular dating analyses were performed using either a uniform

748   (U) or log-normal (L) prior distribution on the calibrated node ages. The "L approach" used

749   here considers that minimum ages are generally more accurate and reliable than maximum

750   ages because younger fossils are generally more abundant and precisely dated than older

751   fossils as a consequence of taphonomy and dating methods (Crees et al., 2019). As expected,

752   the divergence times estimated with the "L approach" are more recent than those estimated

753   with the "U approach" (**Figures 3 and 4**). The values calculated with the two approaches are

754   given for all the nodes described below.

755

756   The age estimated for the MRCA of Carnivora was 52.7 Mya with the "U approach"

757   and 46.7 Mya with the "L approach", and both are younger than previous estimates published

758   in Eizirik et al. (2010; 59.2 Mya) and Nyakatura & Bininda-Emonds (2012; 64.9 Mya).

759   However, our dating estimates fit well with the end of the warmest period of the Cenozoic

760   era, from the Palaeocene–Eocene Thermal Maximum (PETM) at 56 Mya to the Early Eocene

761   Climatic Optimum (EECO) at around 53-50 Mya (Zachos et al., 2001; Zachos et al., 2008;

762   Kirtland Turner et al., 2014). In the fossil record, this period was marked by the first

763   appearances of Primates, Perissodactyla and Cetartiodactyla in North America and Europe, as

764   well as Carnivoraforms, a group formed by the crown group Carnivora plus the stem family

765   'Miacidae' (Spaulding and Flynn, 2012; Hooker 2015; Solé et al., 2016). During the early

766   Eocene greenhouse world, rainforests spread on Earth from pole to pole (Sluijs et al., 2006;

767   Pross et al., 2012), suggesting that early carnivorans were small arboreal species.

768   Biogeographically, Carnivoraforms diversified in the three Laurasian continents during the

769   early Eocene, with a possible origin in the late Paleocene of Asia (Solé et al., 2016). Crown

770   carnivorans originated in one of the three continents of the Northern Hemisphere. To know

771   which one, new fossils need to be discovered in the middle Eocene to fill the gap between the

772   Carnivoraforms of the early Eocene and the oldest carnivorans of the late Eocene (Solé et al.,

773   2016).

774   The divergence times estimated for basal relationships in the Caniformia are similar to

775   those published in Eizirik et al. (2010) but much younger than those published in Nyakatura

776   & Bininda-Emonds (2012): Caniformia = 48.0-42.5 Mya *versus* 48.2 Mya and 61.2 Mya

777   respectively; Arctoidea = 43.4-38.3 Mya *versus* 42.6 Mya and 47.8 Mya. For more recent

778   nodes, our estimates are similar to the two previous studies: Musteloidea = 37.4-33.0 Mya

779   *versus* 32.0 Mya and 35.7 Mya; Mustelidae+Procyonidae = 34.4-30.3 Mya *versus* 27.4 Mya

780   and 31.6 Mya; and Pinnipedia = 27.2-22.9 Mya *versus* 24.5 Mya and 22.4 Mya. By contrast,

781   the ages inferred for the families are generally much younger in Eizirik et al. (2010) (**Figure**

782   **4**), a result potentially explained by a poor species sampling (two species of Ursidae, three

783   species of Canidae, etc.) and the low phylogenetic signal of nuclear protein-coding genes for

784   the most recent nodes.

785   The ages estimated for basal divergences in the Feliformia are much younger than in

786   Eizirik et al. (2010) and Nyakatura & Bininda-Emonds (2012): Feliformia = 34.4-31.1 Mya

787   *versus* 44.5 and 53.2 Mya; Feloidea = 27.4-24.7 Mya *versus* 33.3 and 52.9 Mya; and

788   Viverroidea = 28.2-25.8 Mya *versus* 37.4 and 31.8 Mya. These results may be explained by

789   the use of different calibration points: Feloidea = 34-20 Mya *versus* >28.5 Mya in Eizirik et

790   al. (2010); Felidae = 20-14 Mya *versus* >31.15 Mya in Nyakatura & Bininda-Emonds (2012);

791   and Viverridae = 34-14 Mya versus >25.72 Mya in Nyakatura & Bininda-Emonds (2012).

792   The minimum age constraints used by Nyakatura & Bininda-Emonds (2012) for Felidae and

793   Viverridae seem however problematic because they were not extracted from the fossil record,

794   but from the supertree of Bininda-Emonds et al. (2007). By contrast, the minimum boundary

795   of 28.5 Mya used for Feloidea in Eizirik et al. (2010) was interpreted from early Oligocene

796   fossils assumed to be the oldest felids according to McKenna and Bell (1997). In Meredith et

797   al. (2011), who used a similar minimum boundary for Feloidea (> 28.3 Mya), the divergence

798   times estimated for Feliformia were found to be highly variable between their eight molecular

799   dating analyses, with mean ages between 35.81 and 43.07 Mya. Three early Oligocene fossils

800   were used as calibration points in this study: *Proailurus* and *Stenogale*, which were assumed

801   to be stem Felidae; and *Palaeoprionodon*, which was supposed to be a stem Prionodontidae.

802   However, the phylogenetic positions of these fossils remain uncertain. Indeed, the three fossil

803   genera formed the sister-group of Feloidea + Viverroidea + *Herpestides antiquus*† in the

804   phylogenetic analyses of Solé et al. (2014; 2016). In the classification of Hunt (2001),

805   *Palaeoprionodon* was included in the subfamily Prionodontinae with *Prionodon* and other

806   extant viverrid genera, such as *Genetta* and *Pioana*. Although *Prionodon* was treated as a

807   member of the family Viverridae by Hunt (2001) and previous authors, it was then found to

808   be the sister-group of the Felidae by Gaubert & Veron (2003) which was confirmed by more

809   recent studies (e.g. Eizirik et al., 2010; Nyakatura & Bininda-Emonds, 2012; Meredith et al.,

810   2011; **Figure 2**). In Nyakatura & Bininda-Emonds (2012), *Paleoprionodon* is used as a

811   calibration point for the Viverridae (based on Hunt & Tedford, 1993, according to Bininda-

812   Emonds et al., 2007), although it is suggested to be either close to *Prionodon* or a stem

813   Feliformia (see e.g. Hunt, 2001; Veron, 2010; Solé et al., 2014).

814   Our dating estimates suggests that the basal split between *Nandinia* and other genera

815   of Feliformia occurred at the Eocene/Oligocene transition (34 Mya), when a brutal and global

816   cooling of 5°C resulted in the extinction of many taxa and the appearance of several modern

817   mammal lineages (Russell & Tobien, 1986). Accordingly, *Nandinia* may be the descendant of

818   forest-adapted ancestors, whereas the other lineage of Feliformia may have evolved in

819   response to this important climatic change by adapting to more open vegetation.

820

Conclusion

821

822   Based on a large taxonomic sampling (220 taxa represented by 2,442 mitogenomes)

823   and 22 fossil calibration points (having both minimum and maximum age boundaries and a

824   position based on recent fossil revisions and phylogenies), our study proposes a new time

825   scaled phylogeny for Carnivora and provides further insights into the evolutionary history of

826   this order. The age estimates for the Carnivora and the two suborders Caniformia and

827   Feliformia fit well with global changes corresponding to the appearance of other mammal

828   lineages. Moreover, our phylogenetic results, although largely similar to other recent

829   phylogenies, suggest several taxonomic changes that would need to be confirmed using

830   nuclear data.

831

Acknowledgments

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1314 **Table 1. Maximum (Max) and minimum (Min) age calibrations (in millions**

1315 **of years ago, Mya) used for molecular dating analyses** (with either uniform or

1316 log-normal prior distributions [U/L] on calibrated node ages interpreted from the

1317 fossil record).

1318

Most recent common ancestor	Max	Distribution	Min	References and justification
Ferae	85	N, M = 75	65	www.timetree.org ; split *Canis* / *Manis* (13 studies)
Caniformia				
Canidae				
(*Canis, Cuon, Lycaon*)	12	U/L, M = 1.5	6	Tedford et al. (2009)
(*Vulpes, Nyctereutes*)	34	U/L, M = 6.25	9	Tedford et al. (2009)
Ursidae	34	U/L, M = 5.75	11	Abella et al. (2012), Banyue & Zhanxiang (2003), Bonis et al. (2019)
(*Arctotherium, Tremarctos*)	10	U/L, M = 5.75	3	Soibelzon et al. (2005)
Ursus maritimus† from Svalbard (GU573488)	0.11	N, M = 0.12	0.13	Lindqvist et al. (2010)
Pinnipedia	34	U/L, M = 3.75	19	Rybczynski et al. (2009), Berta et al. (2018), Dewaele et al. (2018)
Otarioidea	23	U/L, M = 2.00	15	Berta et al. (2018)
Phocidae	23	U/L, M = 2.75	12	Berta et al. (2018)
Mustelidae				
(Guloninae … Mustelinae)	27.6	U/L, M = 2.825	16.3	Sato et al. (2009), Valenciano et al. (2020)
(Lutrinae, Ictonychinae)	27.6	U/L, M = 3.85	12.2	Sato et al. (2009), Grohé et al. (2013)
Feliformia				
Feloidea	34	U/L, M = 3.5	20	Werdelin et al. (2010)
Felidae	20	U/L, M = 1.5	14	Werdelin et al. (2010)
(*Acinonyx, Puma*)	14	U/L, M = 2.65	3.4	Werdelin et al. (2010), Werdelin & Peigné (2010)
(*Leptailurus … Profelis*)	14	U/L, M = 2.5	4	Werdelin & Peigné (2010)
(*Caracal, Profelis*)	14	U/L, M = 2.5	4	Werdelin et al. (2010)
(*Panthera, Neofelis*)	14	U/L, M = 2.0125	5.95	Tseng et al. (2014)
Viverroidea				
Viverridae				
(Genettinae, Viverrinae)	34	U/L, M = 5.00	14	Werdelin & Peigné (2010)
(**Herpestidae, Eupleridae**)	34	U/L, M = 3.375	20.5	Werdelin & Peigné (2010)
(*Helogale, Crossarchus*)	20.5	U/L, M = 3.625	6	Werdelin & Peigné (2010)
(*Galerella … Cynictis*)	20.5	U/L, M = 3.275	7.4	Werdelin & Peigné (2010)
Hyaenidae				
(*Hyaena, Parahyaena*)	9.5	U/L, M = 1.475	3.6	Ferretti (2007), Werdelin & Peigné (2010)

1319 Abbreviations: L = Log Normal; M = Mean age; N = Normal; U: Uniform.

1320 **Figures**

1321 **Figure 1. Variation in base composition of the mitogenomes of Carnivora**

1322 The *PCG-DNA* dataset was used to calculate the frequency of the four bases (A, C, G and T)

1323 at each of the three codon positions, and the 12 variables measured were then summarized by

1324 a principal component analysis (PCA). The main graph represents the individual factor map

1325 based on 220 taxa. The families of Carnivora are highlighted by different colours. The small

1326 circular graph at the top left represents the variables factor map.

1327

1328 **Figure 2. Phylogeny of Carnivora based on mitogenomes**

1329 The Bayesian tree was reconstructed using the *mtDNA* dataset (220 taxa and 14,892 bp) and

1330 GTR+I+G model. Dash branches indicate nodes supported by posterior probability (PP) <

1331 0.95.

1332 Black circles indicate nodes that are also monophyletic in the two following trees: Bootstrap

1333 50% majority-rule consensus tree reconstructed from the MRP matrix of the SuperTRI

1334 analysis; and Bayesian tree obtained from the analysis of the *mtDNA-Tv* dataset and

1335 JC69+I+G model. Grey circles show nodes that are not found to be monophyletic with one of

1336 the two methods detailed above. White circles indicate nodes that are not monophyletic in

1337 both *mtDNA-Tv* and SuperTRI bootstrap consensus trees.

1338 No information was provided for the nodes highly supported by the SuperTRI analysis, i.e.

1339 which were found monophyletic in all the 10 Bayesian trees reconstructed from the 10 half-

1340 overlapping sub-datasets of the *mtDNA* dataset. For nodes less supported by the SuperTRI

1341 analysis, the number of Bayesian trees (< 10) showing the nodes is indicated.

1342 Species names follow the classification of the IUCN (2020); the taxa written in red highlight

1343 the taxonomic issues discussed in the main text.

1344

1345

1346   **Figure 3. A molecular timescale for carnivoran evolution**

1347   The estimates of divergence time were calculated under BEAST v.2.4.7 using the GTR+I+G

1348   model on the *mtDNA* dataset. The asterisks show the calibration points used for molecular

1349   estimation (see Table 1 for details). The chronogram, mean ages (values in black) and

1350   associated 95% confidence intervals (grey bars) were inferred using a uniform prior

1351   distribution for fossil calibration points ("U approach"). For comparison, the values in blue

1352   are mean ages estimated using a log normal prior distribution for fossil calibration points ("L

1353   approach"; see main text for details and discussion). Species names follow the classification

1354   of the IUCN (2020); the name of the taxa written in red have been changed following our

1355   results which suggest these taxonomic changes; see the discussion for details.

1356

1357   **Figure 4. Comparison with published chronograms on Carnivora**

1358   The mean divergence times were here estimated with two approaches for the prior distribution

1359   on the calibrated node ages: (1) a uniform distribution between maximum and minimum

1360   boundaries ("U approach", blue histograms); and (2) a log-normal distribution ("L approach",

1361   green histograms; see main text for details). The results were compared with mean ages

1362   inferred in Eizirik et al. (2010; red histograms) and Nyakatura & Bininda-Emonds (2012;

1363   orange histograms).

1364

1365   # Supporting Information

1366   **S1 Appendix. Origin of the sequences used in this study**

1367   **S2 Appendix. Primers**

1368   **S3 Appendix. Base composition**

1369   **S4 Appendix. Distances**

1370   **S5 Appendix. Bayesian tree reconstructed using the *mtDNA-Tv* dataset (220 taxa and**

1371   **14,892 bp) and JC69+I+G model**

1372   **S6 Appendix. Bootstrap 50% majority-rule consensus tree reconstructed from the MRP**

1373   **matrix of the SuperTRI analysis**

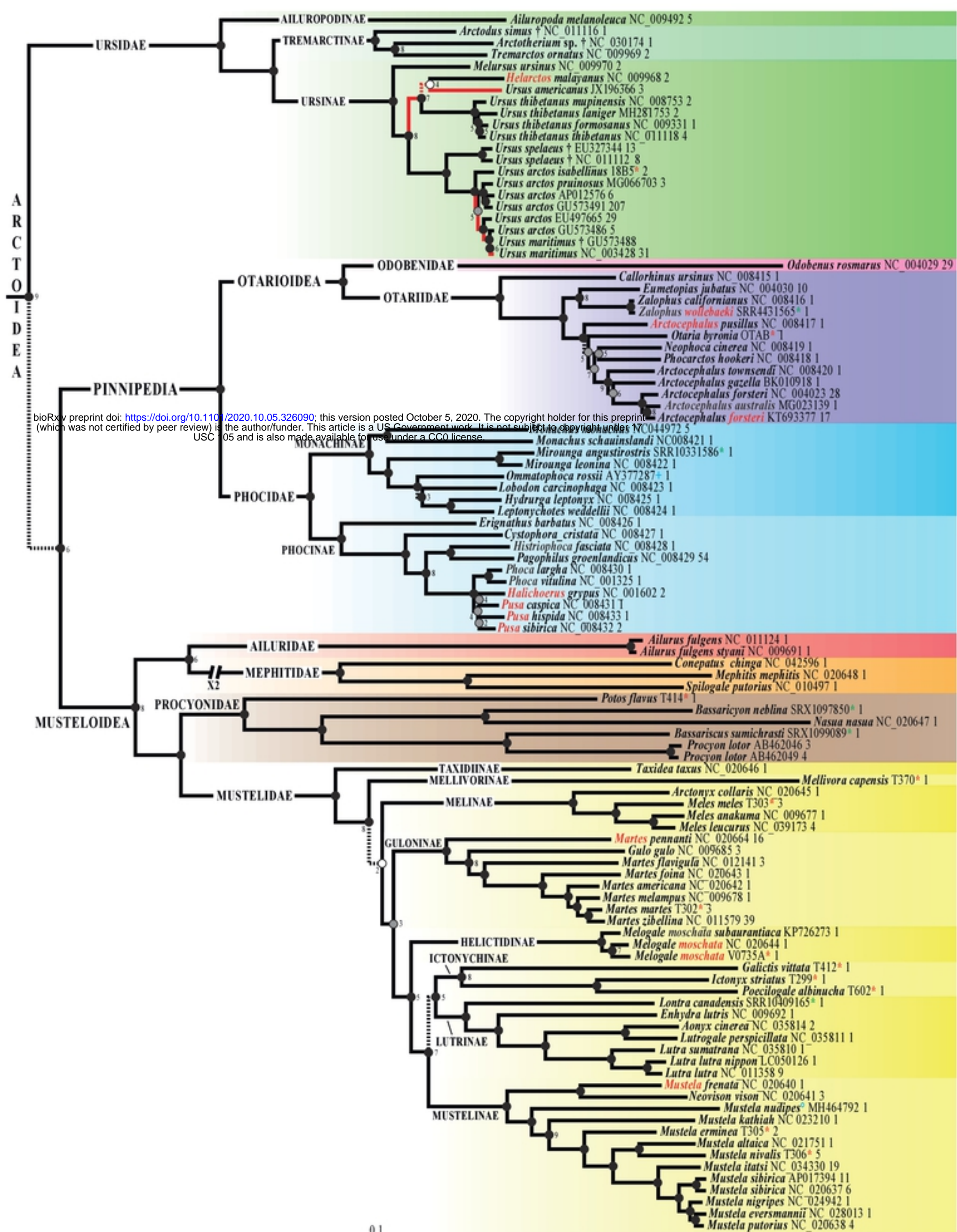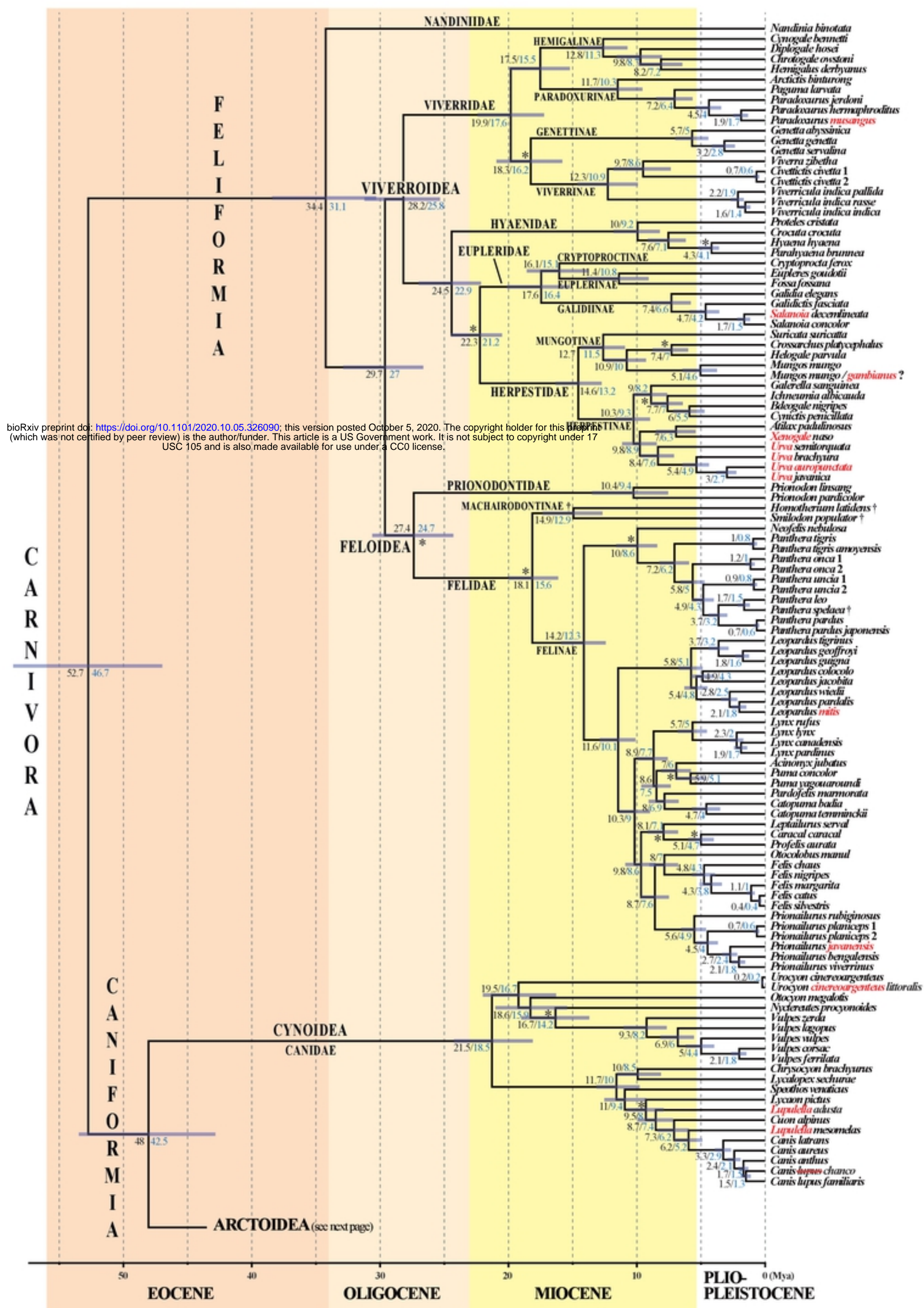1374   **S7 Appendix. Cytochrome b phylogeny of the genus *Melogale***

Figure 1

Figure 2A

Figure 2B

Figure 3A

Figure 3B

Figure 4