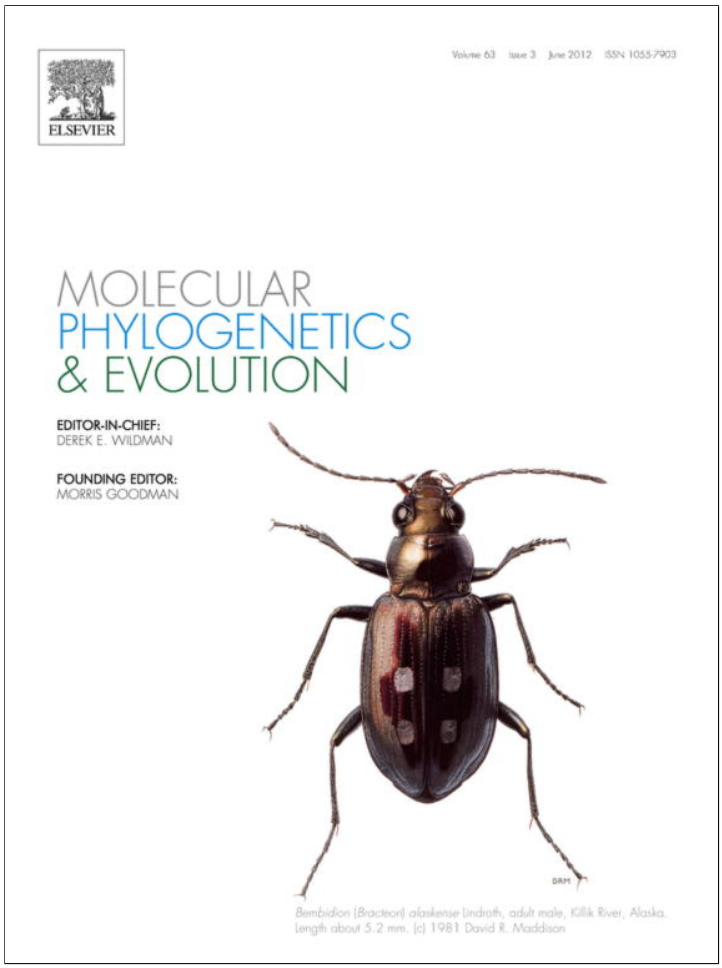

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Evolutionary and biogeographic history of weasel-like carnivorans (Musteloidea) ☆

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ABSTRACT

We analyzed a concatenated (8492 bp) nuclear–mitochondrial DNA data set from 44 musteloids (including the first genetic data for *Lyncodon patagonicus*) with parsimony, maximum likelihood, and Bayesian methods of phylogenetic and biogeographic inference and two Bayesian methods of chronological inference. Here we show that Musteloidea emerged approximately 32.4–30.9 million years ago (MYA) in Asia, shortly after the greenhouse–icehouse global climate shift at the Eocene–Oligocene transition. During their Oligocene radiation, which proceeded wholly or mostly in Asia, musteloids diversified into four primary divisions: the Mephitidae lineage separated first, succeeded by Ailuridae and the divergence of the Procyonidae and Mustelidae lineages. Mustelidae arose approximately 16.1 MYA within the Mid-Miocene Climatic Optimum, and extensively diversified in the Miocene, mostly in Asia. The early offshoots of this radiation largely evolved into badger and marten ecological niches (Taxidiinae, Melinae, Mellivorinae, Guloninae, and Helictidinae), whereas the later divergences have adapted to other niches including those of weasels, polecats, minks, and otters (Mustelinae, Ictonychinae, and Lutrinae). Notably, and contrary to traditional beliefs, the morphological adaptations of badgers, martens, weasels, polecats, and minks each evolved independently more than once within Mustelidae. Ictonychinae (which is most closely related to Lutrinae) arose approximately 9.5–8.9 MYA, most likely in Asia, where it diverged into the Old World Ictonychini (*Vormela*, *Poecilictis*, *Ictonyx*, and *Poecilogale*) and New World Lyncodontini (*Lyncodon* and *Galictis*) lineages. Ictonychini presumably entered Africa during the Messinian Salinity Crisis (at the Miocene–Pliocene transition), which interposed the origins of this clade (approximately 6.5–6.0 MYA) and its African *Poecilictis–Ictonyx–Poecilogale* subclade (approximately 4.8–4.5 MYA). Lyncodontini originated approximately 2.9–2.6 MYA at the Pliocene–Pleistocene transition in South America, slightly after the emergence of the Panamanian land bridge that provided for the Great American Biotic Interchange. As the genera *Martes* and *Ictonyx* (as currently circumscribed) are paraphyletic with respect to the genera *Gulo* and *Poecilogale*, respectively, we propose that *Pekania* and *Poecilictis* be treated as valid genera and that "*Martes*" *pennanti* and "*Ictonyx*" *libyca*, respectively, be assigned to these genera.

1. Introduction

The weasel-like carnivorans (Musteloidea) include weasels, otters, martens, badgers, and relatives (Mustelidae); raccoons and their kin (Procyonidae); the red panda (Ailuridae); and skunks and stink badgers (Mephitidae; e.g., Delisle and Strobeck, 2005; Flynn et al., 2005; Fulton and Strobeck, 2006, 2007; Sato et al., 2006, 2009; Árnason et al., 2007; Yonezawa et al., 2007). With its 84 living species classified into 33 genera (Wozencraft, 2005; the Japanese otter, *Lutra nippon*, is considered extinct—Sasaki, 2009), Musteloidea encompasses ~30% of the extant carnivoran species diversity, which makes this clade the most species-rich superfamily within the order Carnivora. Musteloids are widespread in Eurasia, Africa, and the Americas, and also occur in New Zealand

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following human-mediated introductions in the late nineteenth century. Musteloids have adapted to a variety of climatic and biotic conditions, being found today in a broad and diverse spectrum of habitats spanning from tropical rainforest to arctic tundra, and from desert to inland waterways and coastal sea waters. They exhibit diverse locomotor and dietary habits, with not only terrestrial forms but also largely arboreal, fossorial, and aquatic specialists, with diets ranging from strictly carnivorous to vegetarian (see Macdonald, 2006). All this renders Musteloidea a fascinating and challenging taxon for evolutionary and biogeographic investigations.

The most species-rich, ecomorphologically diverse, and widely distributed musteloid family is Mustelidae (e.g., Wolsan, in press), which makes this family particularly well-suited for addressing evolutionary and biogeographic questions. Within Mustelidae, recent multilocus DNA studies (Koepfli and Wayne, 2003; Fulton and Strobeck, 2006; Koepfli et al., 2008; Wolsan and Sato, 2010) have provided sound evidence for a close phylogenetic relationship between some Old World (mostly African) polecats and weasels (*Ictonyx*, *Poecilogale*, and *Vormela*) and the New World (South and southern North American) grisons (*Galictis*). Given the large distance separating the current distributions of these two groups of mustelids, their evolutionary and biogeographic history is especially intriguing. A conspicuous feature shared by these mustelids is a contrastingly colored pelage. For most of these species, defensive behaviors with threat displays and excretion of pungent musk from anal glands have been reported, suggesting that the primary adaptive value of their striking coloration lies in warning potential predators (Pocock, 1909; Koepfli et al., 2008). To refer to a clade uniting both groups, Fulton and Strobeck (2006; followed by Koepfli et al., 2008) adopted the subfamilial name Galictinae Reig, 1956, whereas Wolsan and Sato (2010) applied Ictonychinae Pocock, 1922 because it has nomenclatural priority (International Commission on Zoological Nomenclature, 1999, Article 23). The latter name is therefore used here.

Extensive research during recent decades (Schmidt-Kittler, 1981; Wolsan, 1993; Ledje and Árnason, 1996; Dragoo and Honeycutt, 1997; Flynn and Nedbal, 1998; Bininda-Emonds et al., 1999; Flynn et al., 2000, 2005; Koepfli and Wayne, 2003; Sato et al., 2003, 2004, 2006, 2009; Fulton and Strobeck, 2006, 2007; Árnason et al., 2007; Koepfli et al., 2007, 2008; Yonezawa et al., 2007; Harding and Smith, 2009; Eizirik et al., 2010; Wolsan and Sato, 2010; and others) has considerably extended and refined knowledge on the evolutionary history of Musteloidea. The monophyly of this taxon has been demonstrated conclusively and many internal phylogenetic relationships have likewise been convincingly resolved. The degree of consensus among published estimates of divergence times for particular lineages has also improved recently. Nevertheless, there are important aspects of musteloid phylogeny and its chronology that still await clarification, such as the pattern and timing of early mustelid diversification and the phylogenetic placement of the Patagonian weasel (*Lyncodon patagonicus*).

In contrast to the evolutionary history, the biogeographic history of Musteloidea has not been extensively investigated and is not well understood. A comprehensive study of Mustelidae (Koepfli et al., 2008) provided insight into this family's historical biogeography, but the power of inference in that study was limited by the fact that the method used for ancestral-area reconstruction did not allow polymorphous characters, and therefore species distributed on two or more continents were assigned to one of them on potentially arbitrary grounds.

To shed more light on the pattern and timing of the evolutionary and biogeographic diversification of Musteloidea, we first prepared a dataset of concatenated nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) sequences from 44 musteloids and two outgroup species, each 8492 bp in aligned length. We then applied parsimony, maximum likelihood (ML), and Bayesian methods of phylogenetic and biogeographic inference and two different Bayesian methods of chronological inference. Special consideration has been given to the initial radiation of Musteloidea and the diversification of Mustelidae with particular reference to Ictonychinae. Specifically, we report the first genetic data for *Lyncodon patagonicus* and show that this species is an ictonychine. In addition, we provide the first rigorous analytical ancestral-area reconstruction of Musteloidea and a complete species-level reconstruction of the evolutionary and biogeographic history of the extant Ictonychinae.

2. Material and methods

2.1. Sampling

The nucleotide sequences obtained were from 12 protein-coding exons and four noncoding introns of nine nDNA genes and from a protein-coding mtDNA gene (Table 1). The sequence data were either newly obtained (DDBJ/EMBL/GenBank accessions AB285330–AB285332, AB305635, and AB564020–AB564112) or derived from previous studies (Supplementary Tables S1 and S2). Altogether, 45 wild species and one domestic form (*Mustela furo*) of the arctoid Carnivora were sampled; the sampling included 44 members of the ingroup Musteloidea plus a pinniped (*Phoca largha*) and an ursid (*Melursus ursinus*) as a collective outgroup (Supplementary Table S1). Selection of this outgroup was based on multiple lines of evidence indicating that Pinnipedia (seals, sea lions, walrus) and Ursidae (bears) are the closest extant relatives of Musteloidea (e.g., Wolsan, 1993; Wyss and Flynn, 1993; Delisle and Strobeck, 2005; Flynn et al., 2005; Fulton and Strobeck, 2006; Sato et al., 2006, 2009; Árnason et al., 2007; Rybczynski et al., 2009; Schröder et al., 2009; Eizirik et al., 2010).

2.2. Laboratory techniques

Total genomic DNA was extracted from tissue samples using a standard phenol–chloroform procedure (Sambrook and Russell, 2001). The PCR amplification of DNA from *Mellivora capensis* was preceded by whole-genome amplification with the illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Little Chalfont, UK). All PCR reactions were conducted in an automated thermal cycler (model PC 808, Astec, Fukuoka, Japan) with the following conditions: a 3-min denaturation period at 94 °C followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s; this was followed by an extension period at 72 °C for 10 min. Each 50-μl reaction mixture contained 10× Ex Taq buffer, 2 mM $MgCl_2$, 0.2 mM dNTP mix, 1.25 U of Ex Taq (Hot Start version) polymerase (Takara, Shiga, Japan), 0.8 μM of each primer, and 0.1–0.5 μg of genomic DNA. Amplification was performed through nonnested (for *CHRNA1*, *FES*, *GHR*, and *RHO*) or nested (for *APOB*, *BRCA1*, *MT-CYB*, *RAG1*, *RBP3*, and *VWF*) PCR reactions, using two new (vWF-F281-mustelids [5′-TGGTGCCCCCCACGGAAGGC-3′] and vWF-R1432-mustelids [5′-TCTCCAGCTCCTGCGGGTCGG-3′]) and 37 published primers (Supplementary Table S3). A 1-μl aliquot of each reaction mixture after the first nested PCR was used as a template for the second nested PCR. Sequencing was carried out with the Big Dye Terminator (version 3.1) Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan). The raw sequence data were generated on an ABI3130 automated sequencer (Applied Biosystems, Tokyo, Japan).

2.3. Phylogenetic analyses

2.3.1. Sequence alignment and supermatrix assembly

Sequences were aligned via multiple alignment in DNASIS Pro version 2.6 (Hitachi Software Engineering, Tokyo, Japan) following

Table 1
Genomic location and aligned length of the DNA segments analyzed in this study.

Genome	Gene symbol and name*	Gene region	Aligned length (bp)
Nuclear	*APOB*, apolipoprotein B (including Ag(x) antigen)	Exon 26	963
	BRCA1, breast cancer 1, early onset	Exon 11	1049
	CHRNA1, cholinergic receptor, nicotinic, alpha 1 (muscle)	Exon 10, intron 9	390
	FES, feline sarcoma oncogene	Exons 14 and 15, intron 14	462
	GHR, growth hormone receptor	Exons 9 and 10, intron 9	711
	RAG1, recombination activating gene 1	Exon 1	1095
	RBP3, retinol binding protein 3, interstitial	Exon 1	1188
	RHO, rhodopsin	Exons 3 and 4, intron 3	288
	VWF, von Willebrand factor	Exon 28	1206
Mitochondrial	*MT-CYB*, mitochondrially encoded cytochrome b	Total gene	1140

* Gene symbols and names are those recommended for the homologous human locus by the Human Genome Organisation (http://www.genenames.org/, last accessed January 19, 2011).

the similarity criterion (Simmons, 2004). The total number of pairwise differences between compared sequences with regard to base substitutions and gaps (insertions and deletions, all constrained to be multiples of three bases in protein-coding sequences) was minimized (Zurawski and Clegg, 1987). Equal costs were assumed for gap opening and extension vs. substitutions, but lower costs for substitutions in the cases of ties. For each tie between a transition and a transversion, the transition was selected.

To assess the amount of phylogenetic incongruence among the gene genealogies, we compared ML single-gene tree topologies and found that they were largely congruent in terms of >70%-bootstrap-supported clades (Supplementary Fig. S1). We then assembled the aligned sequence data in a phylogenetic matrix containing 390,632 character-data cells (46 taxa × 8492 characters), of which 25,822 (6.6%) were coded as missing data. The missing data corresponded to the unavailable sequence data and inferred gaps. All phylogenetic analyses were conducted on this supermatrix (available under study accession No. S11504 in TreeBASE; http://www.treebase.org/, last accessed January 19, 2011). We note that coding gaps alternatively as fifth character states for each base position regardless of the gap length (e.g., Giribet and Wheeler, 1999) under parsimony did not alter the topologies of the inferred trees except that *Mustela lutreola* was paired with *M. furo* (with a bootstrap frequency of 91%).

2.3.2. Parsimony analysis

The parsimony phylogenetic analysis was performed in TNT version 1.1 (Goloboff et al., 2008). Trees were obtained from heuristic searches with 10^3 random-addition sequence replicates and tree bisection–reconnection (TBR) branch swapping supplemented by a TBR round on the resulting shortest-length trees. Additional searches were conducted using the sectorial-searches, tree-drifting, and tree-fusing algorithms (Goloboff, 1999). Support for the hypothesized clades was quantified with nonparametric bootstrap frequencies (Felsenstein, 1985) as well as symmetric-resampling frequencies and symmetric-resampling frequency differences (Goloboff et al., 2003). All indices were calculated on the basis of 2.5×10^3 pseudoreplicates, each consisting of a heuristic search using 10^2 random-addition sequence replicates and TBR branch swapping.

2.3.3. Maximum-likelihood analysis

The ML phylogenetic analysis was executed in GARLI version 0.96 (Zwickl, 2006). The best-fit model of base substitutions (GTR + I + Γ; Lanave et al., 1984) was determined with the Akaike Information Criterion (AIC; Akaike, 1973) in Modeltest version 3.7 (Posada and Crandall, 1998). Using this model, heuristic searches were performed via five independent runs of the genetic algorithm, each with an ML stepwise-addition starting tree and 2×10^4 generations. Nonparametric bootstrap frequencies were computed from 10^2 pseudoreplicates of an as-is addition sequence with TBR branch swapping.

2.3.4. Bayesian analysis

The Bayesian phylogenetic analysis was conducted in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) with the *prset ratepr = variable* option in effect (Marshall et al., 2006). The best-fit models of base substitution were chosen independently for each gene partition using AIC in MrModeltest version 2.2 (Nylander, 2004). The following models were adopted: GTR + Γ (Lanave et al., 1984) for the *APOB*, *BRCA1*, *FES*, *GHR*, and *RHO* partitions; GTR + I + Γ for the *MT-CYB*, *RBP3*, and *VWF* partitions; K80 + Γ (Kimura, 1980) for the *CHRNA1* partition; and SYM + I + Γ (Zharkikh, 1994) for the *RAG1* partition. Model parameters were estimated as part of the analysis. Gene partitions were unlinked. Two independent runs of Metropolis-coupled MCMC (Markov-chain Monte Carlo) were conducted. Each run consisted of four Markov chains, one cold and three incrementally heated, which started from a random tree. The chains were run for 3×10^7 generations and sampled every 10^2 generations. The first 1.5×10^5 sampled trees were discarded as burn-in. Inspection of the parameter files generated in both runs with Tracer version 1.5 (Drummond and Rambaut, 2007) showed that log likelihood (ln *L*) scores had converged on a stationary distribution within the burn-in period. Potential scale reduction factors for the monitored parameters were near 1.0.

2.4. Chronological analyses

2.4.1. Multidivtime analysis

The Multidivtime analysis was performed using the Bayesian relaxed-clock method first proposed by Thorne et al. (1998) and further developed in Kishino et al. (2001) and Thorne and Kishino (2002). We first inferred the optimal tree topology for each of the 10 gene partitions (Table 1) separately by running ML phylogenetic analyses under the model and its parameters assessed with AIC in Modeltest 3.7 and using heuristic searches with as-is addition sequence and TBR branch swapping in PAUP* version 4.0b10 (Swofford, 2002). The ML estimates of base frequencies, the transition-to-transversion rate ratio, and the shape parameter of the discrete gamma distribution of rates among sites for each gene partition were next computed under the F84 + Γ model (Felsenstein and Churchill, 1996) with Baseml (within PAML version 4.2; Yang, 2007). The output files from Baseml were then transformed with Paml2modelinf (contained in Multidistribute version 9/25/03; Thorne, 2003) into the input file for Estbranches (also within Multidistribute), which in turn was employed to calculate the ML of branch lengths and to generate their variance–covariance matrix for each gene partition. Finally, the output from Estbranches was used to approximate the posterior distributions of substitution

rates and divergence times in Multidivtime (within Multidistribute) using MCMC. The Markov chains were run for 3×10^6 generations. Parameters were sampled every 10^2 generations after a burn-in of 7.5×10^5 generations. Three independent runs of Multidivtime were performed. The strict consensus of the two 50% majority-rule consensus trees obtained from both runs of the Bayesian (MrBayes) phylogenetic analysis was used in Estbranches and Multidivtime. The fossil-based minimum clade ages were applied as minimum-bound priors in Multidivtime.

2.4.2. BEAST analysis

The BEAST analysis was conducted with the Bayesian uncorrelated lognormal relaxed-clock model implemented in BEAST version 1.6.1 (Drummond et al., 2006; Drummond and Rambaut, 2007). We first generated the BEAST input file with BEAUti version 1.6.1 (Drummond and Rambaut, 2007). Each of the 10 gene partitions (Table 1) was allowed to have its own independent base-substitution model and parameters. The best-fit models were inferred using AIC in Modeltest 3.7. When an optimal model was not available in BEAUti 1.6.1, we selected a similar but more complex near-optimal model (Huelsenbeck and Rannala, 2004). The models eventually used were the same as in the Bayesian (MrBayes) phylogenetic analysis except that TrNef + Γ (Tamura and Nei, 1993) instead of K80 + Γ was assigned to the *CHRNA1* partition. The Yule process of speciation was applied as a tree prior. The fossil calibrating information was incorporated in the form of lognormal prior age distributions in line with the recommendations of Ho (2007) and Ho and Phillips (2009). Each of the fossil-based minimum clade ages was set as the zero offset of the lognormal distribution to represent the minimum bound. The means and standard deviations of the lognormal distributions were set to 1 million years each. We next performed five independent MCMC runs of 10^7 generations each in BEAST 1.6.1. Each run was sampled every 10^3 generations. We then inspected each BEAST log file with Tracer 1.5 to confirm if the parameters converged to the stationary distribution and were sufficiently sampled. After removing the initial 25% of samples from each run as burn-in, the post-burn-in samples from the five runs were combined. All effective sample size values for parameters of the time to the most recent common ancestor exceeded 200, with the exception of two clades (Musteloidea and Mephitidae), for which these values were 144 and 132, respectively.

2.4.3. Fossil constraints

The estimates of minimum divergence times inferred from the fossil record were applied to constrain the ages of three clades. Two of these clades are major crown clades within Ictonychinae. One is the *Lyncodon–Galictis* clade (which we refer to as Lyncodontini, using a tribal name derived from the subfamilial name Lyncodontinae coined by Pocock, 1922) and the other is the *Ictonyx–Poecilogale–Vormela* clade (hereafter referred to as Ictonychini). The third constrained clade is a deep-level clade within Musteloidea (the crown clade of procyonids and mustelids).

The constraint put on the age of Lyncodontini derived from a comparison of ages assigned to the first (lowest stratigraphic) occurrences of *Lyncodon* and *Galictis*. The geologically oldest fossil known of *Lyncodon* is a skull of †*L. bosei* described in Pascual (1958). The skull comes from a site in the Ensenada Formation, Argentina (Soibelzon et al., 2008a). This site is correlated with the geomagnetic polarity chron C1r1n and thus corresponds to an age within a range of 1.07–0.99 million years ago (MYA; Soibelzon et al., 2008b). In turn, the geologically oldest species of *Galictis* is †*G. sorgentinii* (Cione and Tonni, 1995) known from a partial mandible described in Reig (1957). This fossil is referred to the Vorohuean, a subage of the Marplatan South American Land Mammal Age (Cione and Tonni, 1995). Woodburne et al. (2006) correlate the Vorohuean to ~3.0–2.4 MYA. Accordingly, we adopted 2.4 MYA as the minimum age of Lyncodontini.

The age of Ictonychini was constrained on the basis of the first occurrence of this clade represented by the record of †*Baranogale helbingi* from Podlesice, Poland (Kowalski, 1959; Petter, 1987; Wolsan, 1989; Spassov, 2001). This fossil site is the reference locality of the European Neogene mammal chronological unit MN 14 (de Bruijn et al., 1992). The unit itself is regarded in Agustí et al. (2001) as a biostratigraphic zone that spans 4.9–4.2 MYA. We therefore assumed a minimum age of 4.2 MYA for Ictonychini.

The constraint imposed on the age of the crown clade of procyonids and mustelids was assessed based on geological dating of the first occurrences of †*Pseudobassaris riggsi* and †*Plesictis plesictis* (Wolsan, 1993 and references therein). We considered †*Pseudobassaris riggsi* the geologically oldest known stem procyonid (after Wolsan, 1993; Wolsan and Lange-Badré, 1996; Sato et al., 2009) and treated †*Plesictis plesictis* as the oldest known stem mustelid (following Wolsan, 1999; Sato et al., 2003, 2009). The first occurrences of these species date to ages within intervals of 30.3–27.6 MYA and 24.7–23.3 MYA, respectively (Sato et al., 2009 and references therein), which yielded a minimum of 27.6 MYA for the procyonid–mustelid clade.

It is of note that a recent total-evidence analysis (Finarelli, 2008) recovered †*Pseudobassaris* as a stem arctoid, and †*Plesictis* as sister to *Phoca vitulina* (Pinnipedia). The morphological character partition and taxon sampling used in this analysis, however, can account for these unusual placements. For instance, several basicranial characters whose apomorphic states suggest procyonid or procyonid–mustelid affinities of †*Pseudobassaris* were either not included (character 9 of Wolsan, 1993) or coded as plesiomorphic (characters 17, 24, 26, and 30 of Finarelli, 2008), although these characters are indeed invariably or variably apomorphic in this genus (Wolsan, 1993; Wolsan and Lange-Badré, 1996; Sato et al., 2003). If these characters were coded with the apomorphic states, †*Pseudobassaris* would likely be removed to a position within the procyonid–mustelid clade. The only representatives of the total clade (crown clade plus its paraphyletic stem) of procyonids sampled by Finarelli (2008) in addition to †*Pseudobassaris* were two extant species (*Procyon lotor* and *Potos flavus*) whose morphological (particularly dental) characteristics are modified in many respects from those of the early members of the total clade. Inclusion of other stem or early crown procyonids (e.g., †*Broiliana nobilis*; Wolsan, 1993) could suggest more links between †*Pseudobassaris* and the extant procyonids.

We also note that Wang et al. (2005) recovered †*Pseudobassaris* as a stem mustelid, rather than a stem procyonid (Wolsan, 1993; Sato et al., 2009). Importantly, however, even though the inferred placements of †*Pseudobassaris* differ between these studies, both placements are in agreement with our use of †*Pseudobassaris riggsi* to calibrate the procyonid–mustelid clade.

To examine how the age of †*Pseudobassaris riggsi* corresponds to an independent age estimate for the procyonid–mustelid divergence, we ran an additional BEAST analysis applying the same procedure, models, and settings as in the original BEAST analysis except that the age of the procyonid–mustelid divergence was constrained with a minimum of 23.3 MYA based on the first occurrence of the stem mustelid †*Plesictis plesictis* and, additionally, the age of the musteloid–pinniped divergence was constrained with a minimum of 33.7 MYA based on the first occurrence of the stem musteloid †*Mustelavus priscus* (Sato et al., 2009 and references therein). The results of this analysis (Supplementary Table S4) are highly congruent to those obtained with our original BEAST and Multidivtime analyses. The 95% credibility interval for the age of the procyonid–mustelid divergence estimated in this additional analysis (30.3–23.4 MYA) encompasses the geological age of †*Pseudobassaris riggsi* (which lies between 30.3 and 27.6 MYA;

Sato et al., 2009 and references therein). We note that the 95% credibility intervals for the age of the procyonid–mustelid divergence obtained in two recent dating analyses using other DNA and taxon samplings and other fossil calibrations (Eizirik et al., 2010) also embrace the age of †*P. riggsi*.

2.5. Biogeographic analyses

2.5.1. Distributional dataset

All of the 45 sampled wild arctoid species (Supplementary Table S1) were scored for presence or absence in each of five discrete areas corresponding to continents (Africa, Asia, Europe, North America, and South America) according to the recent geographic distribution of these species (Wozencraft, 2005). Multiple areas were assigned to species occurring in two or more continents. Recent human-mediated range expansions were not taken into consideration. *Mustela furo* was treated as of unknown distribution. This distributional dataset (Fig. 1) was used in all biogeographic analyses.

Admittedly, our biogeographic analyses have limitations related to the fact that the distributional dataset does not include extinct musteloids and because reconstructions of ancestral areas based on extant species alone may differ from those that also contain extinct species. Indeed, ancestral-area analyses on extant taxa that do not include extinct taxa can potentially be inaccurate. However, analyses that include extinct taxa can also lead to inaccurate results because of the incompleteness of the fossil record (Lieberman, 2002).

2.5.2. Parsimony analysis

The parsimony biogeographic analysis was carried out with Sankoff optimization (Sankoff and Rousseau, 1975) in TNT 1.1. The costs of symmetric transitions between continents were weighted on the basis of current and former post-Eocene (Asian–North American and African–European) intercontinental land connections as follows: one step, Africa–Asia, Africa–Europe, Asia–Europe, Asia–North America, and North America–South America; two steps, Africa–North America, Asia–South America, and Europe–North America; and three steps, Africa–South America, and Europe–South America. The distributional dataset was first optimized on either of the two alternative equally most-parsimonious trees obtained from the parsimony phylogenetic analysis, and then an ancestral-area mapping common to both trees was generated by selecting the *common mapping* option. Where the ancestral area for a clade was subject to more than one interpretation, the concerned continents were identified using the *recons* option.

2.5.3. Maximum-likelihood analysis

The ML biogeographic analysis was conducted under the dispersal–extinction–cladogenesis model in Lagrange version 2.0.1 (Ree and Smith, 2008). The present and former post-Eocene land links among the five continents were taken into account by scaling the symmetric dispersal rates between Africa and Asia, Africa and Europe, Asia and Europe, Asia and North America, and North America and South America to 1.0; those between Africa and North America, Asia and South America, and Europe and North America to 0.5; and those between Africa and South America and between Europe and South America to 0.33. The topology and branch lengths with the highest ln *L* score among the five runs of the ML phylogenetic analysis were applied.

2.5.4. Bayesian analysis

The Bayesian biogeographic analysis was performed using a Bayesian model-averaging approach implemented in the BayesMultistate program contained in the BayesTraits (version 1.0) package (Pagel and Meade, 2006, 2007). We employed two sets of trees, which were analyzed separately. Both sets consisted of the last 5×10^4 post-burn-in trees sampled in a different run of the Bayesian (MrBayes) phylogenetic analysis. Ancestral areas for clades with a posterior probability of <1.0 were estimated by applying the most-recent-common-ancestor method of Pagel et al. (2004). Preliminary analyses were completed to adjust the magnitude of the rate-coefficient proposals (*ratedev* parameter) until the acceptance rates of proposed changes achieved 20–40%. We then conducted five independent reversible-jump MCMC runs for both sets of trees. Sampling was conducted every 10^2 generations, with chains being propagated for 10^7 generations and the first 5×10^4 sampled trees discarded. Stasis of ln *L* was confirmed with Tracer 1.5. The posterior probabilities resulting from the runs that showed the highest harmonic mean ln *L* for either tree set were averaged for each clade.

3. Results

3.1. Phylogenetic inference

The parsimony (Fig. 1), ML (Fig. 2), and Bayesian (Fig. 3) phylogenetic analyses resulted in trees with largely congruent topologies, with the majority of clades being consistently corroborated with strong support. Minor differences concerned the placements of *Mellivora capensis* (ML vs. parsimony and Bayesian analyses), *Gulo gulo* relative to *Martes flavigula* (parsimony vs. ML and Bayesian analyses), and *Mustela sibirica* relative to *Mustela itatsi* (Bayesian vs. parsimony and ML analyses). Additionally, a parsimony trichotomy was recovered within *Mustela* and a Bayesian tetratomy within *Martes* (both of these were uniformly resolved in the other two analyses).

All phylogenetic analyses (Figs. 1–3) strongly supported a clade composed of *Mydaus javanensis* and *Mephitis mephitis* (Mephitidae, clade 46) as the earliest offshoot of Musteloidea, followed by *Ailurus fulgens* (Ailuridae) sister to the common clade of raccoons (*Procyon*; Procyonidae, clade 43) and Mustelidae (clade 41). Within Mustelidae, *Taxidea taxus* (Taxidiinae) was recovered as sister to the rest of the family. *Arctonyx collaris* and the species of *Meles* are closely related (Melinae, clade 39). The martens (*Martes*) and *Gulo gulo* are more crownward and also closely related (Guloninae, clade 28). The genus *Martes* is paraphyletic relative to *Gulo*, with *Martes pennanti* strongly supported as sister to all other sampled gulonines. Still more crownward, *Melogale moschata* (Helictidinae) is sister to the robustly-supported clade containing the weasels, polecats, and minks of *Mustela* and *Neovison* (Mustelinae, clade 13) and a clade composed of otters (Lutrinae, clade 8) and the weasels, polecats, and grisons of Ictonychinae (clade 3). A sister relation between Ictonychinae and Lutrinae (as well as all relationships within both subfamilies) was well supported by each of the phylogenetic analyses (Figs. 1–3). Ictonychinae was divided into a clade consisting of the grisons (*Galictis*) and *Lyncodon patagonicus* (Lyncodontini, clade 1) and a clade composed of *Vormela peregusna*, *Poecilogale albinucha*, and two species of *Ictonyx* (Ictonychini, clade 4). *Ictonyx striatus* and *Poecilogale albinucha* are more closely related to each other than either is to *Ictonyx libyca*, rendering *Ictonyx* a paraphyletic genus.

3.2. Chronological inference

The age estimates resulting from the Multidivtime and BEAST chronological analyses were highly correlated ($r^2 = 0.99$), with close correspondence found for most divergences (Fig. 3). The initial musteloid radiation involving the separation into lineages leading to Mephitidae, Ailuridae, Procyonidae, and Mustelidae

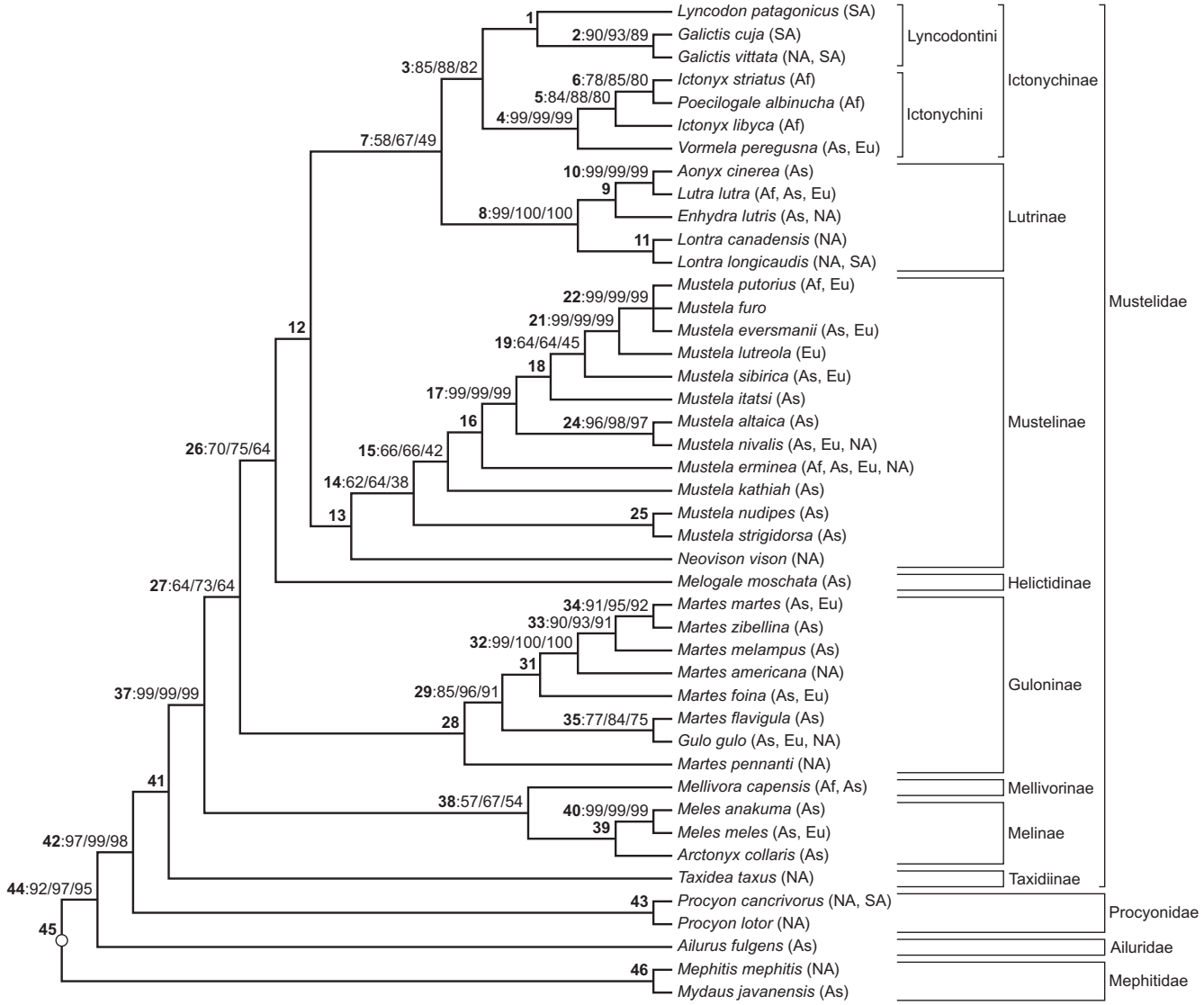

Fig. 1. Parsimony cladogram of Musteloidea. This is the strict consensus of the two alternative equally most-parsimonious trees (length, 7754 steps; consistency index for informative characters, 0.375; retention index, 0.619) found in the parsimony phylogenetic analysis. The placement of the root is indicated with an open circle (outgroup species are not shown). Numbers at nodes are the respective clade numbers for clades with the 100% values of all support indices or, for the remaining clades, the respective clade numbers followed by the values of bootstrap frequency, symmetric-resampling frequency, and symmetric-resampling frequency difference (all in percentages and in this order). Areas of recent geographic distribution for wild species (assigned in the distributional dataset used in biogeographic analyses) are indicated in parentheses: Af, Africa; As, Asia; Eu, Europe; NA, North America; SA, South America. The circumscriptions of relevant suprageneric taxa are shown to the right.

(divergences 45, 44, and 42) was estimated to have occurred early in the Oligocene. The origin of Mephitidae (divergence 46) was dated to the Early (Multidivtime analysis) or early Middle (BEAST analysis) Miocene. In turn, the emergence of Mustelidae (divergence 41) dates back to near the Early/Middle Miocene boundary. The lineages for all of the mustelid subfamilies had their origin in the Miocene. The inception of Ictonychinae (divergence 3) was dated to the early Late Miocene, and that of Ictonychini (divergence 4) to the twilight of the Miocene. The radiation of African Ictonychini (divergences 5 and 6) was estimated to have transpired in the Pliocene, whereas that of Lyncodontini (divergences 1 and 2) was placed at the Pliocene–Pleistocene transition.

3.3. Biogeographic inference

The parsimony, ML, and Bayesian ancestral-area reconstructions were largely concordant and indicated that much of the present-day diversity of musteloids originated in Asia (Table 2). All biogeographic analyses consistently pointed to Asia as the center of origin for Musteloidea and also for 17 of its subclades. Only eight clades were unequivocally of non-Asian ancestry. These are *Procyon* and *Lontra* (both reconstructed in all biogeographic analyses to be of North American origin), Lyncodontini and *Galictis* (consistently of South American origin), clades 5 and 6 within Ictonychini (consistently of African origin), and clades 21 and 22 within *Mustela* (consistently of European origin). Ancestral areas estimated for 11 clades differed among the three analytical methods, but those favoring Asia prevailed in nine cases.

4. Discussion

4.1. Initial musteloid radiation

4.1.1. Branching order

Deep-level phylogenetic relationships within Musteloidea (particularly the relative position of Ailuridae and Mephitidae) have, until recently, remained unresolved and the cause of contention or ambiguity (e.g., Agnarsson et al., 2010; Morlo and Peigné,

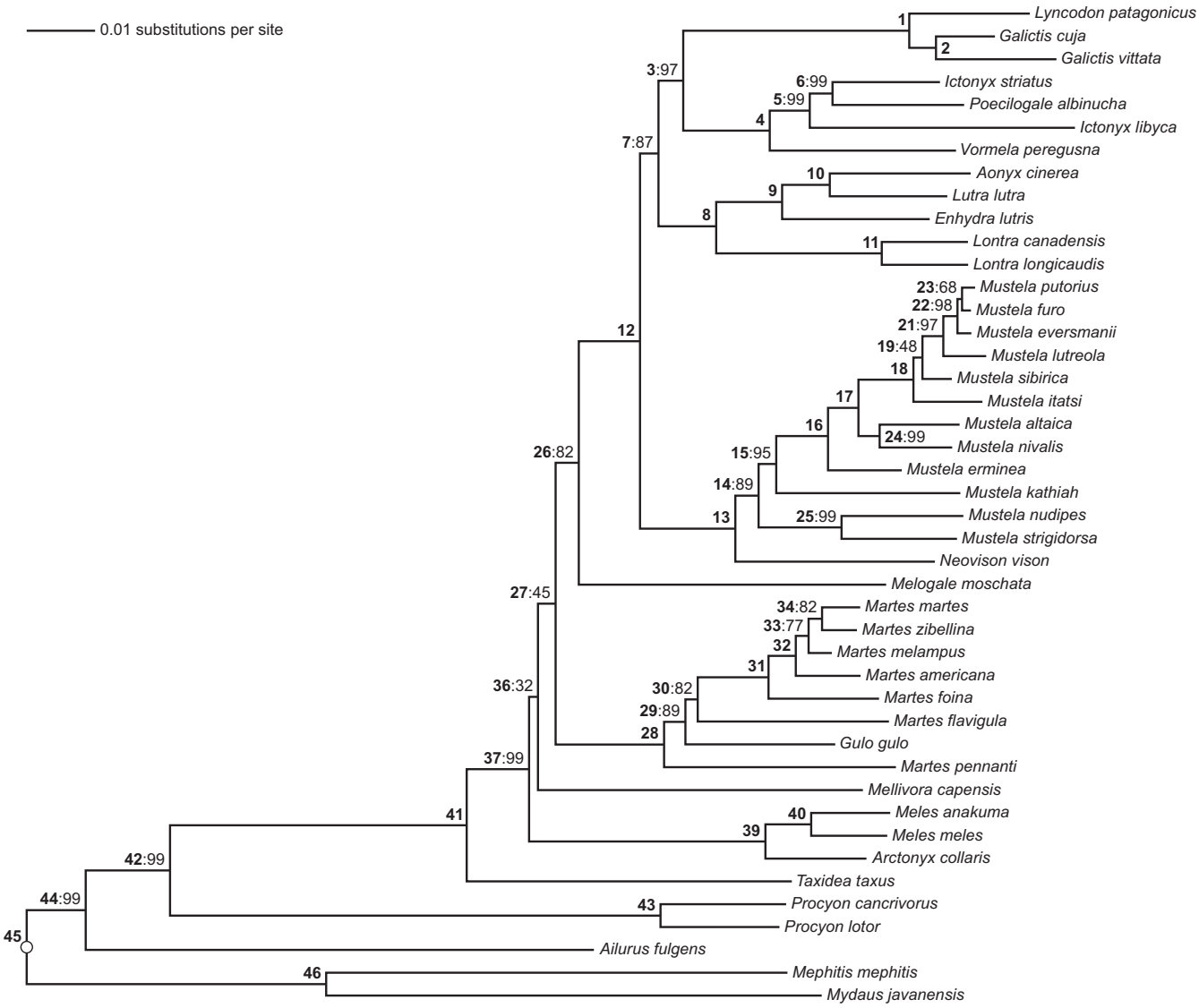

Fig. 2. Maximum likelihood (ML) phylogram of Musteloidea. The tree with the highest log likelihood (−51157.3706) observed across the five runs of the ML phylogenetic analysis is presented. The placement of the root is indicated with an open circle (outgroup species are not shown). Numbers at nodes are the respective clade numbers for clades supported with a bootstrap frequency of 100% or, for the remaining clades, the respective clade numbers followed by the value of bootstrap frequency (%).

2010; Salesa et al., 2011). That Mephitidae and Ailuridae are successively more closely related to a clade containing Procyonidae and Mustelidae was first suggested by Sato et al. (2006) and later, independently, by Fulton and Strobeck (2006) based on evidence from nDNA sequences. Robust support for this hypothesis (congruent across parsimony and probabilistic methods of phylogenetic inference and diverse statistical tests of topology) was first reported by Sato et al. (2009) on the basis of a larger set of nDNA sequences. Eizirik et al. (2010) verified this result using still more nDNA data. The present study further reinforces this conclusion with consistently strong support from diverse analyses conducted on a combined nDNA and mtDNA data set (Figs. 1–3). We note that the strongest overall support for this hypothesis comes from Sato et al. (2009) and the present work, which may be due to the fact that these two studies have employed the most complete musteloid sampling.

4.1.2. Timing and duration

The initial radiation of musteloids was estimated to have occurred during a ~2.5–4-million-year time interval in the Oligocene (~32.4–28.4 MYA with Multidivtime analysis and ~30.9–28.4 MYA with BEAST analysis; Fig. 3). Most previous analyses applying a multilocus molecular dating approach have inferred similar interval lengths (~3–4.5 million years) and similar age estimates for the involved divergences (31.4–28.4 MYA, Sato et al., 2009; ~33.1–29.0 MYA, Yonezawa et al., 2007; 33.8–29.4 MYA and 32.0–27.4 MYA, Eizirik et al., 2010). Conversely, Árnason et al.'s (2007) analysis suggested a markedly older age for the origin of Musteloidea and consequently a longer interval of 6.5 million years (~35.5–29.0 MYA) for the initial musteloid radiation.

How do these molecular age estimates compare with the fossil record? The first appearance of the earliest known musteloid (†*Mustelictis olivieri*; Sato et al., 2009) dates to 32.8–30.9 MYA, the earliest known stem procyonid (†*Pseudobassaris riggsi*; Wolsan, 1993; Wolsan and Lange-Badré, 1996; Sato et al., 2009) to 30.3–27.6 MYA, the earliest known ailurid (†*Amphictis ambigua*; Ginsburg, 1999; Wolsan, 1999) to 25.6–24.0 MYA, the earliest known stem mustelid (†*Plesictis plesictis*; Wolsan, 1999; Sato et al., 2003, 2009) to 24.7–23.3 MYA, and the earliest known total-clade mephitid (†*Miomephitis pilgrimi*; Wolsan, 1993; Ginsburg, 1999) to 20.3–17.6 MYA (Sato et al., 2009 and references therein). Comparisons of these geological age ranges with our molecular estimates of

Clade	Multidivtime analysis		BEAST analysis	
	Posterior mean (MYA)	95% posterior interval (MYA)	Posterior mean (MYA)	95% posterior interval (MYA)
1	2.89	3.76–2.42	2.61	2.88–2.42
2	2.03	2.86–1.34	1.67	2.17–1.17
3	9.53	11.35–7.95	8.95	10.32–7.65
4	6.48	8.22–4.97	6.01	7.23–4.78
5	4.85	6.35–3.58	4.46	5.51–3.46
6	4.27	5.65–3.10	3.44	4.47–2.49
7	10.24	12.11–8.63	9.80	11.24–8.44
8	8.30	10.11–6.74	7.37	8.69–6.07
9	5.76	7.29–4.45	4.60	5.59–3.60
10	4.00	5.30–2.90	3.16	3.99–2.37
11	2.25	3.20–1.48	1.43	2.01–0.93
12	10.60	12.48–8.99	10.49	12.01–9.03
13	7.13	8.74–5.77	6.56	7.75–5.45
14	6.30	7.82–5.01	5.67	6.69–4.69
15	5.56	7.01–4.33	5.17	6.13–4.24
16	3.97	5.14–3.00	3.40	4.10–2.73
17	3.19	4.25–2.32	2.62	3.17–2.11
18	1.60	2.38–1.01	1.52	1.87–1.18
20	1.47	2.21–0.90	1.48	1.83–1.14
21	0.60	1.16–0.24	1.22	1.54–0.91
22	0.25	0.56–0.06	0.61	0.86–0.38
23	0.17	0.44–0.02	0.41	0.61–0.22
24	2.60	3.55–1.84	2.00	2.55–1.52
25	3.46	4.63–2.48	2.89	3.74–2.09
26	12.06	14.11–10.30	12.50	14.23–10.78
27	12.65	14.72–10.83	13.30	15.11–11.49
28	7.90	9.64–6.41	6.51	7.85–5.32
29	7.30	8.98–5.85	5.41	6.49–4.34
30	6.56	8.21–5.13	4.95	5.99–3.95
31	3.63	4.84–2.61	2.46	3.08–1.85
32	1.72	2.50–1.10	1.65	2.12–1.21
37	13.60	15.75–11.71	13.49	15.35–11.78
38	12.67	14.89–10.67	12.55	14.59–10.53
39	3.28	4.45–2.30	2.70	3.47–1.96
40	1.94	2.83–1.22	1.81	2.41–1.23
41	16.13	18.44–14.08	16.07	18.12–14.05
42	28.35	30.38–27.62	28.35	29.80–27.62
43	3.87	5.39–2.62	2.96	4.08–1.94
44	30.50	33.19–28.53	30.32	32.71–27.68
45	32.36	35.53–29.87	30.86	34.86–27.65
46	20.21	23.50–17.19	15.60	20.55–11.78

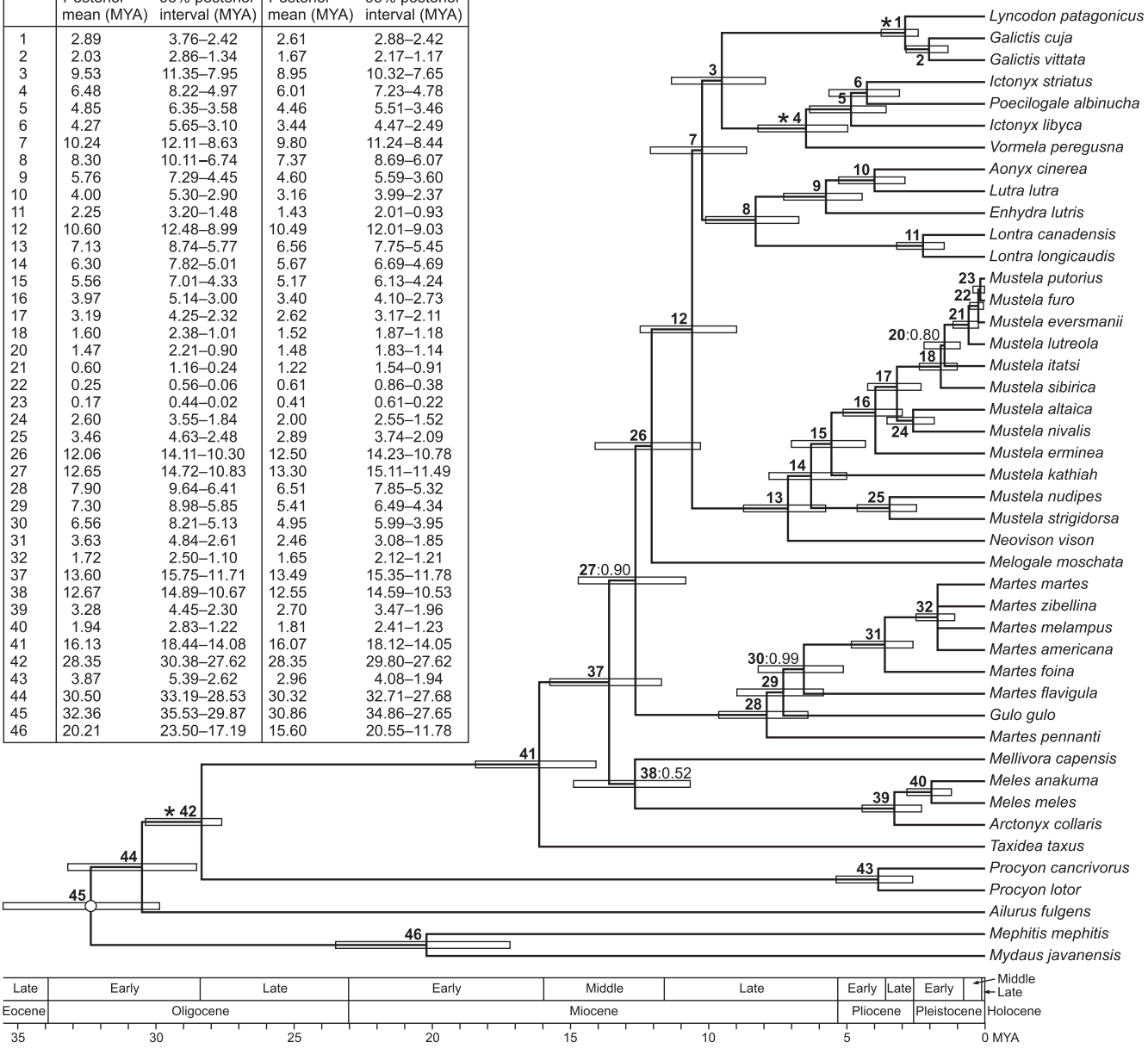

Fig. 3. Bayesian chronogram of Musteloidea. The branching topology is the strict consensus of the two 50% majority-rule consensus trees (harmonic mean log likelihoods, −49421.75 and −49406.85; average standard deviation of split frequencies, 0.0022) derived from both MrBayes runs (Bayesian phylogenetic analysis). The placement of the root is indicated with an open circle (outgroup species are not shown). Numbers at nodes are the respective clade numbers for clades with 1.00 posterior probabilities received from both MrBayes runs or, for the remaining clades, the respective clade numbers followed by the value of posterior probability (when posterior probabilities differed between both MrBayes runs, their mean is given). Stars indicate clades with ages constrained by fossils. The ages of clades correspond to their posterior means averaged across the three runs of the Multidivtime chronological analysis (the maximum differences between posterior means for a clade across the three Multidivtime runs ranged from 0.003 to 0.039 MYA [million years ago] with a median of 0.013 MYA). Uncertainty in clade ages is indicated by horizontal bars on clade origins: bar lengths equate to the 95% posterior intervals of clade ages maximized across the three Multidivtime runs (i.e., limited by the maximum and minimum interval values of all the runs). The results of the BEAST chronological analysis based on the combined post-burn-in samples from the five BEAST runs are presented in the table (inset) for comparison with the Multidivtime results.

divergence times yield estimates for the lengths of ghost lineages (gaps in the fossil record). These fall within ranges of 10.6–14.8 million years for the total clade of mephitids, 4.7–6.5 million years for Ailuridae (total clade), 3.7–5.1 million years for the total clade of mustelids, 0–1.5 million years for Musteloidea (crown clade), and 0–0.75 million years for the total clade of procyonids.

4.1.3. Geographic location

Our biogeographic analyses consistently indicated an Asian origin of Musteloidea. This agrees with fossil evidence that also points to an Asian center of early musteloid diversification (Sato et al., 2009). Fossil data suggest that musteloids initially entered Europe as early as 32.8–30.9 MYA (†*Mustelictis*) followed by several waves of musteloid dispersal from Asia to Europe during the Oligocene and later, which are exemplified by such genera as †*Pseudobassaris*, †*Amphictis*, †*Plesictis*, †*Bathygale*, †*Franconictis*, †*Stromeriella*, †*Angustictis*, †*Broiliana*, †*Paragale*, and †*Plesiogale* (reviewed in Wolsan, 1993). The first documented wave of musteloid immigration to North America is provided by †*Zodiolestes* (reviewed in Baskin, 1998) and occurred as late as ~23 MYA in the Early Miocene

Table 2
Ancestral areas for the clades of Musteloidea (depicted in Figs. 1–3) as favored by the parsimony, maximum likelihood, and Bayesian biogeographic analyses.

Clade	Parsimony	Maximum likelihood	Bayesian inference
1	South America	South America	South America
2	South America	South America	South America
3	Asia	Asia	Africa
4	Asia	Africa and Asia	Africa
5	Africa	Africa	Africa
6	Africa	Africa	Africa
7	Asia	Asia	Africa
8	Asia	Asia and North America	Asia
9	Asia	Asia	Asia
10	Asia	Asia	Asia
11	North America	North America	North America
12	Asia	Asia	Asia
13	Asia	Asia and North America	Asia
14	Asia	Asia	Asia
15	Asia	Asia	Asia
16	Asia	Asia	Asia
17	Asia	Asia	Asia
18	Asia	Asia	Europe
19	Asia or Europe	Asia and Europe	n.a.
20	n.a.	n.a.	Europe
21	Europe	Europe	Europe
22	Europe	Europe	Europe
23	n.a.	Europe	Africa
24	Asia	Asia	Asia
25	Asia	Asia	Asia
26	Asia	Asia	Asia
27	Asia	Asia	Asia
28	Asia	Asia and North America	Asia
29	Asia	Asia	Asia
30	n.a.	Asia	Asia
31	Asia	Asia	Asia
32	Asia	Asia and North America	Asia
33	Asia	Asia	n.a.
34	Asia	Asia	n.a.
35	Asia	n.a.	n.a.
36	n.a.	Asia	n.a.
37	Asia	Asia	Asia
38	Asia	n.a.	Asia
39	Asia	Asia	Asia
40	Asia	Asia	Asia
41	Asia or North America	Asia and North America	Asia
42	Asia or North America	North America	Asia
43	North America	North America	North America
44	Asia	Asia	Asia
45	Asia	Asia	Asia
46	Asia	Asia and North America	Asia

n.a., not applicable.

(Tedford et al., 2004). We note that the endemic North American †*Mustelavus*, †*Promartes*, †*Oligobunis*, and †*Megalictis* (including †*Aelurocyon* and †*Paroligobunis*; Hunt and Skolnick, 1996), all considered musteloids in Baskin (1998), represent the paraphyletic musteloid stem rather than the crown clade Musteloidea (Sato et al., 2009).

4.2. Timing and location of mustelid origin

The origin of Mustelidae was dated here to ~16.1 MYA (during the Early–Middle Miocene transition). This estimate is close to the nDNA-based ages of 16.3 MYA (Sato et al., 2009) and 15.6 MYA (Eizirik et al., 2010), though the latter authors also obtained a younger date of 13.0 MYA using a second dating method. Other estimates of this divergence event inferred by the application of multilocus molecular dating are considerably older and include ~20.2 MYA based on mitochondrial nucleotide (Yonezawa et al., 2007) and amino acid (Árnason et al., 2007) sequences, and 26.2–20.9 MYA based on combined nDNA and mtDNA sequences (Koepfli et al., 2008). We note that †*Plesictis plesictis*, whose approximate geological age (24 MYA) was used by Koepfli et al. (2008) to constrain the age of Mustelidae in their chronological analyses, is a stem rather than crown mustelid (Sato et al., 2009), which may account for older dates recovered in their analyses.

Whether Asia or North America is the ancestral continent for Mustelidae remains to be conclusively resolved. Although our Bayesian biogeographic analysis supported Asia, the parsimony analysis yielded an equivocal result, while the ML analysis favored an area involving both continents. Previous ML analyses similarly resulted in equivocal ancestral-area reconstructions (Koepfli et al., 2008).

4.3. Establishment and interrelationships of mustelid subfamilies

Recent hypotheses based on anatomical data (Bryant et al., 1993) or on a combination of anatomical and mtDNA data or mtDNA data alone (Dragoo and Honeycutt, 1997; Marmi et al., 2004) have postulated a mellivorine affinity for *Taxidea taxus* (Taxidiinae) or have placed this species in a sister relationship to *Meles* alone or together with *Arctonyx collaris* (Melinae). Our results reject these hypotheses and instead strongly support Taxidiinae as sister to all other mustelids, corroborating in this respect the phylogenetic reconstruction inferred in Koepfli et al. (2008).

Our findings also concur with recent observations (e.g., Koepfli and Wayne, 2003; Fulton and Strobeck, 2006; Koepfli et al., 2008; Sato et al., 2009; Wolsan and Sato, 2010) that *Meles* and *Arctonyx* are sister taxa within Melinae, and that the extant species of *Martes* and *Gulo* are closely related within Guloninae. Although the monophylies of Melinae and Guloninae are well grounded, the relative placement of both subfamilies remains uncertain. Our phylogenetic analyses placed Melinae outside a clade containing Guloninae and more-crownward mustelids (Figs. 1–3). Support for this relationship was, however, relatively weak. Most of the former multilocus molecular analyses have recovered the same phylogenetic arrangement between these two subfamilies (Koepfli and Wayne, 2003; Sato et al., 2003, 2006, 2009; Marmi et al., 2004; Fulton and Strobeck, 2006; Sato, 2006; Árnason et al., 2007; Yonezawa et al., 2007; Schröder et al., 2009; Eizirik et al., 2010; Ki et al., 2010; Wolsan and Sato, 2010; Yamada and Masuda, 2010), whereas others placed Guloninae outside a clade containing Melinae and the more-crownward mustelids (Sato et al., 2006; Yu and Zhang, 2006; Yu et al., 2008), hypothesized a sister relation between Melinae and Guloninae (Koepfli and Wayne, 2003; Yu et al., 2004; Fulton and Strobeck, 2006; Koepfli et al., 2008; Wolsan and Sato, 2010), or failed to resolve the relationship between the two subfamilies (Sato et al., 2004; Fulton and Strobeck, 2006; Yu et al., 2008).

The phylogenetic placement of *Mellivora capensis* (Mellivorinae) also remains contentious and its resolution awaits further research. Notably, a position sister to all mustelids except Taxidiinae, hypothesized by Koepfli et al. (2008), was not supported. Our analyses instead weakly recovered Mellivorinae as sister to either Melinae (parsimony and Bayesian inference) or all mustelids except Melinae and Taxidiinae (ML), albeit with only weak support in each case. The latter relationship was also weakly supported in a Bayesian analysis of *MT-CYB* sequences (Agnarsson et al., 2010).

The ferret–badgers (*Melogale*, Helictidinae) were strongly recovered as sister to a clade composed of Mustelinae, Ictonychinae, and Lutrinae (Figs. 1–3). This observation agrees with the results of earlier phylogenetic investigations exploring sequence data from multiple DNA loci (Koepfli and Wayne, 2003; Sato et al., 2004, 2006, 2009; Fulton and Strobeck, 2006; Sato, 2006; Koepfli et al., 2008; Wolsan and Sato, 2010), but contradicts a hypothesis based on anatomical characters, which instead proposes *Melogale* as sister to all other mustelids and mephitids (Bryant et al., 1993).

The monophyly of the mustelines, ictonychines, and lutrines was also strongly supported, corroborating results obtained in other molecular studies (Dragoo and Honeycutt, 1997; Koepfli and Wayne, 2003; Flynn et al., 2005; Fulton and Strobeck, 2006; Rozhnov et al., 2006; Koepfli et al., 2008; Harding and Smith, 2009; Agnarsson et al., 2010; Eizirik et al., 2010; Wolsan and Sato, 2010), which conflict with competing (largely morphology-based) hypotheses (e.g., Bryant et al., 1993; Baryshnikov and Abramov, 1998; Bininda-Emonds et al., 1999). Previous molecular investigations have suggested that ictonychines are most closely related to either mustelines (Fulton and Strobeck, 2006; Rozhnov et al., 2006; Harding and Smith, 2009; Agnarsson et al., 2010) or lutrines (Dragoo and Honeycutt, 1997; Koepfli and Wayne, 2003; Fulton and Strobeck, 2006; Eizirik et al., 2010; Wolsan and Sato, 2010), or are sister to a hypothesized clade composed of mustelines and lutrines (Dragoo and Honeycutt, 1997; Flynn et al., 2005; Koepfli et al., 2008). Support for these phylogenetic associations was, however, not very strong. In contrast, our analyses provided relatively strong support for a close relationship between Ictonychinae and Lutrinae to the exclusion of Mustelinae.

4.4. Timing and location of ictonychine diversification

Most of the time estimates within Ictonychinae obtained by Koepfli et al. (2008) are younger than our estimates and fall within the ranges of 8.2–7.9 MYA (vs. our ~9.5–8.9 MYA) for the origin of Ictonychinae, 4.6–4.0 MYA (vs. our ~6.5–6.0 MYA) for the rise of Ictonychini, 3.5–3.0 MYA (vs. our ~4.8–4.5 MYA) for the beginning of the crown clade of African Ictonychini, and 2.7–2.2 MYA (vs. our ~4.3–3.4 MYA) for the separation of the lineages for *Ictonyx striatus* and *Poecilogale albinucha*. Conversely, the previous estimates of 3.0–2.8 MYA (Koepfli et al., 2008) and 5.6 and 6.6 MYA (Harding and Smith, 2009) for the split between *Galictis cuja* and *G. vittata* (the latter study based on *MT-CYB* data alone) are older than our estimates (~2.0 and ~1.7 MYA).

Although our results agree with those of Koepfli et al. (2008) that Ictonychinae originated in the Old World rather than in the New World, it remains unresolved where specifically this event occurred. Koepfli et al.'s (2008) ML biogeographic analyses supported Eurasia, whereas our analyses favored either Asia (parsimony and ML) or Africa (Bayesian inference). Where Ictonychini arose is also uncertain. Our analyses favored Asia (parsimony) or Africa (Bayesian inference) or suggested that the ancestral range extended onto both continents (ML). The results of Koepfli et al. (2008) are also equivocal in this regard. As the origins of Ictonychinae and Ictonychini date back to the Late Miocene (Fig. 3), the presence of ictonychines in the Late Miocene fossil record in Eurasia (e.g., †"*Baranogale*" *adroveri*; Petter, 1964, 1987) and North America (e.g., †*Cernictis hesperus*; Baskin, 1998), and the fact that no pre-Pliocene ictonychine has been reported from Africa (McKenna and Bell, 1997; de Bonis, 2008) suggest Asia, rather than Africa, as the ancestral area for both clades. It should be noted, however, that the African fossil record has been examined less extensively than those of Eurasia and North America, and that the Miocene African mustelids are not well known.

5. Conclusions

5.1. Early Musteloidea

Our phylogenetic results indicate that early crown musteloids diversified into four primary divisions: the Mephitidae lineage separated first, succeeded by Ailuridae and finally by the divergence of the Procyonidae and Mustelidae lineages. Previous investigations have either supported this hypothesis (Fulton and Strobeck, 2006; Sato et al., 2006, 2009; Eizirik et al., 2010) or generated alternative hypotheses (reviewed in Sato et al., 2009 and Morlo and Peigné, 2010). These alternative hypotheses, however, have received, at most, weak support or conflicted with each other across the applied methods of analysis. We therefore reject these alternative hypotheses.

There are two competing hypotheses regarding the center of origin for the crown clade Musteloidea. One proposes Asia (Sato et al., 2009), whereas the other favors North America (Yonezawa et al., 2007). Our biogeographic results reject the latter hypothesis and corroborate the Asian origin, which also agrees with the fossil record (see Section 4.1.3).

The results of our chronological analyses suggest that the inception (~32.4–30.9 MYA) and initial radiation of Musteloidea postdated a rapid change in global climate during the Eocene–Oligocene transition (~33.5 MYA), which marked a dramatic shift from "greenhouse" to "icehouse" conditions. This global climate change was accompanied by substantial climatic, environmental, and biotic alterations in Asia and over other parts of the Northern Hemisphere (Meng and McKenna, 1998; Dupont-Nivet et al., 2007; Eldrett et al., 2009; and references therein).

5.2. Mustelidae

Our findings suggest that the crown clade Mustelidae emerged ~16.1 MYA within a period of global warmth known as the Mid-Miocene Climatic Optimum (~17–15 MYA; Zachos et al., 2001) and also indicate that early crown mustelids underwent an extensive Miocene diversification, which proceeded largely in Asia. The survivors of the early divergences of this diversification (Taxidiinae, Melinae, Mellivorinae, Guloninae, and Helictidinae) are mostly represented by badgers and martens. The later divergences gave rise to the most-crownward mustelids (Mustelinae, Ictonychinae, and Lutrinae), which have adapted to other ecological niches and mostly include weasels, polecats, minks, and otters.

With the exception of the honey badger (*Mellivora capensis*), which has primarily been classified in its own subfamily (Mellivorinae), all other extant badgers were long regarded as closely related to each other and accordingly included in a common badger subfamily dubbed Melinae (e.g., Macdonald, 1985). Similarly, all martens have been grouped in a single genus (*Martes*); the weasels, polecats, and minks have, until recently, been united with the martens and wolverines in a subfamily referred to as Mustelinae; and the otters have been retained in their own subfamily Lutrinae (e.g., Wozencraft, 2005). Although the monophyly of all otters (both extant and extinct) remains to be established, our findings are congruent with earlier observations (e.g., Bryant et al., 1993; Koepfli and Wayne, 2003; Fulton and Strobeck, 2006; Koepfli et al., 2008; Wolsan and Sato, 2010) that the living otters are monophyletic. Our results, however, also clearly indicate that the badgers, martens, weasels, polecats, and minks are each not monophyletic (Figs. 1–3). Specifically, the badgers are polyphyletic and the martens are paraphyletic with respect to the wolverines. In turn, weasels and polecats are scattered within Mustelinae (here restricted to encompass only two extant genera, *Mustela* and *Neovison*) and Ictonychinae, while a mink is found within both *Mustela* and *Neovison*. One or more of these conclusions have also gained strong support in studies by Koepfli and Wayne (2003), Flynn et al. (2005), Fulton and Strobeck (2006), Koepfli et al. (2008), Sato et al. (2009), and Wolsan and Sato (2010). As a consequence, throughout this paper we use the subfamilial name Melinae in a restricted sense to denote a monophyletic group of true badgers containing *Arctonyx collaris* and the *Meles* species, which is in accord with the phylogenetic definition of Melinae provided in Wolsan and Sato (2010). Although the species nomenclature in the present paper follows Wozencraft (2005) for consistency with the currently prevailing taxonomy, so that all martens are conventionally referred to the genus *Martes* as traditionally conceived, we

recommend that the subgenus *Pekania* be elevated to the rank of genus to accommodate the fisher (*Martes pennanti*), and that the genus *Martes* be confined to a monophyletic group of species that includes the remaining extant martens.

Although the sister relation between Taxidiinae and the rest of Mustelidae and that between Helictidinae and a clade containing Mustelinae, Ictonychinae, and Lutrinae are well grounded based on the findings of this and other recent studies (e.g., Koepfli et al., 2008), the pattern of phylogenetic relationships among Melinae, Mellivorinae, and Guloninae remains ambiguous and its resolution requires further study. Our results provide relatively strong support for a close relationship between Ictonychinae and Lutrinae to the exclusion of Mustelinae, making this phylogenetic arrangement the best supported hypothesis at present for relations among these subfamilies.

5.3. Ictonychinae

Our phylogenetic results corroborate the hypothesis that the Old World Ictonychini and New World Lyncodontini are monophyletic (Koepfli and Wayne, 2003; Fulton and Strobeck, 2006; Koepfli et al., 2008; Wolsan and Sato, 2010). Our study additionally elucidates the phylogenetic position of *Lyncodon patagonicus*, clearly revealing that *Lyncodon* and *Galictis* are sister taxa. Alternative hypotheses about the relationships of ictonychines (e.g., Bryant et al., 1993; Baryshnikov and Abramov, 1998; Bininda-Emonds et al., 1999; Agnarsson et al., 2010) are thus rejected.

Our phylogenetic reconstruction of Ictonychini agrees with that of Koepfli et al. (2008), with both studies yielding strong support for a position of *Ictonyx libyca* outside a clade containing *Ictonyx striatus* and *Poecilogale albinucha*. We therefore recommend that *I. libyca* be assigned to *Poecilictis*, a genus in which this species was often included previously (e.g., Macdonald, 1985; Petter, 1987; Bryant et al., 1993; Baryshnikov and Abramov, 1998; Spassov, 2001). Our recommendation is contrary to that of Koepfli et al. (2008), who proposed inclusion of all three species in the genus *Ictonyx*.

The results of our chronological and biogeographic analyses indicate that the crown clades Ictonychinae and Ictonychini originated ~9.5–8.9 MYA and ~6.5–6.0 MYA, respectively, in Asia or Africa. The fossil record suggests Asia, rather than Africa, as the initial centers of diversification for both clades (see Section 4.4). If it was indeed Asia, then Ictonychini entered Africa probably as late as during the Messinian Salinity Crisis, ~6.0–5.3 MYA, when the Mediterranean Sea became isolated from the Atlantic Ocean and largely evaporated (Krijgsman et al., 1999). This is also suggested by our findings that point to the rise of the *Ictonyx–Poecilogale* clade after the Messinian Salinity Crisis, ~4.8–4.5 MYA, in Africa. Our results also reveal that the origin of the crown clade Lyncodontini (~2.9–2.6 MYA in South America) postdated the complete emergence of the Panamanian isthmus (~3.7–3.1 MYA; Duque-Caro, 1990), which offered a land bridge for faunal exchange between North and South America, an event termed the Great American Biotic Interchange (Woodburne, 2010).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2012.02.025.

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