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Phylogenetic Relationships and Divergence Times among Mustelids (Mammalia: Carnivora) Based on Nucleotide Sequences of the Nuclear Interphotoreceptor Retinoid Binding Protein and Mitochondrial Cytochrome *b* Genes

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ABSTRACT—Phylogenetic relationships among 20 species-group taxa of Mustelidae, representing Mustelinae (*Mustela*, *Martes*, *Gulo*), Lutrinae (*Enhydra*), and Melinae (*Meles*), were examined using nucleotide sequences of the nuclear interphotoreceptor retinoid binding protein (IRBP) and mitochondrial cytochrome *b* genes. Neighbor-joining and maximum-parsimony phylogenetic analyses on these genes separately and combined were conducted. While IRBP performed better than cytochrome *b* in recovering more-inclusive clades, cytochrome *b* demonstrated more resolving power in recovering less-inclusive clades. Strong support was found for a close affinity of *Enhydra* with *Mustela* to the exclusion of *Martes* and *Gulo* (causing Mustelinae to be paraphyletic); the most-basal position of *Mustela vison* within *Mustela*, followed by *Mustela erminea*; an association of *Mustela lutreola*, *Mustela itatsi*, *Mustela sibirica*, and the subgenus *Putorius* (including *Mustela putorius* and *Mustela eversmanii*), to the exclusion of *Mustela nivalis* and *Mustela altaica*; and a basal position of *Mustela itatsi* to a clade containing *Mustela sibirica* and *Putorius*. Whereas cytochrome *b* strongly supported *Mustela lutreola* as the sister species to *Putorius*, IRBP strongly supported its basal placement to the *Mustela itatsi*-*Mustela sibirica*-*Putorius* clade. The low level of sequence divergence in cytochrome *b* between *Mustela lutreola* and *Putorius* is therefore a result of interspecific mitochondrial introgression between these taxa, rather than a recent origin of *Mustela lutreola* in a close relationship to *Putorius*. Time estimates inferred from IRBP and cytochrome *b* for mustelid divergence events are mostly in agreement with the fossil record.

Key words: molecular phylogeny, Mustelidae, cytochrome *b* gene, nuclear IRBP gene, divergence time

INTRODUCTION

Mustelidae is the largest and most-diverse family among carnivoran mammals (order Carnivora). There are 66 (including *Mustela itatsi*) mustelid species extant today. They are usually classified in 25 genera and six subfamilies (e.g., Wozencraft, 1993). These subfamilies are Mustelinae (weasels, martens, and their allies), Lutrinae (otters), Melinae (badgers), Mellivorinae (honey badger), Taxidiinae (American badger), and Mephitinae (skunks). Mustelids are widely distributed geographically and occur throughout Eurasia, Africa, and America (introduced into New Zealand). They have adapted to very varied climatic and biotic conditions and are found in habitats that range from the arctic tundra to tropical rainforest and from deserts to inland waterways and even the open sea (e.g., Macdonald, 1985; Nowak, 1991). In many ecosystems of the Northern Hemisphere, mustelids are the most-common predatory mammals.

The increasing interest in mustelids and their phylogeny is therefore not surprising. There is an extensive bibliography related to phylogenetic relationships within this family, including studies based on either morphological grounds

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(neontology: e.g., Wozencraft, 1989; Bryant *et al.*, 1993; Wyss and Flynn, 1993; paleontology: e.g., Wolsan, 1993a, 1999; Baskin, 1998; Ginsburg and Morales, 2000) or genetic grounds (karyology: e.g., Graphodatsky *et al.*, 1976; Couturier and Dutrillaux, 1986; Obara, 1991; serum immunology: e.g., Seal *et al.*, 1970; Belyaev *et al.*, 1984; Taranin *et al.*, 1991; protein electrophoresis: e.g., Hartl *et al.*, 1988; O'Brien *et al.*, 1989; Dragoo *et al.*, 1993; DNA-DNA hybridization: e.g., Árnason and Widegren, 1986; Lushnikova *et al.*, 1989; Wayne *et al.*, 1989; amino-acid sequencing: Hashimoto *et al.*, 1993; Stanhope *et al.*, 1993; nucleotide sequencing: see the next paragraph for references), or integrating morphological and genetic data (Vrana *et al.*, 1994; Dragoo and Honeycutt, 1997; Bininda-Emonds *et al.*, 1999). Despite this considerable accumulation of information, the mustelid phylogeny remains an issue of uncertainty and controversy. Although the fossil record is the only source of direct evidence of past organismal history and uniquely contributes to phylogeny reconstruction by dating divergence times and providing access to denser taxon sampling than is otherwise possible (Smith and Littlewood, 1994; Smith, 1998; and references therein), paleontological data have largely been ignored by students of mustelid phylogeny.

Recent attention in phylogenetic relationships among mustelids has focused on nucleotide-sequence data (Dragoo *et al.*, 1993; Hosoda *et al.*, 1993, 1997, 1999, 2000; Masuda and Yoshida, 1994a, b; Vrana *et al.*, 1994; Ledje and Árnason, 1996a, b; Carr and Hicks, 1997; Dragoo and Honeycutt, 1997; Koepfli and Wayne, 1998, 2001; Davison *et al.*, 1999, 2000a, 2001; Demboski *et al.*, 1999; Emerson *et al.*, 1999; Kurose *et al.*, 1999a, b, 2000, 2001; Cassens *et al.*, 2000; Flynn *et al.*, 2000). Most of these studies come from analysis of variation in nucleotide sequences of mitochondrial genes. An additional, relatively untapped, source of phylogenetic information is the coding sequences of single-copy nuclear loci. Single-copy nuclear genes are an ideal source of phylogenetic information because homologous single-copy genes from different species are in all likelihood orthologous—that is, derived from a single common ancestral gene through a series of speciation events—and therefore suitable for the cladistic examination of relationships among species lineages (e.g., Stanhope *et al.*, 1992, 1996).

The gene encoding interphotoreceptor retinoid binding protein (IRBP) is a single-copy nuclear gene (Bridges *et al.*, 1986; Stanhope *et al.*, 1992, 1996; Springer *et al.*, 1997). This gene is expressed in eyes of vertebrates (Bridges *et al.*, 1986), where it functions in the transfer of retinoids during light- and dark-phase adaptation (Fong *et al.*, 1990; Pepperberg *et al.*, 1993). Variation in nucleotide sequences of this gene has proved useful for elucidating higher-level relationships among mammals (Stanhope *et al.*, 1992, 1996; Springer *et al.*, 1997, 1999, 2001; Jansa and Voss, 2000). Recently, this gene has also been employed in studies of lower-level mammalian relationships (Jansa and Voss, 2000; Serizawa *et al.*, 2000; Suzuki *et al.*, 2000).

This paper is the first report on nucleotide sequences of IRBP in Mustelidae. Here we present results on phylogenetic relationships among 20 species-group taxa of this family, inferred from nucleotide sequences of IRBP and the mitochondrial cytochrome *b* gene, the latter obtained from DNA databases. We also examine differences in performance between the two genes in recovering clades at different taxonomic levels. A new, well-documented fossil-based time estimate for the mustelid-procyonid divergence is used to calibrate the molecular clocks of the mustelid IRBP and cytochrome *b* genes. By means of this calibration we estimate dates for divergence events within Mustelidae.

MATERIALS AND METHODS

Sampling

A fragment of the first exon of IRBP, 1188 base pairs (bp) in length (sites 337–1530 in the human reference sequence [Fong *et al.*, 1990], except for sites 1318–1323 that are absent from all mustelids), was sequenced from 20 individuals representing 19 species-group taxa, five genera, and three subfamilies of Mustelidae and one species of Procyonidae (Table 1). The complete nucleotide sequences of cytochrome *b* (1140 bp) from 19 species-group taxa of Mustelidae and the procyonid, as well as nucleotide sequences of IRBP and cytochrome *b* from two species of the carnivoran suborder Feliformia and the order Rodentia (Table 1), were obtained from the DDBJ/EMBL/GenBank International Nucleotide Sequence Database.

A 3-bp fragment of IRBP (sites 1311–1313 in the human reference sequence [Fong *et al.*, 1990]) was absent from all examined representatives of *Mustela*. This fragment was therefore excluded from phylogenetic analyses, which finally included 1185 bp of IRBP and 1140 bp of cytochrome *b*, each for 19 species-group taxa of Mustelidae and an outgroup species. The composite nucleotide-sequence data consisted of a total of 2325 bp (1185 bp from IRBP and 1140 bp from cytochrome *b*) for each of 18 species-group taxa of Mustelidae (for which the data on the two genes were available) and an outgroup species.

As the outgroup in all phylogenetic analyses, the procyonid *Procyon lotor* was used on the basis of the proposed sister-group relationship between Mustelidae and Procyonidae, supported by evidence from both genetics and morphology (Bininda-Emonds *et al.*, 1999; Flynn *et al.*, 2000; and references therein).

DNA isolation, amplification, and sequencing

DNA was extracted from tissues preserved in ethanol by the conventional phenol-chloroform method. The amplification was performed via nested polymerase chain reactions (PCRs), using an automated thermal cycler (model PJ 2000, TAKARA). Each first PCR mix contained 10 mM Tris (pH 8.3); 50 mM KCl; 0.01% gelatin; 0.1% Triton X-100; 2.5 mM $MgCl_2$; 0.2 mM dNTP mix; 0.05 μM of each primer (1 pmol of each primer per reaction); 0.5 units of Amplitaq DNA polymerase (ABI, Applied Biosystems); and 0.1–0.5 μg of template total genomic DNA in a total volume of 20 μl. Thermal cycling parameters of the first PCR were as follows: denaturation at 94°C for 1 min, annealing and extension at 70°C for 3 min each (Stanhope *et al.*, 1992). A 1-μl aliquot of each reaction mixture after the first PCR was used as a template for the second PCR in a 20-μl reaction mixture with the same reagents except for the concentration of $MgCl_2$ (which was 1.875 mM) and the primer pairs. The second PCR was performed under the following conditions: denaturation at 96°C for 30 sec, annealing at 50°C for 30 sec, extension at 60°C for 30 sec. In the first PCR, a 1.3-kb fragment of IRBP was amplified using primers +IRBP217 and –IRBP1531

Table 1. Taxon and gene sampling, with DDBJ/EMBL/GenBank International Nucleotide Sequence Database accession numbers. For new accessions (this paper), localities of the vouchers are provided.

Taxon	Accession no.; locality or reference	
	IRBP	Cytochrome *b*
Carnivora		
Caniformia		
Mustelidae		
Mustelinae		
Gulo gulo (Wolverine)	AB082962; Sakhalin, Russia	AB051245; Hosoda *et al*., 2000
Martes americana (American Marten)	AB082963; Maine, USA	AB051234; Hosoda *et al*., 2000
Martes flavigula (Yellow-Throated Marten)	AB082964; Primorye, Russia	AB051235; Hosoda *et al*., 2000
Martes foina (Beech or Stone Marten)	AB082965; Thuringia, Germany	AB051236; Hosoda *et al*., 2000
Martes martes (Pine Marten)	AB082966; Moscow, Russia	AB051237; Hosoda *et al*., 2000
Martes melampus (Japanese Marten)	AB082967; Wakayama, Honshu, Japan	AB051238; Hosoda *et al*., 2000
Martes zibellina (Sable)	–	AB012360; Kurose *et al*., 1999a
Mustela altaica (Mountain Weasel)	AB082968; Altai region, Russia	AB051239; Hosoda *et al*., 2000
Mustela erminea (Ermine or Stoat)	AB082969; Hokkaido, Japan	AB051240; Hosoda *et al*., 2000
Mustela eversmanii (Steppe Polecat)	AB082970; Chita region, Russia	AB026102; Kurose *et al*., 2000
Mustela itatsi (Japanese Weasel)	AB082971; Aomori, Honshu, Japan	AB026104; Kurose *et al*., 2000
Mustela lutreola (European Mink)	AB082972; Novosibirsk, Russia	AB026105; Kurose *et al*., 2000
Mustela nivalis (Weasel)	AB082973; Aomori, Honshu, Japan	AB051241; Hosoda *et al*., 2000
Mustela putorius furo (Domestic Ferret)	AB082974; experimental animal	AB026103; Kurose *et al*., 2000
Mustela putorius putorius (European Polecat)	AB082975; Moscow, Russia	AB026107; Kurose *et al*., 2000
Mustela sibirica (Siberian Weasel)	AB082976; Wakayama, Honshu, Japan	AB051242; Hosoda *et al*., 2000
Mustela vison (American Mink)	AB082977; Hokkaido, Japan*	AF057129; Koepfli and Wayne, 1999
Lutrinae		
Enhydra lutris (Sea Otter)	AB082978; Alaska, USA	AB051244; Hosoda *et al*., 2000
Melinae		
Meles meles anakuma (Japanese Badger)	AB082980; Miyazaki, Kyushu, Japan	–
Meles meles meles (European Badger)	AB082979; Thuringia, Germany	X94922; Ledje and Arnason, 1996a
Procyonidae		
Procyon lotor (Raccoon)	AB082981; Miyazaki, Kyushu, Japan*	X94930; Ledje and Arnason, 1996a
Feliformia		
Felis catus (Domestic Cat)	Z11811; Stanhope *et al*., 1992	U20753; Lopez *et al*., 1996
Rodentia		
Apodemus speciosus (Large Japanese Field Mouse)	AB032856; Serizawa *et al*., 2000	AB032849; Serizawa *et al*., 2000

* An introduced population.

(Stanhope *et al*., 1992). In the second PCR, three segments were amplified against the product of the first PCR, using the three primer sets: R +IRBP335 and U –IRBP734 (Serizawa *et al*., 2000); R +IRBP724 (5'-CAGGAAACAGCTATGACCCCTGCACGTGGACACCATCT-3') and U –IRBP1145 (5'-TGTAAAACGACGGCCAGTGCGGTCCACCAGCGTGTAGT-3'); and R +IRBP1110 and U –IRBP1530 (Serizawa *et al*., 2000). Numbers in the primer names designate the position of the 3' end of the primer in the human reference sequence (Fong *et al*., 1990). The prefixes "+" and "–" refer to the reading and complementary strands, respectively.

The sequencing of the product of the second PCR was carried out according to the manufacturer's instructions, using either a Dye Primer or Big Dye Primer Cycle Sequencing Kit (ABI) and run on an ABI 373A or ABI 310 automated sequencer.

Phylogenetic analyses

Phylogenetic analyses were performed by two methods, neighbor-joining (NJ; Saitou and Nei, 1987) and maximum parsimony (MP; Swofford and Olsen, 1990), both implemented by using PAUP* version 4.0b10 (Swofford, 1998). The NJ analyses employed matrices of genetic distances generated by using the Kimura two-parameter method (Kimura, 1980). The MP analyses were conducted using 100 heuristic tree-bisection reconnection searches in which the input order of taxa was randomized. All NJ analyses and the MP analyses of IRBP sequences were based on equally weighted nucleotide substitutions. For MP analyses of cytochrome *b* sequences, the following character weightings were used: (1) equally weighted nucleotide substitutions (MP_{TiTv}), and (2) transversions only at third positions of codons and all nucleotide substi-

tutions at first and second positions (MP_{-3Ti}). The weighting decreasing the transition bias was employed to minimize the effects of saturation.

For either gene, a χ^2-test of homogeneity was implemented by using PAUP* 4.0b10 to test the assumption of base-compositional homogeneity. Prior to combining the two genes into single analyses, the incongruence length difference test (also termed the partition homogeneity test; Mickevich and Farris, 1981; Farris *et al*., 1995) was performed to test significance of incongruence between the two data sets.

Bootstrap proportions (BS; Felsenstein, 1985) were obtained by generating 1000 heuristic replicates with PAUP* 4.0b10, each consisting of 100 heuristic tree-bisection reconnection searches in which the input order of taxa was randomized. The decay index (DI; also known as the Bremer support, branch support, clade stability, length difference; Bremer, 1988, 1994) was calculated using TreeRot version 2b (Sorenson, 1999).

Estimation of divergence time

Divergence times were estimated assuming the constancy of the rate of molecular change over time (molecular-clock hypothesis; Zuckerkandl and Pauling, 1965). To test this hypothesis, the two-cluster test (Takezaki *et al*., 1995) was performed.

Gene-specific rates of nucleotide substitution were calibrated using our fossil-based time estimate for the mustelid-procyonid split (inferred from the first stratigraphic appearances of these families) and the average Kimura two-parameter genetic distance between *Procyon lotor* and the mustelid species-group taxa sequenced. Divergence times between mustelid species-group taxa were estimated by means of the gene-specific rate of nucleotide substitution and the Kimura two-parameter genetic distance between these taxa. Divergence times between mustelid clades were estimated by using the rate of nucleotide substitution and the average Kimura two-parameter genetic distance between the sequenced species-group taxa contained in these clades. For the cytochrome *b* gene, transversion distances only were used to avoid saturation problems caused by transitions.

RESULTS

Nucleotide variation

IRBP

The studied individuals of *Martes americana* and *Meles meles meles* showed heterozygosity (G-C, silent substitution) at sites 1209 and 1434, respectively (sites according to the human reference sequence [Fong *et al*., 1990]). Each of the 10 individuals of *Mustela* shows a single indel of 3-bp deletion, which corresponds to sites 1311–1313 in the human reference sequence (Fong *et al*., 1990). Excepting the three sites, there are 158 (13.3%) variable sites among the remaining 1185 sites from the mustelids examined, including 31 (19.6%), 18 (11.4%), and 109 (69.0%) sites at first, second, and third codon positions, respectively. Of these variable sites, 70 (44.3%) are phylogenetically informative, including nine (12.9%), five (7.1%), and 56 (80.0%) sites at first, second, and third codon positions, respectively. The mean frequencies of the four bases are as follows: A, 17.7%; C, 31.9%; G, 32.0%; and T, 18.4%. The null hypothesis of homogeneity in base composition across the mustelid taxa was not rejected by the χ^2-test (P>0.05).

Nucleotide substitutions and Kimura two-parameter genetic distances among the 1185 bp from the mustelids studied range from, respectively, one and 0.08% between the two subspecies of *Mustela putorius* to, respectively, 51 and 4.45% between *Mustela itatsi* and *Meles meles meles* (Table 2). The value of the genetic distance between the two subspecies of *Meles meles* is 0.51%. Among the nine species of *Mustela*, the genetic distances range from 0.17% (*M. putorius furo* vs. *M. sibirica*) to 2.49% (*M. itatsi* vs. *M. vison*), but the maximum value is only 1.89% (*M. erminea* vs. *M. itatsi*) when *Mustela vison* is excluded from comparison. Among martens, the distances range from 0.42 to 0.76% (*M. melampus* vs. *M. foina* and *M. martes*, respectively) within the subgenus *Martes*, and to 1.71% (*M. flavigula* vs. any of *M. martes, M. melampus*, and *M. americana*) within the genus. The minimum genetic distance between species of different genera is 0.85% (*Martes foina* vs. *Gulo gulo*), and the minimum distance between species of different subfamilies is 2.50% (*Gulo gulo* vs. *Meles meles anakuma*).

Cytochrome b

There was no length variation in nucleotide sequences of the cytochrome *b* gene from the mustelids studied. Of the 1140 bp sequenced, 454 (39.8%) sites are variable, including 95 (20.9%), 27 (5.9%), and 332 (73.1%) sites at first, second, and third codon positions, respectively. Of the variable sites, 336 (74.0%) are phylogenetically informative, including 66 (19.6%), 16 (4.8%), and 254 (75.6%) sites at first, second, and third codon positions, respectively. The mean frequencies of the four bases are as follows: A, 29.0%; C, 29.9%; G, 13.5%; and T, 27.5%. The null hypothesis of homogeneity in base composition across the mustelid taxa was not rejected by the χ^2-test (P>0.05).

Nucleotide substitutions and Kimura two-parameter genetic distances among the 1140 bp from the mustelids examined range from, respectively, three and 0.26% between *Mustela putorius furo* and *Mustela eversmanii* to, respectively, 197 and 20.28% between *Mustela nivalis* and *Meles meles* (Table 3). The value of the genetic distance between the two subspecies of *Mustela putorius* is 0.62%. Among the nine species of *Mustela*, the genetic distances range from 0.26% (*M. putorius furo* vs. *M. eversmanii*) to 15.27% (*M. nivalis* vs. *M. vison*); however, when *Mustela vison* is excluded, the maximum distance is only 11.03% (*M. itatsi* vs. *M. altaica*). Among species of martens, the distances range from 2.70% (*M. martes* vs. *M. melampus*) to 9.91% (*M. foina* vs. *M. zibellina*) within the subgenus *Martes*, and to 15.23% (*M. flavigula* vs. *M. americana*) within the genus. The minimum genetic distance between species of different genera is 12.46% (*Mustela erminea* vs. *Martes melampus*), and the minimum distance between species of different subfamilies is 13.37% (*Mustela erminea* vs. *Enhydra lutris*).

Phylogenetic inference

IRBP

A tree that resulted from NJ analysis of the IRBP nucleotide sequences (Fig. 1A) and the strict consensus of the 16

Table 2. Numbers of base-pair differences (above diagonal) and Kimura two-parameter percentage genetic distances (below diagonal) among partial nucleotide sequences of IRBP (1185 bp) from mustelids and *Procyon lotor*.

	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*Gulo gulo*	–	12	17	10	11	11	39	37	41	44	39	34	39	40	39	34	37	29	31	63
2	*Martes americana*	1.02	–	20	6	8	7	43	42	47	50	46	40	45	46	45	41	39	31	31	68
3	*Martes flavigula*	1.45	1.71	–	19	20	20	43	43	47	48	46	42	45	46	45	38	42	37	39	70
4	*Martes foina*	0.85	0.51	1.62	–	8	5	42	42	46	49	45	39	44	45	44	37	37	31	31	66
5	*Martes martes*	0.94	0.68	1.71	0.68	–	9	41	41	45	48	44	38	43	44	43	38	40	32	34	67
6	*Martes melampus*	0.94	0.59	1.71	0.42	0.76	–	42	42	46	49	45	39	44	45	44	39	39	31	31	67
7	*Mustela altaica*	3.38	3.73	3.73	3.65	3.56	3.64	–	17	13	16	13	7	11	12	11	24	39	47	50	86
8	*Mustela erminea*	3.21	3.65	3.73	3.65	3.56	3.65	1.45	–	21	22	21	16	19	20	19	24	36	43	45	86
9	*Mustela eversmanii*	3.56	4.09	4.09	4.00	3.91	4.00	1.11	1.80	–	9	13	11	4	5	4	26	41	47	50	91
10	*Mustela itatsi*	3.83	4.37	4.18	4.28	4.19	4.27	1.37	1.89	0.77	–	16	14	7	8	7	29	42	48	51	91
11	*Mustela lutreola*	3.38	4.00	4.00	3.91	3.82	3.91	1.11	1.80	1.11	1.37	–	10	11	12	11	25	40	47	50	87
12	*Mustela nivalis*	2.94	3.47	3.64	3.38	3.29	3.37	0.59	1.37	0.94	1.20	0.85	–	9	10	9	21	35	44	47	82
13	*Mustela putorius furo*	3.38	3.91	3.91	3.82	3.73	3.82	0.94	1.62	0.34	0.59	0.94	0.76	–	1	2	24	39	45	48	89
14	*Mustela putorius putorius*	3.47	4.00	4.00	3.91	3.82	3.91	1.02	1.71	0.42	0.68	1.02	0.85	0.08	–	3	25	40	46	49	90
15	*Mustela sibirica*	3.38	3.91	3.91	3.82	3.73	3.82	0.94	1.62	0.34	0.59	0.94	0.77	0.17	0.25	–	24	39	45	48	89
16	*Mustela vison*	2.94	3.56	3.29	3.20	3.29	3.38	2.06	2.06	2.23	2.49	2.14	1.80	2.06	2.14	2.06	–	36	39	43	82
17	*Enhydra lutris*	3.21	3.38	3.65	3.20	3.47	3.38	3.38	3.11	3.55	3.64	3.46	3.02	3.37	3.46	3.37	3.11	–	47	47	88
18	*Meles meles anakuma*	2.50	2.67	3.20	2.67	2.76	2.67	4.09	3.74	4.09	4.18	4.09	3.82	3.91	4.00	3.91	3.38	4.10	–	6	71
19	*Meles meles meles*	2.68	2.67	3.38	2.67	2.94	2.67	4.37	3.92	4.36	4.45	4.36	4.09	4.18	4.27	4.18	3.73	4.10	0.51	–	72
20	*Procyon lotor*	5.57	6.03	6.22	5.84	5.94	5.93	7.72	7.73	8.20	8.20	7.81	7.34	8.00	8.10	8.01	7.35	7.94	6.31	6.40	–

Table 3. Numbers of base-pair differences (above diagonal) and Kimura two-parameter percentage genetic distances (below diagonal) among the complete nucleotide sequences of cytochrome *b* (1140 bp) from mustelids and *Procyon lotor*.

	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*Gulo gulo*	–	152	142	149	142	143	147	160	149	168	163	167	162	169	164	177	175	168	185	216
2	*Martes americana*	15.25	–	152	103	58	53	63	147	143	150	162	154	152	150	148	151	172	170	180	218
3	*Martes flavigula*	14.12	15.23	–	152	140	135	147	166	152	169	163	169	176	170	167	173	181	175	181	208
4	*Martes foina*	14.86	9.90	15.22	–	94	89	103	147	149	167	168	167	156	168	163	168	180	164	181	209
5	*Martes martes*	14.09	5.35	13.87	8.95	–	30	31	143	134	146	156	150	155	147	142	155	179	167	178	216
6	*Martes melampus*	14.19	4.86	13.29	8.43	2.70	–	45	137	129	139	154	143	142	140	135	146	174	160	177	219
7	*Martes zibellina*	14.68	5.84	14.69	9.91	2.79	4.11	–	147	139	149	160	153	154	150	145	162	176	175	184	220
8	*Mustela altaica*	15.98	14.46	16.70	14.47	14.01	13.33	14.47	–	80	87	114	93	82	88	87	92	146	166	195	198
9	*Mustela erminea*	14.72	14.02	15.05	14.71	13.02	12.46	13.58	7.49	–	94	91	91	89	95	94	91	128	137	180	200
10	*Mustela eversmanii*	16.97	14.83	17.05	16.86	14.38	13.58	14.73	8.20	8.95	–	64	9	92	3	4	41	140	154	182	199
11	*Mustela itatsi*	16.35	16.20	16.31	16.95	15.53	15.26	15.98	11.03	8.62	5.94	–	59	95	65	63	60	136	153	176	202
12	*Mustela lutreola*	16.85	15.29	17.05	16.86	14.83	14.03	15.19	8.82	8.63	0.80	5.45	–	91	12	13	40	141	152	184	201
13	*Mustela nivalis*	16.20	15.04	17.83	15.49	15.39	13.90	15.28	7.68	8.41	8.72	9.02	8.62	–	93	92	95	153	161	197	209
14	*Mustela putorius furo*	17.07	14.82	17.15	16.97	14.48	13.68	14.83	8.30	9.04	0.26	6.04	1.06	8.82	–	7	42	141	155	183	200
15	*Mustela putorius putorius*	16.50	14.62	16.82	16.40	13.94	13.14	14.28	8.20	8.94	0.35	5.84	1.15	8.72	0.62	–	45	139	153	178	197
16	*Mustela sibirica*	18.03	14.93	17.50	16.95	15.39	14.35	16.22	8.72	8.63	3.73	5.54	3.63	9.04	3.82	4.10	–	141	161	176	208
17	*Mustela vison*	17.86	17.41	18.52	18.39	18.26	17.63	17.90	14.48	12.48	13.85	13.37	13.96	15.27	13.95	13.72	13.94	–	162	184	215
18	*Enhydra lutris*	17.01	17.19	17.87	16.49	16.83	16.02	17.81	16.71	13.37	15.32	15.18	15.09	16.09	15.42	15.19	16.11	16.29	–	191	216
19	*Meles meles meles*	19.01	18.34	18.44	18.50	18.12	17.96	18.84	20.02	18.21	18.51	17.78	18.76	20.28	18.62	18.04	17.76	18.74	19.61	–	226
20	*Procyon lotor*	22.55	22.78	21.41	21.63	22.55	22.89	23.05	20.18	20.44	20.31	20.71	20.56	21.54	20.42	20.08	21.44	22.32	22.39	23.89	–

shortest trees that resulted from MP analysis of these sequences (Fig. 1B) consistently indicate that (1) *Enhydra* and *Mustela* are more closely related to each other than either is to *Martes* or *Gulo*, causing the subfamily Mustelinae to be paraphyletic (NJ BS; MP BS/DI = 100; 100/8); (2) *Gulo* and *Martes* are phylogenetically closer to each other than

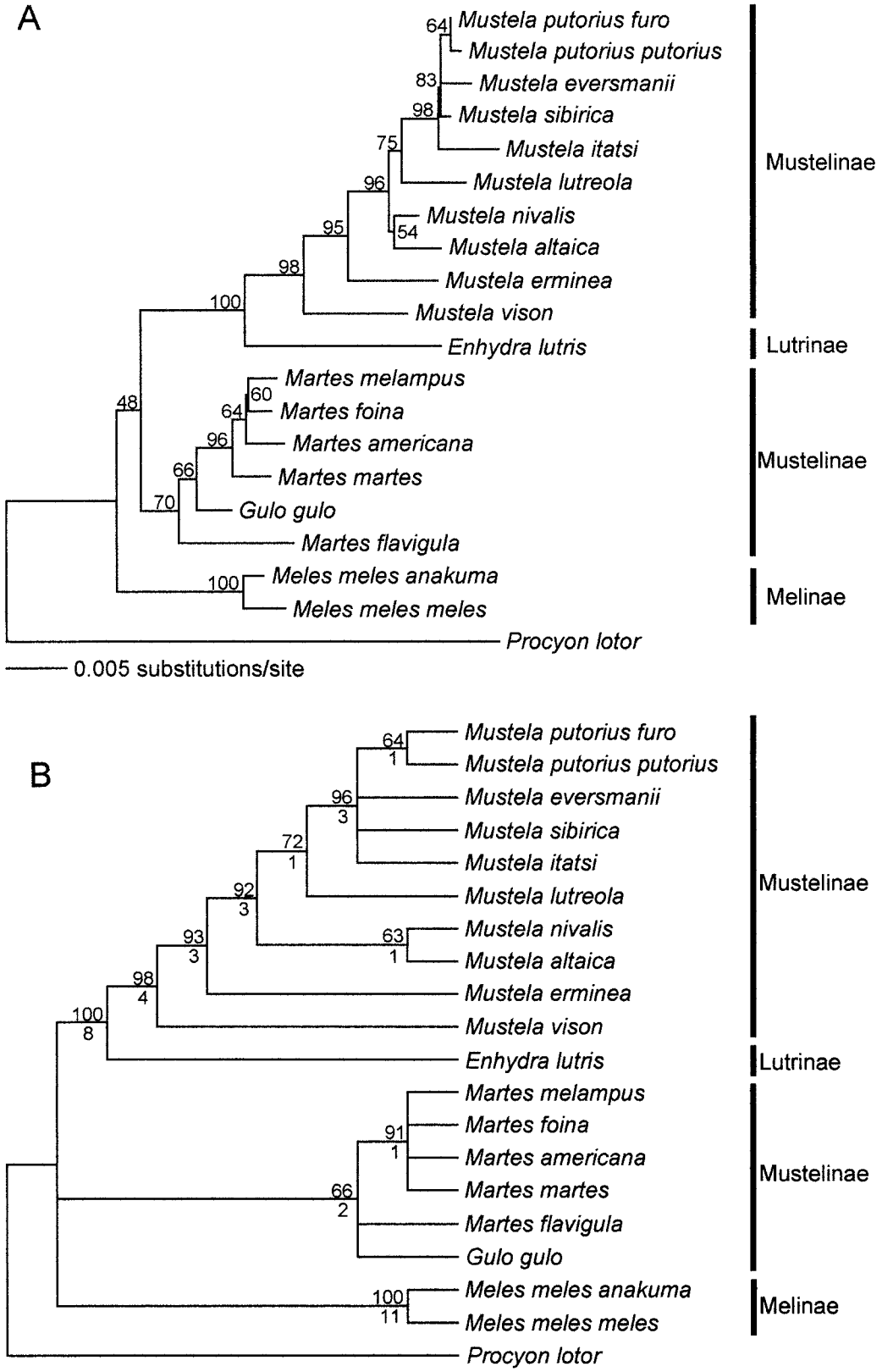

Fig. 1. Phylogenetic relationships among mustelids, based on partial nucleotide sequences of IRBP (1185 bp). **A**, tree resulted from neighbor-joining analysis. The horizontal length of each branch is proportional to the number of nucleotide substitutions per site. Numbers at branches are percentage bootstrap values in support of adjacent nodes. **B**, strict consensus of the 16 shortest trees (length, 218 steps; consistency index, 0.83; retention index, 0.88) resulted from maximum-parsimony analysis. Numbers above branches are percentage bootstrap values in support of adjacent nodes; numbers below branches are the decay indices.

either is to any of the remaining genera under study (70; 66/2); (3) the subgenus *Martes* (*M. martes, M. melampus, M. americana, M. foina*) is monophyletic (96; 91/1); (4) *Mustela* is monophyletic (98; 98/4), with *Mustela vison* (95; 93/3) and *Mustela erminea* (96; 92/3) as successively more closely related to a clade containing the remaining studied species of the genus; (5) within the last clade, large-sized species (*M. lutreola, M. itatsi, M. sibirica, M. eversmanii, M. putorius*) and small-sized species (*M. nivalis, M. altaica*) are contained in separate subclades (75;72/1 and 54; 63/1; respectively); (6) within the subclade of large-sized species, *Mustela lutreola* is basal to the remaining species (98; 96/3); and (7) *Mustela putorius furo* is more closely related to *Mustela putorius putorius* than to *Mustela eversmanii* (64; 64/1).

The phylogenetic position of Melinae is uncertain. While NJ analysis provided weak evidence (BS = 48) of a basal position of *Meles* with respect to a clade encompassing *Enhydra, Mustela, Martes*, and *Gulo*, MP analysis supported a trichotomy among *Meles*, the *Enhydra-Mustela* clade, and the *Martes-Gulo* clade. There is also inconsistent evidence for phylogenetic relationships among *Martes* subgenera and *Gulo*. Whereas NJ analysis placed *Gulo* in a sister-group relationship with the subgenus *Martes*, causing the genus *Martes* to be paraphyletic (BS = 66), MP analysis supported a trichotomy among *Gulo* and the subgenera *Martes* and *Charronia* (*Martes flavigula*). Interrelationships among species of the subgenus *Martes* are also equivocal. Although NJ analysis linked *Martes foina* with *Martes melampus* (BS = 60), recognizing *Martes americana* and *Martes martes* as successively more distant outgroups to this clade, MP analysis supported a polytomy among the four species. Finally, the placement of *Mustela itatsi* is equivocal and there is no support for the monophyly of the subgenus *Putorius* (*Mustela putorius, Mustela eversmanii*). While NJ analysis nested *Mustela itatsi* as basal to a trichotomy of *Mustela sibirica, Mustela eversmanii*, and *Mustela putorius* (BS = 83), MP analysis supported a tetrachotomy among the four species.

Cytochrome b

A tree that resulted from NJ analysis of the nucleotide sequences of the cytochrome *b* gene (Fig. 2A) and the strict consensus of the two shortest trees that resulted from MP_{TiTv} analysis of these sequences (Fig. 2B), as well as the strict consensus of the 12 shortest trees that resulted from the MP_{-3Ti} analysis (Fig. 2C), all uniformly indicate that (1) Mustelinae is paraphyletic relative to *Enhydra*; (2) *Gulo* and *Martes* are more closely related to each other than either is to any of the remaining genera under study (NJ BS; MP_{TiTv} BS/DI; MP_{-3Ti} BS/DI = 75; 57/4; 70/2); (3) the subgenus *Martes* (*M. martes, M. zibellina, M. melampus, M. americana, M. foina*) is monophyletic (100; 100/15; 92/5), with *Martes foina* as basal to the remaining species (100; 100/15; 72/2), and *Martes martes* and *Martes zibellina* as sister species (92; 92/9; 79/2); (4) the genus *Mustela* is monophyletic (72; 56/3; 88/5), with *Mustela vison* (100; 96/10; 97/6) and *Mustela erminea* (48; 69/2; 59/1) as successively more closely related to a clade comprising the sister subclades recognized by the IRBP analyses, one including large-sized species (100; 99/14; 97/6) and the other including small-sized species (77; 75/3; 53/1); and (5) the subgenus *Putorius* is monophyletic (86; 94/4; 60/1).

There is conflicting evidence for placement of Melinae. Although maximum-parsimony analyses consistently recognized *Meles* as basal to a clade containing *Enhydra, Mustela, Martes*, and *Gulo* (MP_{TiTv} BS/DI = 56/10; MP_{-3Ti} BS/DI = 88/7), NJ analysis linked this genus with the *Martes-Gulo* clade, causing Mustelinae to be paraphyletic (BS = 48). Both NJ and MP_{-3Ti} analyses nested *Enhydra* as the sister taxon to *Mustela* (NJ BS = 63; MP_{-3Ti} BS/DI = 70/1), but MP_{TiTv} analysis placed *Enhydra* in the sister-group position to the *Martes-Gulo* clade, albeit with very weak support (BS/DI = 21/1). Interrelationships among *Martes* subgenera and *Gulo* are also equivocal. While NJ (BS = 73) and MP_{TiTv} (BS/DI = 55/5) analyses recognized *Gulo* as the sister taxon to the subgenus *Charronia*, causing the genus *Martes* to be paraphyletic, MP_{-3Ti} analysis supported a trichotomy among *Gulo* and the subgenera *Charronia* and *Martes*. Within the last subgenus, there is discrepant evidence for placement of *Martes melampus* and *Martes americana*. Although NJ and MP_{TiTv} analyses nested *Martes melampus* and *Martes americana* as successively more closely related to the *Martes martes-Martes zibellina* clade (NJ BS = 94 and 92, respectively; MP_{TiTv} BS/DI = 87/5 and 92/9, respectively), MP_{-3Ti} analysis supported a trichotomy among this clade, *Martes melampus*, and *Martes americana*. Within the clade of large-sized *Mustela*, NJ and MP_{TiTv} analyses were consistent in placing *Mustela itatsi, Mustela sibirica*, and *Mustela lutreola* as successively more closely related to the subgenus *Putorius* (NJ BS = 95, 100, and 86, respectively; MP_{TiTv} BS/DI = 74/2, 100/9, and 94/4, respectively), but MP_{-3Ti} analysis supported a tetrachotomy among this subgenus and the three species. Within *Putorius*, in turn, NJ analysis united *Mustela putorius furo* with *Mustela eversmanii* to the exclusion of *Mustela putorius putorius* (BS = 70), but maximum-parsimony analyses supported a trichotomy among these taxa.

Combined IRBP and cytochrome b

The incongruence length difference test did not reject the null hypothesis of homogeneity in phylogenetic signal between the IRBP and cytochrome *b* nucleotide sequences, enabling them to be combined into a single analysis. The NJ analysis of the concatenated nucleotide sequences of the two genes resulted in a tree presented in Fig. 3A. The MP_{TiTv} analysis of these sequences yielded a single shortest tree (Fig. 3B), and the MP_{-3Ti} analysis revealed six shortest trees which produced a strict consensus tree shown in Fig. 3C. A number of phylogenetic observations can be made from these trees. First, a basal position of *Meles* in relation to the remaining mustelids used in this study is weakly supported by NJ analysis (BS = 47), moderately supported by MP_{TiTv} analysis (BS/DI = 78/7), and strongly supported by MP_{-3Ti} analysis (BS/DI = 99/11). Second, there is strong

Fig. 2. Phylogenetic relationships among mustelids, based on the complete nucleotide sequences of cytochrome *b* (1140 bp). **A**, tree resulted from neighbor-joining analysis. The horizontal length of each branch is proportional to the number of nucleotide substitutions per site. Numbers at branches are percentage bootstrap values in support of adjacent nodes. **B**, strict consensus of the two shortest trees (length, 1155 steps; consistency index, 0.50; retention index, 0.57) resulted from maximum-parsimony analysis with all nucleotide substitutions included. Numbers above branches are percentage bootstrap values in support of adjacent nodes; numbers below branches are the decay indices. **C**, strict consensus of the 12 shortest trees (length, 413 steps; consistency index, 0.59; retention index, 0.68) resulted from maximum-parsimony analysis with third-position transitions excluded. Numbers above branches are percentage bootstrap values in support of adjacent nodes; numbers below branches are the decay indices.

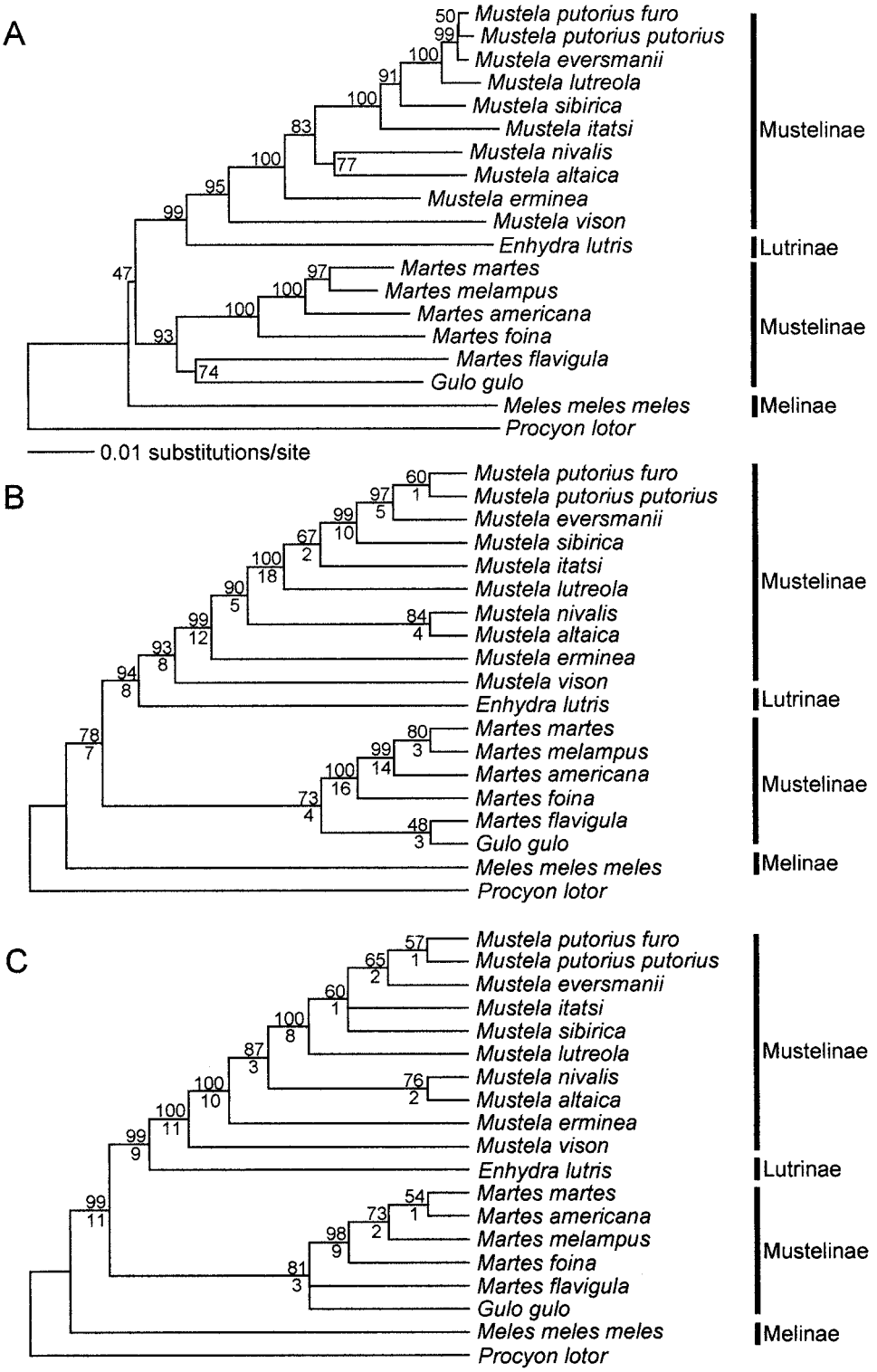

Fig. 3. Phylogenetic relationships among mustelids, based on concatenated nucleotide sequences of IRBP and cytochrome *b* (2325 bp). **A**, tree resulted from neighbor-joining analysis. The horizontal length of each branch is proportional to the number of nucleotide substitutions per site. Numbers at branches are percentage bootstrap values in support of adjacent nodes. **B**, single shortest tree (length, 1352 steps; consistency index, 0.56; retention index, 0.60) resulted from maximum-parsimony analysis with all nucleotide substitutions included. Numbers above branches are percentage bootstrap values in support of adjacent nodes; numbers below branches are the decay indices. **C**, strict consensus of the six shortest trees (length, 627 steps; consistency index, 0.67; retention index, 0.73) resulted from maximum-parsimony analysis with cytochrome *b* third-position transitions excluded. Numbers above branches are percentage bootstrap values in support of adjacent nodes; numbers below branches are the decay indices.

support (NJ BS = 99; MP_{TiTv} BS/DI = 94/8; MP_{-3Ti} BS/DI = 99/9) for a sister-group relationship between *Enhydra* and *Mustela*, causing Mustelinae to be paraphyletic. Third, the genera *Gulo* and *Martes* are moderately to relatively strongly supported as the closest relatives (NJ BS = 93; MP_{TiTv} BS/DI = 73/4; MP_{-3Ti} BS/DI = 81/3), but details of this relationship are equivocal. While NJ and MP_{TiTv} analyses linked *Gulo* with *Martes flavigula*, causing the genus *Martes* to be paraphyletic (NJ BS = 74; MP_{TiTv} BS/DI = 48/3), MP_{-3Ti} analysis supported a trichotomy among *Gulo*, *Martes flavigula*, and the subgenus *Martes*. Fourth, the monophyly of the subgenus *Martes* is strongly supported (NJ BS = 100; MP_{TiTv} BS/DI = 100/16; MP_{-3Ti} BS/DI = 98/9). Fifth, the most-basal position of *Martes foina* in the subgenus *Martes* is strongly supported by NJ (BS = 100) and MP_{TiTv} (BS/DI = 99/14) analyses, but relatively weakly supported by MP_{-3Ti} analysis (BS/DI = 73/2). Sixth, interrelationships among other species of the subgenus *Martes* are equivocal. While NJ and MP_{TiTv} analyses moderately to strongly supported a close relationship between *Martes martes* and *Martes melampus* to the exclusion of *Martes americana* (NJ BS = 97; MP_{TiTv} BS/DI = 80/3), MP_{-3Ti} analysis provided weak support (BS/DI = 54/1) for a basal position of *Martes melampus* with respect to a clade containing *Martes martes* and *Martes americana*. Seventh, the monophyly of *Mustela* is strongly supported (NJ BS = 95; MP_{TiTv} BS/DI = 93/8; MP_{-3Ti} BS/DI = 100/11), with *Mustela vison* strongly supported (NJ BS = 100; MP_{TiTv} BS/DI = 99/12; MP_{-3Ti} BS/DI = 100/10) and *Mustela erminea* less strongly supported (NJ BS = 83; MP_{TiTv} BS/DI = 90/5; MP_{-3Ti} BS/DI = 87/3) as successively more closely related to a clade encompassing the remaining studied species of the genus. Eighth, within the last clade, there are two separate subclades recognized by the individual IRBP and cytochrome *b* analyses: the strongly supported subclade of large-sized species (NJ BS = 100; MP_{TiTv} BS/DI = 100/18; MP_{-3Ti} BS/DI = 100/8) and the moderately supported subclade of small-sized species (NJ BS = 77; MP_{TiTv} BS/DI = 84/4; MP_{-3Ti} BS/DI = 76/2). Ninth, within the subclade of large-sized species, interrelationships among the subgenus *Putorius* and other species are equivocal. While NJ analysis strongly supported *Mustela lutreola* as the sister taxon to *Putorius* (BS = 100), maximum-parsimony analyses supported this species, albeit relatively weakly (MP_{TiTv} BS/DI = 67/2; MP_{-3Ti} BS/DI = 60/1), as the most basal within the subclade. Moreover, *Mustela itatsi* was recognized by NJ (BS = 91) and MP_{TiTv} (BS/DI = 99/10) analyses as less closely related to *Putorius* than is *Mustela sibirica*, but MP_{-3Ti} analysis supported a trichotomy among the three taxa. Tenth, the monophyly of the subgenus *Putorius* is strongly supported by NJ (BS = 99) and MP_{TiTv} (BS/DI = 97/5) analyses, but relatively weakly supported by MP_{-3Ti} analysis (BS/DI = 65/2). Finally, *Mustela putorius furo* and *Mustela putorius putorius* are relatively weakly supported as the closest relatives (NJ BS = 50; MP_{TiTv} BS/DI = 60/1; MP_{-3Ti} BS/DI = 57/1).

Performance of IRBP versus cytochrome *b*

Nucleotide sequences of IRBP appear to contain more resolving power than those of cytochrome *b* in recovering more-inclusive mustelid clades. Although our analyses of IRBP were unable to completely or strongly resolve phylogenetic relationships of *Meles*, they resulted in much greater support—than analyses of cytochrome *b*—for the sister-group status of *Enhydra* to *Mustela*, the monophyly of the genus *Mustela*, and a basal position of *Mustela erminea* with respect to a clade containing *Mustela nivalis*, *Mustela altaica, Mustela lutreola, Mustela itatsi, Mustela sibirica*, and the subgenus *Putorius* (compare Figs. 1 and 2). On the other hand, nucleotide sequences of cytochrome *b* were considerably more efficient than those of IRBP in recovering less-inclusive clades, such as those within the subgenus *Martes* and within the clade of large-sized *Mustela*. In addition, analyses employing both transitions and transversions of cytochrome *b* (Figs. 2A, B; 3A, B) resulted in much better resolved relationships among closely related species than analyses excluding third-position transitions of this gene (Figs. 2C, 3C). On the other hand, analyses that excluded cytochrome *b* third-codon-position transitions generally performed better than analyses using total nucleotide substitutions of this gene in recovering more-inclusive clades.

A comparison of per site substitution rates between nucleotide sequences of IRBP and cytochrome *b* (Fig. 4) indicates that cytochrome *b* transitions are likely to be saturated among supraspecific taxa. This is in contrast to cytochrome *b* transversions which appear to accumulate with time approximately proportionally to IRBP total substitutions as far back as the caniform-feliform divergence. Nevertheless, interordinal comparisons (Carnivora vs. Rodentia) indicate saturation also in cytochrome *b* transversions (Fig. 4B).

One factor that affects the efficacy of the two genes and the two types of cytochrome *b* nucleotide substitutions in resolving phylogenetic relationships at different taxonomic levels is apparently the rate of substitution. Although the higher rates of substitution in cytochrome *b* and in its transitions—as compared to the lower rates of substitution in IRBP and in cytochrome *b* transversions, respectively—provide more phylogenetically informative characters over shorter look-back times, which promotes increase in resolution at lower taxonomic levels, they also result in saturation at reduced time spans, which decreases resolution at higher taxonomic levels.

Dating of divergence events

Calibration of substitution rates

The earliest mustelid known is *Plesictis* (Wolsan, 1999). Its mustelid nature is evidenced by an array of cranial and dental synapomorphies of this family (e.g., Wolsan, 1993a), including the mustelid suprameatal fossa (*sensu* Wolsan, 1992, 1993a, b, 1994, 1996, 1999; Wolsan and Lange-Badré, 1996; not Schmidt-Kittler, 1981). The first stratigraphic appearance of this genus is in the upper Oligocene strata of Cournon, France, where remains of *Plesictis plesic-*

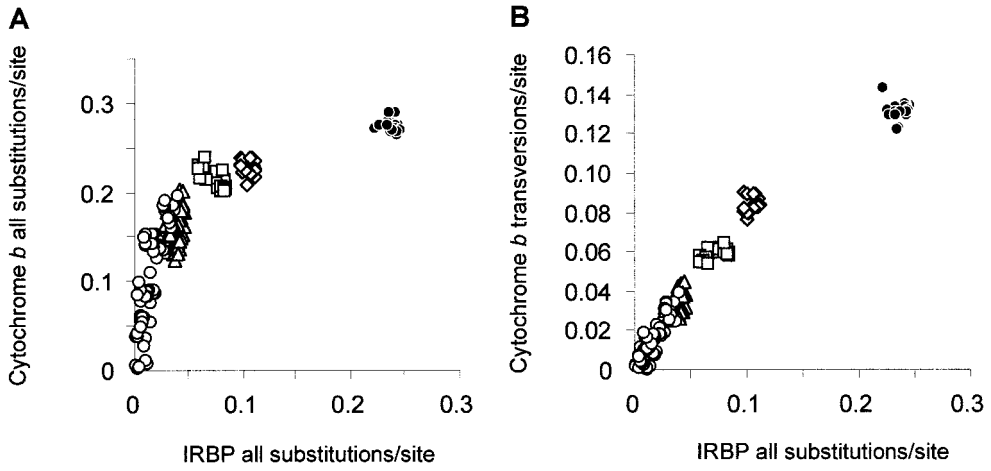

Fig. 4. Comparison of per site substitution rates between partial IRBP (1101 bp) and complete cytochrome *b* (1140 bp) nucleotide sequences as estimated by using the Kimura two-parameter method. **A**, number of per site transitions and transversions for IRBP against the number of per site transitions and transversions for cytochrome *b*. **B**, number of per site transitions and transversions for IRBP against the number of per site transversions for cytochrome *b*. Open circles are for pairwise comparisons among mustelid species-group taxa. Open triangles are for pairwise comparisons among species-group taxa of different mustelid genera. Open squares are for pairwise comparisons between species-group taxa of Mustelidae and *Procyon lotor* (Procyonidae). Open diamonds are for pairwise comparisons between species-group taxa of Mustelidae (Caniformia) and *Felis catus* (Feliformia). Filled circles are for pairwise comparisons between mustelid species-group taxa (Carnivora) and *Apodemus speciosus* (Rodentia). Mustelids used in the comparisons are those listed in Table 1 except *Martes zibellina* and *Meles meles anakuma*.

tis were found (Laizer and Parieu, 1839, their *Mustela plesictis*; Pomel, 1853; Viret, 1929; their *Plesictis genetoïdes*). These remains are referred to a level between the European Paleogene Mammal Reference Levels MP 28 and 29 (e.g., Hugueney, 1997), which corresponds to an age between 24.3 and 24.7 Ma (megannum; Schmidt-Kittler *et al*., 1997, and references therein).

The earliest procyonid known is *Pseudobassaris* (Wolsan, 1993a, 1997a, b, 1998, 1999). The procyonid status of this genus is indicated by the middle-ear synapomorphies of this family, the procyonid suprameatal fossa (*sensu* Wolsan, 1992, 1993a, b, 1994, 1996, 1997a, b, 1998, 1999; Wolsan and Lange-Badré, 1996; not Schmidt-Kittler, 1981) and the procyonid epitympanic recess (Wolsan, 1998, 1999). *Pseudobassaris* first appears in the late Oligocene of Belgarric 1 and Belgarrite 4A, France, from where *Pseudobassaris riggsi* has been recorded (Wolsan and Lange-Badré, 1996, and references therein). The fossil faunal assemblages of these localities are assigned to levels intermediate between the European Paleogene Mammal Reference Levels MP 24 and 25 (e.g., BiochroM'97, 1997) dated at 28.5 and 28.0 Ma, respectively (Schmidt-Kittler *et al*., 1997, and references therein). Thus, the earliest record of Procyonidae is older than that of Mustelidae.

The morphological characteristics of *Pseudobassaris* are congruent with the primitive procyonid morphology inferred from character analysis of the early Miocene to present-day representatives of this family (Wolsan, 1997a, b, 1998). The procyonid synapomorphies of *Pseudobassaris* are of variable occurrence within this genus (Wolsan and Lange-Badré, 1996; Wolsan, 1997a, b, 1998). Furthermore, cranial features synapomorphic for the mustelid-procyonid clade (lack of the alisphenoid canal; separation of the posterior carotid foramen from the posterior lacerate foramen by the caudal entotympanic; posterior inflation of the caudal entotympanic in between the posterior lacerate and stylomastoid foramina [Wolsan, 1993a]) are also variably present within *Pseudobassaris*, so that the primitive conditions characteristic of musteloids basal to the mustelid-procyonid clade also occur (Wolsan and Lange-Badré, 1996; Wolsan, 1997b, 1998). All this indicates that *Pseudobassaris* is very close to mustelid-procyonid split. Therefore, taking into account that *Pseudobassaris* dates to an age between 28.0 and 28.5 Ma, we assume the latter date as the time of divergence between Mustelidae and Procyonidae.

The constancy of IRBP and cytochrome *b* evolutionary rates for mustelids through NJ trees (Figs. 1A, 2A) was found to be moderate by the two-cluster test. Assuming the constancy in evolutionary rates of the two genes within Mustelidae, the divergence time of 28.5 Ma between Mustelidae and Procyonidae indicates an approximate rate of 0.0025 nucleotide substitutions per site per million of years for the mustelid IRBP and an approximate rate of 0.0021 transversions per site per million of years for the mustelid cytochrome *b*.

Estimates of divergence dates

Total substitutions of IRBP and transversions of cytochrome *b* suggest that the lineage of *Meles* diverged from those leading to *Enhydra, Mustela, Martes*, and *Gulo* about 14.5 or 18.1 million years (Myr) ago, respectively, with the lineages of the two studied subspecies of *Meles meles* separating from each other approximately 2 Myr ago (Table 4). The divergence between a clade containing *Enhydra* and

Mustela and that comprising *Gulo* and *Martes* is dated at 14.7–14.8 Ma. The split between the lineages of *Enhydra* and *Mustela* is placed at 13.5–14.1 Ma. The divergence of the *Gulo* lineage from those of martens, in turn, is suggested to be at 3.8–5.8 Ma by IRBP total substitutions, but cytochrome *b* transversions indicate a substantially older date of 6.5–8.1 Ma. Correspondingly, the origin of the subgenus *Martes* is placed at 3.8–6.8 Ma based on IRBP, but an age of 7.8–8.1 Ma is indicated by cytochrome *b*. A date of about 430 ka (kiloannum) for the divergence between *Martes martes* and *Martes zibellina*, or their lineages, is inferred from cytochrome *b* transversions.

Within the genus *Mustela*, the lineage of *Mustela vison* extends as far back as 8.5–9.9 Ma and that of *Mustela erminea* as far back as 3.9–6.7 Ma. The divergence between the clades of small- and large-sized species dates to 4.0–4.8 Ma. The split between the lineages of *Mustela nivalis* and *Mustela altaica* is placed at 2.4 Ma by IRBP, but a considerably older date of 5.6 Ma is suggested by cytochrome *b* transversions. *Mustela itatsi* and *Mustela sibirica*, or their lineages, appear to diverge from each other within a time span from 1.7 to 2.4 Ma.

The subgenus *Putorius* originated about 1.0–1.1 Myr ago. The time of divergence between *Mustela putorius* and *Mustela eversmanii* is estimated at 1.5 Ma on the basis of IRBP, but cytochrome *b* transversions indicate a considerably younger date of approximately 430 ka. Age estimates for the divergence of the *Mustela putorius furo* lineage also substantially differ between the genes. Whereas IRBP dates this event at about 340 ka, cytochrome *b* transversions suggest a date of approximately 860 ka.

DISCUSSION

Nuclear versus mitochondrial genes in phylogenetic reconstruction

Nuclear genes have been found to have more resolving power than mitochondrial genes in recovering ordinal and supraordinal mammalian taxa (Springer *et al*., 2001). Our results indicate that the difference in performance between nuclear and mitochondrial genes, although less well pronounced, can also be seen at lower taxonomic levels. Our analyses of the nuclear IRBP generally performed better than analyses of the mitochondrial cytochrome *b* in recovering more-inclusive mustelid clades. On the other hand, the cytochrome *b* gene demonstrated considerably more resolving power in recovering less-inclusive mustelid clades. For cytochrome *b*, analyses employing both transitions and transversions resulted in much better resolved relationships among closely related species than analyses excluding third-position transitions. On the other hand, more-inclusive mustelid clades were recovered with generally greater efficiency by analyses that excluded cytochrome *b* third-position transitions.

In conclusion, both nuclear and mitochondrial genes and—within the latter—both transitions and transversions are more or less complementary to each other in phylogenetic information. For this reason, analyses based on concatenated nucleotide sequences of the two types of genes, and using a weighting scheme that includes both all substitutions and transversions only at third codon position for mitochondrial genes, appear to provide more reliable estimation of phylogenetic relationships below the family level than analyses based on individual genes alone, and using a single weighting for mitochondrial genes.

Mustelid phylogeny

Relationships among Melinae, Lutrinae, and Mustelinae

Melinae (excluding *Melogale* and *Mydaus*) has recently been hypothesized to be more closely related to either Mustelinae (combined cytochrome *b* and 12S rRNA: Ledje and Árnason, 1996b; morphology: Wozencraft, 1989) or Lutrinae (morphology: Bryant *et al*., 1993; Baskin, 1998); placed in a polytomy with the two subfamilies (12S rRNA: Ledje and Árnason, 1996b; combined genetics and morphology: Bininda-Emonds *et al*., 1999; morphology: Wyss and Flynn, 1993); proposed as an outgroup to the musteline-lutrine clade (cytochrome *b*: Ledje and Árnason, 1996a; Koepfli and Wayne, 1998; combined cytochrome *b*, 12S and 16S rRNA, and morphology: Dragoo and Honeycutt, 1997; α- and β-hemoglobin: Stanhope *et al*., 1993); or linked with some mustelines within the musteline-lutrine clade, thereby causing Mustelinae to be paraphyletic (cytochrome *b*: Masuda and Yoshida, 1994b; 12S rRNA: Emerson *et al*., 1999; combined cytochrome *b* and 12S and 16S rRNA: Dragoo and Honeycutt, 1997; morphology: Abramov and Baryshnikov, 1995; Baryshnikov and Abramov, 1998). Most our analyses corroborated the placement of Melinae basal to a clade encompassing the lutrine *Enhydra* and the mustelines *Mustela*, *Gulo*, and *Martes*, with strongest support yielded by MP_{-3Ti} analysis of the combined genes (Fig. 3C). One analysis only, NJ of cytochrome *b* alone, found evidence, albeit very weak, to suggest a close relationship between Melinae and the *Gulo-Martes* clade to the exclusion of *Mustela* and *Enhydra* (Fig. 2A).

The placement of Lutrinae within Mustelinae, causing the latter subfamily to be paraphyletic, has recently been proposed by an array of both molecular and morphology-based studies (Bryant *et al*., 1993; Masuda and Yoshida, 1994b; Vrana *et al*., 1994; Ledje and Árnason, 1996a; Dragoo and Honeycutt, 1997; Baryshnikov and Abramov, 1998; Koepfli and Wayne, 1998; Emerson *et al*., 1999; Hosoda *et al*., 2000). Most of these studies considered the phylogenetic position of *Enhydra* in relation to both *Mustela* and *Martes* or *Gulo*. The four resultant hypotheses are that (1) *Enhydra* is more closely related to *Mustela* than to *Martes* or *Gulo* (cytochrome *b*: Ledje and Árnason, 1996a; Koepfli and Wayne, 1998; combined cytochrome *b* and 12S and 16S rRNA: Dragoo and Honeycutt, 1997; morphology: Bryant *et al*., 1993); (2) *Enhydra* is more closely related to *Martes* and *Gulo* than to *Mustela* (cytochrome *b*: Hosoda *et al*., 2000); (3) *Enhydra* and *Mustela vison* are successively

more distant outgroups to a clade encompassing *Martes* and the remaining *Mustela* (cytochrome *b*: Masuda and Yoshida, 1994b); and (4) *Enhydra* is basal to a clade containing *Mustela*, *Martes*, and *Gulo* (combined cytochrome *b*, 12S and 16S rRNA, and morphology: Dragoo and Honeycutt, 1997; morphology: Baryshnikov and Abramov, 1998). Only one of our analyses (MP_{TiTv} of cytochrome *b* alone) yielded evidence, albeit very weak, to suggest that *Enhydra* is phylogenetically closer to the *Gulo-Martes* clade than to *Mustela* (Fig. 2B). All other analyses corroborated an association of *Enhydra* and *Mustela* to the exclusion of *Martes* and *Gulo*, with robust support coming from IRBP alone (Fig. 1) and the combined genes (Fig. 3).

Relationships among Gulo, Martes, *and* Mustela

Although Bryant *et al*. (1993) provided morphological evidence to suggest a basal placement of *Martes* with respect to a clade comprising *Gulo* and *Mustela*, all other recent studies of interrelationships among these genera, based on both morphological and genetic data (Abramov and Baryshnikov, 1995; Dragoo and Honeycutt, 1997; Baryshnikov and Abramov, 1998; Koepfli and Wayne, 1998; Bininda-Emonds *et al*., 1999; Hosoda *et al*., 2000), consistently indicate a close relationship between *Gulo* and *Martes* to the exclusion of *Mustela*. The latter interrelationship is confirmed by all our analyses, with strongest support demonstrated by the combined genes (Fig. 3).

Relationships within Martes

The genus *Martes* may be paraphyletic. Of the four recent studies of interrelationships among *Gulo* and species of *Martes*, Koepfli and Wayne (1998, cytochrome *b*) and Bininda-Emonds *et al*. (1999, combined genetic and morphological evidence) supported the monophyletic status of the genus *Martes*, but Baryshnikov and Abramov (1998, morphology) and Hosoda *et al*. (2000, cytochrome *b*) suggested its paraphyly. This paraphyly was due to the hypothesized close relationship of *Gulo* and either *Martes flavigula* and *Martes pennanti* (Hosoda *et al*., 2000) or *Martes flavigula* and some other mustelids (Baryshnikov and Abramov, 1998) to the exclusion of the subgenus *Martes*. While most our analyses indicated weak to moderate support for the paraphyletic status of the genus *Martes* relative to *Gulo* (Figs. 1A; 2A, B; 3A, B), the others were unable to completely resolve phylogenetic relationships of *Gulo* with respect to *Martes flavigula* and the subgenus *Martes*.

The subgenus *Martes* has constantly been regarded as monophyletic (e.g., Anderson, 1970; Bininda-Emonds *et al*., 1999; Hosoda *et al*., 2000), and its monophyly is strongly supported by all our analyses. Within this subgenus, *Martes foina* has generally been recognized as basal to a clade containing the remaining present-day species (Anderson, 1970; Wolsan, 1987; Carr and Hicks, 1997; Bininda-Emonds *et al*., 1999; Hosoda *et al*., 2000). This basal position is supported by our analyses of cytochrome *b* alone and the combined genes, with very strong support coming from the NJ and MP_{TiTv} analyses (Figs. 2A, B; 3A, B). In contrast to these, our NJ analysis of IRBP alone (Fig. 1A) provided support, albeit weak, for a sister-group relationship of *Martes foina* to *Martes melampus*, and the MP analysis of this gene (Fig. 1B) failed to resolve interrelationships within the subgenus.

Martes martes and *Martes zibellina* have mostly been hypothesized as sister species, with *Martes melampus* and *Martes americana* as successively more distant outgroups to this clade (cytochrome *b*: Carr and Hicks, 1997; Hosoda *et al*., 1997, 2000; rDNA: Hosoda *et al*., 1997; morphology: Wolsan, 1987). Our results largely support this hypothesis. Our cytochrome *b* analyses—that is, all our analyses that included *Martes zibellina*—supported the sister-group status of this species to *Martes martes* (Fig. 2), and most our analyses of cytochrome *b* alone (Fig. 2A, B) and the combined genes (Fig. 3A, B) strongly supported *Martes melampus* and *Martes americana* as successively more distant outgroups to the *Martes martes-Martes zibellina* clade. Only one of our analyses (NJ of IRBP alone) yielded support, albeit weak and only in part, for an alternative hypothesis that links *Martes melampus* with *Martes americana* (cytochrome *b*: Carr and Hicks, 1997) and postulates *Martes zibellina* and *Martes martes* as successively more distant outgroups to the *Martes melampus-Martes americana* clade (morphology: Bininda-Emonds *et al*., 1999, based on Anderson's [1970] phylogenetic scenario).

Relationships within Mustela

Although some evidence has been provided to suggest that the genus *Mustela* is paraphyletic relative to *Martes, Vormela*, some lutrines, or *Meles* (cytochrome *b*: Masuda and Yoshida, 1994b; 12S rRNA: Emerson *et al*., 1999; serum immunology: Belyaev *et al*., 1980, 1984; Taranin *et al*., 1991; combined cytochrome *b*, 12S rRNA, and morphology: Vrana *et al*., 1994; morphology: Baryshnikov and Abramov, 1998), there is prevailing evidence from both genetics and morphology (e.g., Dragoo and Honeycutt, 1997; Bininda-Emonds *et al*., 1999; Hosoda *et al*., 2000) in support of the monophyletic status for *Mustela*. Our results confirm the monophyly of this genus, with strongest support demonstrated by IRBP alone (Fig. 1) and the combined genes (Fig. 3).

Mustela vison has long been regarded as closely related to or even conspecific with *Mustela lutreola*. However, overwhelming genetic and morphological evidence have been presented to indicate that the two species are only distantly related, and that *Mustela vison* is indeed an outgroup to a clade comprising all other extant species of the genus (Volobuev and Ternovsky, 1974; Graphodatsky *et al*., 1976; Belyaev *et al*., 1980, 1984; Youngman, 1982; Lushnikova *et al*., 1989; Taranin *et al*., 1991; Masuda and Yoshida, 1994b; Koepfli and Wayne, 1998; Bininda-Emonds *et al*., 1999; Davison *et al*., 1999; Emerson *et al*., 1999; Abramov, 2000a; Hosoda *et al*., 2000; Kurose *et al*., 2000). This basal placement of *Mustela vison* is very strongly sup-

ported by all our analyses.

Our analyses also unanimously indicated a basal position of *Mustela erminea* in relation to a clade encompassing the remaining studied species of the genus except *Mustela vison*, with strongest support coming from the analyses of IRBP alone (Fig. 1). This phylogenetic position of *Mustela erminea* has also been suggested by previous studies of cytochrome *b* nucleotide sequences (Masuda and Yoshida, 1994b; Davison *et al*., 1999; Kurose *et al*., 2000), as well as by evidence from protein electrophoresis (Hartl *et al*., 1988), serum immunology (Taranin *et al*., 1991), karyology (Graphodatsky *et al*., 1976; Obara, 1991), and morphology (Rabeder, 1976). An alternative hypothesis that has recently received some support (cytochrome *b*: Hosoda *et al*., 2000; protein electrophoresis: Hartl *et al*., 1988; karyology: Zima and Král, 1984; combined genetics and morphology: Bininda-Emonds *et al*., 1999; morphology: Youngman, 1982; Abramov, 2000a) associates *Mustela erminea* with *Mustela nivalis* and *Mustela altaica* to the exclusion of *Mustela itatsi, Mustela lutreola, Mustela sibirica*, and the subgenus *Putorius*.

All our analyses supported a sister-group relationship between clades comprising the small-sized species *Mustela nivalis* and *Mustela altaica*, on the one hand, and the large-sized species *Mustela lutreola, Mustela itatsi, Mustela sibirica, Mustela eversmanii*, and *Mustela putorius*, on the other. Strongest support for the monophyletic statuses of the two species groupings come from the concatenated genes (Fig. 3) and NJ and MP_{TiTv} analyses of cytochrome *b* alone (Fig. 2A, B), with the monophyly of the large-sized species being consistently much stronger supported. That the two species groupings are contained in separate clades has already been indicated by both genetic (Volobuev *et al*., 1975; Graphodatsky *et al*., 1976; Zima and Král, 1984; Taranin *et al*., 1991; Hosoda *et al*., 1993, 2000; Masuda and Yoshida, 1994a, b; Davison *et al*., 1999; Kurose *et al*., 2000) and morphological (e.g., Abramov, 2000a) data, as well as by the combined evidence (Bininda-Emonds *et al*., 1999). The only recent source to provide evidence in favor of an alternative relationship is a morphologically based study of Youngman (1982). Although his study identified *Mustela nivalis* and *Mustela altaica* as close relatives, it included their clade within the grouping of the large-sized species.

Mustela itatsi has generally been regarded as conspecific with *Mustela sibirica*, especially by non-Japanese and non-Russian authors. However, notable genetic and morphological evidence have been accumulated (Graphodatsky *et al*., 1979; Watanabe and Kawamoto, 1984; Watanabe *et al*., 1985; Masuda and Yoshida, 1994a, b; Abramov, 2000a, b; Hosoda *et al*., 2000; Kurose *et al*., 2000) in support of the specific distinctness between the two taxa. Our results provide further evidence of this distinctness. Excepting three analyses that failed to resolve relationships between these species, all other analyses indicated a basal placement of *Mustela itatsi* with respect to a clade comprising *Mustela sibirica* and the subgenus *Putorius*, with strongest support yielded by the MP_{TiTv} analysis of the combined genes (Fig. 3B).

The phylogenetic position of *Mustela lutreola* in relation to *Mustela itatsi, Mustela sibirica*, and the species of *Putorius* continues to be problematic. There is conflicting evidence in support of a close affinity of *Mustela lutreola* with *Mustela itatsi* and *Mustela sibirica* to the exclusion of *Putorius* (combined genetics and morphology: Bininda-Emonds *et al*., 1999; morphology: Youngman, 1982); a grouping of *Mustela lutreola* with species of *Putorius* to the exclusion of *Mustela itatsi* and *Mustela sibirica* (combined IRBP and cytochrome *b*: this paper, NJ BS = 100; cytochrome *b*: Davison *et al*., 1999, 2000a; Hosoda *et al*., 2000; Kurose *et al*., 2000; this paper, NJ BS = 100, MP_{TiTv} BS/DI = 100/9; D-loop: Davison *et al*., 2000a; serum immunology: Taranin *et al*., 1991); or a basal placement of *Mustela lutreola* with respect to a clade containing *Mustela itatsi, Mustela sibirica*, and *Putorius* (IRBP: this paper, NJ BS = 98, MP BS/DI = 96/3; combined IRBP and cytochrome *b*: this paper, MP_{TiTv} BS/DI = 67/2, MP_{-3Ti} BS/DI = 60/1). The considerable strengths of support for alternative hypotheses, revealed by our analyses of individual IRBP and cytochrome *b* nucleotide sequences alone, indicate significant conflict in the phylogenetic signal between the two genes in resolving *Mustela lutreola* relationships. As can be inferred from IRBP and cytochrome *b* genetic distances among *Mustela lutreola, Mustela itatsi, Mustela sibirica*, and *Putorius* species-group taxa (Tables 2, 3), this conflict is related to a drastic difference between the two genes in relative levels of sequence divergence between *Mustela lutreola* and species of *Putorius*. The low level of sequence divergence in cytochrome *b* between *Mustela lutreola* and *Putorius* species was interpreted by Davison *et al*. (1999, 2000a, b) as a result of relatively recent speciation of *Mustela lutreola* in a close relationship to species of *Putorius* or the effects of interspecific introgressive hybridization between *Mustela lutreola* and *Putorius*. Our results point to the mitochondrial introgression (including cytochrome *b*), rather than a recent origin of *Mustela lutreola*.

Although some analyses of mitochondrial nucleotide sequences (cytochrome *b*: Davison *et al*., 1999, 2000a; Hosoda *et al*., 2000; D-loop: Davison *et al*., 2000a) found evidence in favor of the *Putorius* paraphyly relative to *Mustela lutreola*, results of other genetically based studies (cytochrome *b*: Kurose *et al*., 2000; karyology: Graphodatsky *et al*., 1976), as well as morphological (e.g., Youngman, 1982; Abramov, 2000a) and combined (Bininda-Emonds *et al*., 1999) evidence indicate that this subgenus is monophyletic. Our analyses of IRBP alone were unable to resolve relationships of *Putorius*, but all our cytochrome *b* and combined analyses uniformly confirmed its monophyly, with strongest support shown by NJ and MP_{TiTv} analyses of the concatenated genes (Fig. 3A, B).

Whereas a close relationship of *Mustela putorius furo* to *Mustela putorius putorius* and *Mustela eversmanii* has probably never been questioned, the particular placement of

Mustela putorius furo within this clade has often been considered controversial (e.g., Blandford, 1987; Davison *et al*., 1999, 2000b) in spite of the fact that compelling evidence from karyology (Zima and Král, 1984, and references therein), morphology (Rempe, 1970; Wolsan, 1993c, d; Kitchener *et al*., 1999), and developmental biology (Volobuev *et al*., 1974; Ternovsky, 1977), as well as additional supporting evidence from genetics (combined cytochrome *b* and D-loop: Davison *et al*., 1999; serum immunology: Belyaev *et al*., 1980), have been presented to indicate that *Mustela putorius furo* and *Mustela putorius putorius* are either conspecific or at least phylogenetically closer to each other than either is to *Mustela eversmanii*. All our analyses of IRBP alone and the concatenated genes weakly but consistently supported this interrelationship. Apparently, the only factual evidence contrary to this placement of *Mustela putorius furo* comes from analyses of cytochrome *b* sequences (Kurose *et al*., 2000; this paper). Analyses of Kurose *et al*. (2000) resulted in weak support for *Mustela putorius furo* as either basal to the *Mustela putorius putorius-Mustela eversmanii* clade or the sister taxon to *Mustela eversmanii*. Our NJ analysis of cytochrome *b* alone also weakly supported a pairing of *Mustela putorius furo* and *Mustela eversmanii* (Fig. 2A), but maximum-parsimony analyses revealed insufficient resolving power of this gene to differentiate among any of the alternative hypotheses of interrelationships among the three taxa.

Timescale for mustelid phylogeny

Origin of Mustelidae

Our fossil-based time estimate for the origin of Mustelidae (mustelid-procyonid split), at 28.5 Ma, is close to Bininda-Emonds *et al*.'s (1999) estimate of 28.1 Ma derived from six literature estimates (median = 28.1 Ma, mean = 29.4 Ma). Other recent time estimates of the mustelid emergence suggested older dates ranging from approximately 31 to 45 Ma (O'Brien *et al*., 1989; Wayne *et al*., 1989, 1991; Garland *et al*., 1993; Flynn, 1996; Byrnes *et al*., 1998; Koepfli and Wayne, 1998; Flynn *et al*., 2000). Most of these estimates were based on either the erroneously assumed (by McKenna and Bell, 1997) early Oligocene age for the mustelids *Plesictis* and *Plesiogale* (which are indeed late Oligocene-early Miocene and early Miocene in age, respectively [e.g., Wolsan, 1993a]) or the alleged mustelid status of the late Eocene or early Oligocene *Mustelavus, Mustelictis* (both following Baskin, 1998), or *Amphicticeps* (following McKenna and Bell, 1997). However, neither *Mustelictis* (contrary to Wolsan, 1992, 1993a; Wolsan and Lange-Badré, 1996) nor *Mustelavus* or *Amphicticeps* have a suprameatal fossa synapomorphic for Mustelidae. Instead, they show primitive shallow suprameatal fossae that are present in musteloids basal to the mustelid-procyonid clade and also occur outside of Musteloidea (e.g., Wolsan, 1999). This, in addition to other cranial and dental features (see, e.g., Schmidt-Kittler, 1981), indicates that *Mustelavus, Mustelictis*, and *Amphicticeps* are neither mustelids nor members of the mustelid-procyonid clade.

Origin of Melinae

The species that have most recently been considered the earliest known melines are the early Miocene *Dehmictis vorax* and *Trochictis artenensis* (Ginsburg and Morales, 2000). The first stratigraphic occurrences of these species are within the European Neogene Land Mammal Zone MN 3 (e.g., Ginsburg and Morales, 2000). This biostratigraphic unit corresponds in age to the interval of 18.0–20.5 Ma (Steininger, 1999, and references therein). This fossil-based minimum age for the origin of Melinae is in close agreement with our estimate of 18.1 Ma inferred from cytochrome *b* transversions for the divergence between the lineage of *Meles* and a clade encompassing the remaining mustelids used in this study (Table 4), and it disagrees with our IRBP estimate of this event at 14.5 Ma. That the latter is an underestimate is also suggested by our other results. This estimate is 0.2–0.3 Myr younger than our IRBP and cytochrome *b* estimates of the divergence time between the *Enhydra-Mustela* and *Gulo-Martes* clades (Table 4), although these clades were recognized by most our phylogenetic analyses as diverged from each other after the emergence of the lineage of *Meles* (Figs. 1A; 2B, C; 3A–C).

Dates of about 7.9 and 13.7 Ma for the divergence of Melinae (excluding *Melogale* and *Mydaus*), suggested by Hosoda *et al*. (1993) and Bininda-Emonds *et al*. (1999), respectively, are too young and contradict both the fossil record and our molecular results.

Divergence between the European and Japanese badger lineages

The earliest known finds referred to *Meles meles* are of middle Pleistocene age and have been recorded from both Europe (e.g., Wolsan, 1993e; Griffiths, 1994; Palombo and Mussi, 2001) and Japan (Kawamura *et al*., 1989), as well as from the mainland Asia (Baryshnikov and Batyrov, 1994). From deposits older than the late Middle Pleistocene and extending as far back as the Late Pliocene, an array of other *Meles* species have been reported (see, e.g., Wolsan, 2001). The morphological and size characteristics of these putative extinct species are well within the variability range observed throughout the current Eurasian distribution of *Meles meles*, which justifies the conclusion that the late Pliocene to present-day badgers are conspecific (Wolsan, 2001). If this is true, then our IRBP estimate of a late Pliocene age, at 2 Ma, for the split between the lineages of the European *Meles meles meles* and the Japanese *Meles meles anakuma* is consistent with the widely accepted view that the two taxa are conspecific. Nevertheless, if *Meles meles* is really no older than the Middle Pleistocene, as traditionally conceived, then our divergence-time estimate provides evidence of specific distinctness between the European and Japanese badgers, a view that has recently received some support from morphology (Baryshnikov and Potapova, 1990; Lüps and Wandeler, 1993; Lynch, 1994;

Table 4. Estimates of divergence times (in millions of years before present) for mustelids, based on a mustelid-procyonid split at 28.5 Ma and Kimura two-parameter genetic distances derived from total substitutions among partial nucleotide sequences of IRBP (1185 bp) and from transversions among the complete nucleotide sequences of cytochrome *b* (1140 bp).

Divergence event	IRBP	Cytochrome *b*
Meles to *Enhydra-Mustela-Gulo-Martes*	14.5	18.1
Meles meles meles to *Meles meles anakuma*	2.0	–
Enhydra-Mustela to *Gulo-Martes*	14.8	14.7
Enhydra to *Mustela*	13.5	14.1
Gulo to *Martes flavigula*	5.8	6.5
Gulo to *subgenus Martes*	3.8	8.1
Subgenus Martes to *Martes flavigula*	6.8	7.8
Martes martes to *Martes zibellina*	–	0.43
Mustela vison to *other Mustela*	8.5	9.9
Mustela erminea to *other Mustela except M. vison*	6.7	3.9
Mustela nivalis-M. altaica to *Mustela itatsi-M. sibirica-M. lutreola-Putorius*	4.0	4.8
Mustela nivalis to *Mustela altaica*	2.4	5.6
Mustela itatsi to *Mustela sibirica*	2.4	1.7
Putorius to *Mustela itatsi*	2.7	1.1
Putorius to *Mustela sibirica*	1.0	1.1
Mustela putorius to *Mustela eversmanii*	1.5	0.43
Mustela putorius putorius to *Mustela putorius furo*	0.34	0.86

Stubbe *et al*., 1998; Abramov, 2001).

Origin of Lutrinae

Four genera have recently been considered to be the oldest lutrines known. These are *Kenyalutra* (e.g., Hunt, 1996; McKenna and Bell, 1997; Made, 1999), *Potamotherium* (Thenius, 1989; Stubbe, 1993), *Mionictis* (= *Larteticti*s; e.g., Willemsen, 1992; Baskin, 1998; Ginsburg, 1999), and *Paralutra* (e.g., McKenna and Bell, 1997; Heizmann and Morlo, 1998; Ginsburg, 1999). However, *Kenyalutra* is indeed a viverrid (Morales *et al*., 2000), *Potamotherium* is a musteloid very distantly related to Lutrinae (e.g., Baskin, 1998), and *Mionictis* is a musteline (Heizmann and Morlo, 1998). Therefore, *Paralutra* appears to be the earliest known actual lutrine. The first stratigraphic appearance of this genus has recently been suggested to be during the Early Miocene (McKenna and Bell, 1997; Ginsburg, 1999, p. 126), but the last author on p. 147, Willemsen (1992), and Heizmann and Morlo (1998) indicate that it is middle Miocene in age and within the European Neogene Land Mammal Zone MN 7+8. This zone covers a time span from 11.1 to 13.5 Ma (Steininger, 1999, and references therein).

Our molecular results correspond well with the fossil record. Our time estimates of the split between the lineages of *Enhydra* and *Mustela*, inferred from IRBP total substitutions (at 13.5 Ma) and cytochrome *b* transversions (at 14.1 Ma), are close to each other and only slightly older than the earliest fossil record of Lutrinae. These dates are also in harmony with our other results, being younger than our estimate of 14.7–14.8 Ma for the divergence between the *Enhydra-Mustela* and *Gulo-Martes* clades.

A similar date for the lutrine origin, at approximately 13–14 Ma, results from Koepfli and Wayne's (1998) study, and Bininda-Emonds *et al*. (1999) place this event between 9.9 and 17.1 Ma. Assuming that Lutrinae is monophyletic, our estimate of 14.7–14.8 Ma for the split between the *Enhydra-Mustela* and *Gulo-Martes* clades indicates that dates of 15–23 and 20–25 Ma proposed for the divergence of Lutrinae by Hosoda *et al*. (2000) and Wayne *et al*. (1989), respectively, are overestimates.

Origin of Mustelinae

The earliest mustelines known are the early Miocene *Plesiogale angustifrons* and *Paragale huerzeleri* (e.g., Schmidt-Kittler, 1981; Wolsan, 1993a). These species have been recorded from the older part of the European Neogene Land Mammal Zone MN 2 (e.g., Hugueney, 1997). This part, MN 2a, corresponds to a span of time from about 21.5 to 22.5 Ma (Schlunegger *et al*., 1996). This minimum age for the origin of Mustelinae, inferred from fossils, is older than the fossil-based minimum divergence ages for Melinae and Lutrinae. It is also older than our IRBP and cytochrome *b* time estimates of the splits between the lineages leading to *Meles* or *Enhydra*, on the one hand, and to mustelines examined, on the other (Table 4). This provides further evidence supporting the paraphyletic status of Mustelinae with respect to Lutrinae, and also indicates that the melines, too, share a musteline ancestor.

Bininda-Emonds *et al*.'s (1999) estimate of 11.4 Ma for the divergence of Mustelinae (which they constrained to be

monophyletic) is clearly an underestimate as indicated by both the fossil record and our molecular results.

Origin of Gulo and the subgenus Martes

The earliest known finds of *Gulo* are from the Pliocene deposits of the Adycha River basin and Udunga, Russia (Sotnikova, 1995; Vangengeim *et al*., 1998). The fossil locality of Udunga dates to around 3.3 Ma and that of the Adycha River basin may be about 0.5 Myr older (Vislobokova *et al*., 2001).

The early Miocene "*Martes" laevidens* has recently been proposed as the oldest known member of the subgenus *Martes* (Anderson, 1994). However, the basicranial anatomy of *"Martes" laevidens* (as evidenced by its only preserved cranium stored under register number 1937 II 13539 in the Bayerische Staatssammlung für Paläontologie und historische Geologie, Munich, Germany) clearly indicates that this species is not congeneric with living martens. Hence, the earliest known actual representative of the subgenus *Martes* appears to be *Martes wenzensis* (Anderson, 1970, 1994; Rabeder, 1976). The earliest record of this species is from the Pliocene deposits of Węże 1, Poland (Wolsan, 1989, and references therein), which date to 3.3–4.0 Ma (Głazek *et al*., 1976; Głazek and Szynkiewicz, 1980).

The fossil-based minimum ages for the origin of *Gulo* and the subgenus *Martes* are in harmony with our molecular results on the time of divergence between *Gulo* and martens and that between the subgenus *Martes* and other mustelines, which are contained within a span that ranges in age from 3.8 to 8.1 Ma (Table 4). Date estimates of about 5 and 5–6 Ma for the divergence of the subgenus *Martes*, derived from cytochrome *b* by Carr and Hicks (1997) and Hosoda *et al*. (1997), respectively, are within this time span. Slightly older divergences of the subgenus *Martes* and the genus *Gulo*, at 8.2 Ma, were postulated by Bininda-Emonds *et al*. (1999). A substantially older date of 10–14 Ma proposed for these events by Hosoda *et al*. (2000) is apparently an overestimate.

Origin of Martes martes *and* Martes zibellina

The earliest undoubted record of *Martes martes* is from the last interglacial period, the Eemian (e.g., Wolsan, 1993e; Anderson, 1994; Kolfschoten, 2000), at around 120 ka (Sánchez Goñi *et al*., 2000). In addition, fossils that may represent this species have also been reported from deposits extending as far back as 400 ka (Wolsan, 1993e). The earliest known finds of *Martes zibellina*, in turn, are late Pleistocene in age (e.g., Vereshchagin and Baryshnikov, 1985; Anderson, 1994) and thus are younger than 130 ka (Kolfschoten and Gibbard, 2000; Sánchez Goñi *et al*., 2000). The fossil record of the extinct *Martes vetus*, which has been considered ancestral to both *Martes martes* and *Martes zibellina* (e.g., Anderson, 1970, 1994; Wolsan, 1989), ranges from almost the beginning of the Pleistocene (which is dated at nearly 1.8 Ma [e.g., Lindsay, 2001]) to about 400 ka, with a possible extension to approximately 300 ka (Wolsan, 1993e). Thus, the paleontological data are in good agreement with our estimate of about 430 ka, inferred from cytochrome *b* transversions, for the split between the species *Martes martes* and *Martes zibellina* or their lineages. In view of the paleontological and molecular evidence presented here, dates of 1.8 and 2–4 Ma postulated for this event by Bininda-Emonds *et al*. (1999) and Hosoda *et al*. (2000), respectively, are overestimates.

Origin of Mustela

The earliest undoubted *Mustela* remains recovered come from lower Pliocene strata dated at 3.4–4.2 Ma (Morlo and Kundrát, 2001). This minimum divergence age for *Mustela*, derived from paleontological data, is close to Hosoda *et al*.'s (1993) and O'Brien *et al*.'s (1989) estimates of this event at less than 3.9 Ma and over 4 Ma, respectively. Nonetheless, the fossil record does not contradict our molecular results, which place the beginning of this genus between 8.5–9.9 Ma (divergence of *Mustela vison*) and 13.5–14.1 Ma (*Enhydra-Mustela* split). Other recent estimates of the *Mustela* origin at 10.4–11.4 Ma (Bininda-Emonds *et al*., 1999), between 8.4–11.6 and 13.7–17.4 Ma (Kurose *et al*., 2000), at 12–20 Ma (Wayne *et al*., 1989), and between 10–14 and 15–23 Ma (Hosoda *et al*., 2000) either closely approach our estimate or suggest a more remote emergence of the genus.

Origin of polecats

Three extant species are currently recognized as members of the subgenus *Putorius*. These are *Mustela putorius* (including *Mustela putorius furo*), *Mustela eversmanii*, and *Mustela nigripes* (e.g., Wolsan, 1993c; Abramov, 2000a; Owen *et al*., 2000). While *Mustela nigripes* first appears at the Early-Middle Pleistocene boundary, about 750–850 thousand years (kyr) ago (Owen *et al*., 2000), the fossil record of *Mustela putorius* and *Mustela eversmanii* commences in the late Middle Pleistocene, about 400 kyr ago (Wolsan, 1993c-e). These data are in close agreement with our estimate of 430 ka inferred from cytochrome *b* transversions for the divergence between *Mustela putorius* and *Mustela eversmanii*. This estimate approaches Kurose *et al*.'s (2000) and Wolsan's (1993c) estimates of this divergence at 520 ka and 600–700 ka, respectively. Our IRBP date of 1.5 Ma for this event, as well as dates of 1–3 Ma (Wayne *et al*., 1991), about 2–3 Ma (O'Brien *et al*., 1989), and 2.8 Ma (Bininda-Emonds *et al*., 1999) are overestimates in view of our IRBP and cytochrome *b* dates of 1.0 and 1.1 Ma, respectively, for the split between the lineages of *Putorius* and *Mustela sibirica* and our cytochrome *b* date of 1.1 Ma for the split between the lineages of *Putorius* and *Mustela itatsi*.

Our results place the origin of the subgenus *Putorius* at approximately 1 Ma (Table 4). This date is in harmony with the fossil-based minimum divergence time (at 750–850 ka) derived from the earliest stratigraphic occurrences of the extant species of *Putorius*. Recent age estimates of the *Putorius* divergence included dates of less than 340 ka

(Hosoda *et al*., 1993), 520 ka to 1.06 Ma (Kurose *et al*., 2000), 2.8 Ma (Bininda-Emonds *et al*., 1999), 2–4 Ma (Hosoda *et al*., 2000), and around 2–5 Ma (O'Brien *et al*., 1989).

Thus, both our results and the majority of recent time estimates of the emergence of *Putorius* contradict the view that the extinct *Mustela stromeri* is a member of this subgenus. *Mustela stromeri* has persistently been considered the earliest known representative of *Putorius* and a probable ancestor of both *Mustela putorius* and *Mustela eversmanii* since it was first described by Kormos (1934). The undoubted records of this poorly known species are from the Late Pliocene, and its questionable finds have also been reported from the Early and Middle Pleistocene (e.g., Wolsan, 1993c, e). The late Pliocene records come from Hungarian localities Beremend 5 and Osztramos 7 (Jánossy, 1986), which date to around 3.1–3.2 Ma (Montuire, 1996). Taking account of this age and in view of our divergence-time estimates (Table 4), the inclusion of *Mustela stromeri* in *Putorius* causes this subgenus to be paraphyletic with respect to both *Mustela itatsi* and *Mustela sibirica*.

Origin of Mustela putorius furo

Both Kurose *et al*.'s (2000) date of 1.06 Ma and our cytochrome *b* date of 860 ka for the split between the lineages of *Mustela putorius furo* and *Mustela putorius putorius* are overestimates in view of our cytochrome *b* date of about 430 ka for the separation between *Mustela putorius* and *Mustela eversmanii*. Our IRBP date of 340 ka for the divergence of the *Mustela putorius furo* lineage, however, is in agreement with both our other divergence-time estimates (Table 4) and the fossil-based minimum age of approximately 400 ka for the emergence of *Mustela putorius* (Wolsan, 1993e).

Domestic Ferret has been recorded since the fourth century BC when Aristotle mentioned it in his work (e.g., Davison *et al*., 1999). It is generally thought to have been domesticated somewhere in the Mediterranean region, although Strabo explicitly indicated its African origin (e.g., Davison *et al*., 1999) and Linnaeus (1758) named this taxon as being from Africa. The earliest African record of *Mustela putorius* is late Pleistocene in age (Aouraghe, 2000) and thus younger than 130 ka (Kolfschoten and Gibbard, 2000; Sánchez Goñi *et al*., 2000). Our IRBP date of 340 ka for the separation between the lineages of *Mustela putorius furo* and *Mustela putorius putorius* is in harmony with this fossil evidence from North Africa and supports the view that Domestic Ferret was domesticated from the African branch of *Mustela putorius*, which diverged from the European branch in the late Middle Pleistocene to immigrate to North Africa.

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