

# Molecular Phylogeny of Arctoids (Mammalia: Carnivora) with Emphasis on Phylogenetic and Taxonomic Positions of the Ferret-badgers and Skunks

Jun J. Sato<sup>1†</sup>, Tetsuji Hosoda<sup>2</sup>, Mięczyław Wolsan<sup>3</sup> and Hitoshi Suzuki<sup>1\*</sup>

<sup>1</sup>Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, Kita-ku, Sapporo 060-0810, Japan

<sup>2</sup>Kiokan High School, 43-1 Komatsubara, Yukawa, Gobo 644-0012, Japan

<sup>3</sup>Institute of Paleobiology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warszawa, and Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679 Warszawa, Poland

**ABSTRACT**—Phylogenetic relationships among the ferret-badger *Melogale moschata*, the skunk *Mephitis mephitis*, and 21 other arctoid carnivorans, representing Mustelidae (Mustelinae: *Mustela*, *Martes*, *Gulo*; Lutrinae: *Enhydra*; Melinae: *Meles*), Procyonidae (*Procyon*), and Ursidae (*Ursus*, *Melursus*), were evaluated through maximum-parsimony phylogenetic analysis of concatenated partial nucleotide sequences of the nuclear recombination-activating gene 1 (RAG1) and gene encoding interphotoreceptor retinoid-binding protein (IRBP). The analysis strongly supports *Melogale* as more closely related to a musteline-lutrine clade (containing *Mustela* and *Enhydra*) than to *Meles* or another musteline clade containing *Martes* and *Gulo* (causing Melinae and Mustelinae, as traditionally circumscribed, to be nonmonophyletic). This, together with known morphological and karyological evidence for nonmeline affinities of *Melogale*, justify the exclusion of the ferret-badgers from the monophyletic Melinae. Therefore, we recommend that *Melogale* be classified in a distinct mustelid subfamily, the monotypic Helictidinae. Our analysis also strongly supports an outgroup position of the skunks to a clade containing Procyonidae and the nonmephitine Mustelidae (causing Mustelidae, as traditionally circumscribed, to be paraphyletic). This position of the skunks agrees with results of most previous genetic studies. However, it is contradicted by known morphological evidence from both living and fossil taxa, as well as genetic evidence from protein electrophoresis. These consistently support the traditional placement of the skunks within the monophyletic Mustelidae (recently in a close relationship to Lutrinae). Therefore, we consider the recent elevation of the skunks to the level of family as premature, and recommend that this clade be left at the subfamily level (Mephitinae) within the family Mustelidae, pending further evidence.

**Key words:** *Melogale*, Melinae, Mephitinae, nuclear RAG1 gene, nuclear IRBP gene

## INTRODUCTION

The ferret-badgers, genus *Melogale*, include four living species (*M. personata*, *M. moschata*, *M. orientalis*, and *M. everetti*) found in southeastern Asia (Wozencraft, 1993, and references therein). Although this genus appears to be only distantly related to *Meles* within Mustelidae, as suggested

by evidence from morphology (e.g., Pocock, 1922; Petter, 1971; Bryant *et al.*, 1993) and karyology (Nie *et al.*, 2002), it is generally classified as a member of the subfamily Melinae, apparently following Simpson's (1945) influential classification. Only few recent authors (Baryshnikov and Abramov, 1997, 1998; Aristov and Baryshnikov, 2001; Sato *et al.*, 2003) explicitly exclude *Melogale* from this subfamily.

The skunks include 10 living species grouped into four genera (the North American *Mephitis* and *Spilogale*, the North and South American *Conepatus*, and the southeast Asian *Mydaus*; e.g., Wozencraft, 1993; Dragoo and Honeycutt, 1997; Dragoo *et al.*, 2003), as well as a number of extinct species and genera, of which the earliest known is

\* Corresponding author: Tel. +81-11-706-2279;

FAX. +81-11-706-2279.

E-mail: htsuzuki@ees.hokudai.ac.jp

† Present address: Laboratory of Animal Cell Technology, Faculty of Life Science and Technology, Fukuyama University, Higashimura-cho, Aza, Sonzo, 985 Fukuyama 729-0292, Japan

the early Miocene European *Miomephitis pilgrimi* (e.g., Ginsburg, 1999). The skunks have traditionally been classified as the subfamily Mephitinae within the family Mustelidae (recently in a close relationship to Lutrinae), primarily based on morphological evidence from extant and extinct taxa (Bininda-Emonds *et al.*, 1999; Wolsan, 1999; and references therein). Recently, however, primarily on the basis of genetic evidence (suggesting an outgroup position of Mephitinae to a clade containing Procyonidae and the non-mephitine Mustelidae), Ledje and Árnason (1996a, b) and Dragoo and Honeycutt (1997) excluded the skunks from the family Mustelidae, and elevated them to the level of family, Mephitidae.

Here we present a hypothesis of phylogenetic relationships among arctoid carnivorans. This hypothesis is derived from a phylogenetic analysis of nucleotide sequences from

two nuclear genes. Special consideration is given to phylogenetic and taxonomic placement of the ferret-badgers and the skunks.

## MATERIALS AND METHODS

### Sampling

A total of 28 species-group taxa (species and subspecies) were examined, of which 23 represented the carnivoran infraorder Arctoidea and five represented the carnivoran infraorder Aeluroidea (Table 1). For each of these species-group taxa, partial nucleotide sequences of two nuclear genes, the recombination-activating gene 1 (RAG1) and the gene encoding interphotoreceptor retinoid-binding protein (IRBP), were obtained by sequencing or extraction from the DDBJ/EMBL/GenBank databases. The RAG1 sequences are a fragment of the exon, 1095 base pairs (bp) in length, corresponding to human sites 1852–2946 (Schatz *et al.*, 1989). The IRBP sequences are a fragment of exon 1, 1188 bp in length, corre-

**Table 1.** Taxon, organism, and gene sampling, with DDBJ/EMBL/GenBank accession numbers

| Infraorder | Taxon               |                        |                                  |                        | Organism                       |                                      | Gene                  |                       |
|------------|---------------------|------------------------|----------------------------------|------------------------|--------------------------------|--------------------------------------|-----------------------|-----------------------|
|            | Family <sup>a</sup> | Subfamily <sup>a</sup> | Species                          |                        | Collection number <sup>b</sup> | Source location                      | RAG1                  | IRBP                  |
|            |                     |                        | Scientific name                  | Common name            |                                |                                      |                       |                       |
| Arctoidea  | Mustelidae          | Lutrinae               | <i>Enhydra lutris</i>            | Sea Otter              | TH257                          | Alaska, USA                          | AB109355 <sup>c</sup> | AB082978 <sup>d</sup> |
|            |                     |                        |                                  |                        |                                |                                      |                       |                       |
|            |                     | Melinae                | <i>Meles meles</i>               | Eurasian Badger        | TH223                          | Thuringia, Germany                   | AB109356 <sup>c</sup> | AB082979 <sup>d</sup> |
|            |                     |                        | <i>Melogale moschata</i>         | Chinese Ferret-badger  | AK703                          | Vietnam                              | AB109357 <sup>c</sup> | AB109330 <sup>c</sup> |
|            |                     |                        |                                  |                        |                                |                                      |                       |                       |
|            |                     | Mephitinae             | <i>Mephitis mephitis</i>         | Striped Skunk          | HTS3                           | Obihiro Zoo                          | AB109358 <sup>c</sup> | AB109331 <sup>c</sup> |
|            |                     |                        |                                  |                        |                                |                                      |                       |                       |
|            |                     | Mustelinae             | <i>Gulo gulo</i>                 | Wolverine              | TH150                          | Sakhalin, Russia                     | AB109340 <sup>c</sup> | AB082962 <sup>d</sup> |
|            |                     |                        | <i>Martes americana</i>          | American Marten        | HS990                          | Maine, USA                           | AB109341 <sup>c</sup> | AB082963 <sup>d</sup> |
|            |                     |                        | <i>Martes flavigula</i>          | Yellow-throated Marten | AK11                           | Primorye, Russia                     | AB109342 <sup>c</sup> | AB082964 <sup>d</sup> |
|            |                     |                        | <i>Martes foina</i>              | Beech Marten           | HS1396                         | Thuringia, Germany                   | AB109343 <sup>c</sup> | AB082965 <sup>d</sup> |
|            |                     |                        | <i>Martes martes</i>             | European Pine Marten   | AK702                          | Moscow, Russia                       | AB109344 <sup>c</sup> | AB082966 <sup>d</sup> |
|            |                     |                        | <i>Martes zibellina</i>          | Sable                  | TH47                           | Hokkaido, Japan                      | AB109345 <sup>c</sup> | AB109329 <sup>c</sup> |
|            |                     |                        | <i>Mustela altaica</i>           | Mountain Weasel        | AK805                          | Altai region, Russia                 | AB109346 <sup>c</sup> | AB082968 <sup>d</sup> |
|            |                     |                        | <i>Mustela erminea</i>           | Ermine                 | TH106                          | Hokkaido, Japan                      | AB109347 <sup>c</sup> | AB082969 <sup>d</sup> |
|            |                     |                        | <i>Mustela eversmanii</i>        | Steppe Polecat         | HS2169                         | Chita region, Russia                 | AB109348 <sup>c</sup> | AB082970 <sup>d</sup> |
|            |                     |                        | <i>Mustela lutreola</i>          | European Mink          | AK13                           | Novosibirsk, Russia                  | AB109349 <sup>c</sup> | AB082972 <sup>d</sup> |
|            |                     |                        | <i>Mustela nivalis</i>           | Least Weasel           | HS686                          | Aomori, Honshu, Japan                | AB109350 <sup>c</sup> | AB082973 <sup>d</sup> |
|            |                     |                        |                                  |                        |                                |                                      |                       |                       |
|            |                     |                        | <i>Mustela putorius furo</i>     | Domestic Ferret        | TH27                           | experimental animal                  | AB109351 <sup>c</sup> | AB082974 <sup>d</sup> |
|            |                     |                        | <i>Mustela putorius putorius</i> | European Polecat       | AK720                          | Moscow, Russia                       | AB109352 <sup>c</sup> | AB082975 <sup>d</sup> |
|            |                     |                        | <i>Mustela sibirica</i>          | Siberian Weasel        | TH98                           | Wakayama, Honshu, Japan              | AB109353 <sup>c</sup> | AB082976 <sup>d</sup> |
|            |                     |                        | <i>Mustela vison</i>             | American Mink          | TH49                           | Hokkaido, Japan <sup>e</sup>         | AB109354 <sup>c</sup> | AB082977 <sup>d</sup> |
|            | Procyonidae         | Procyoninae            | <i>Procyon cancrivorus</i>       | Crab-eating Raccoon    | HS1423                         | Yokohama City Zoo                    | AB109360 <sup>c</sup> | AB109332 <sup>c</sup> |
|            |                     |                        | <i>Procyon lotor</i>             | Northern Raccoon       | KT2994                         | Miyazaki, Kyushu, Japan <sup>e</sup> | AB109359 <sup>c</sup> | AB082981 <sup>d</sup> |
|            | Ursidae             | Ursinae                | <i>Melursus ursinus</i>          | Sloth Bear             | HS1421                         | Yokohama City Zoo                    | AB109362 <sup>c</sup> | AB109334 <sup>c</sup> |
|            |                     |                        | <i>Ursus arctos</i>              | Brown Bear             | HS1420                         | Yokohama City Zoo                    | AB109361 <sup>c</sup> | AB109333 <sup>c</sup> |
| Aeluroidea | Felidae             | Felinae                | <i>Leopardus pardalis</i>        | Ocelot                 | HS1229                         | Yokohama City Zoo                    | AB109363 <sup>c</sup> | AB109335 <sup>c</sup> |
|            |                     |                        |                                  |                        |                                |                                      |                       |                       |
|            |                     | Pantherinae            | <i>Panthera leo</i>              | Lion                   | HS1205                         | Yokohama City Zoo                    | AB109364 <sup>c</sup> | AB109336 <sup>c</sup> |
|            |                     |                        | <i>Panthera pardus</i>           | Leopard                | HS1203                         | Yokohama City Zoo                    | AB109365 <sup>c</sup> | AB109337 <sup>c</sup> |
|            |                     |                        | <i>Panthera tigris</i>           | Tiger                  | HS1201                         | Yokohama City Zoo                    | AB109366 <sup>c</sup> | AB109338 <sup>c</sup> |
|            | Viverridae          | Paradoxurinae          | <i>Paguma larvata</i>            | Masked Palm Civet      | HS1198                         | Yokohama City Zoo                    | AB109367 <sup>c</sup> | AB109339 <sup>c</sup> |
|            |                     |                        |                                  |                        |                                |                                      |                       |                       |

<sup>a</sup> According to Wozencraft's (1993) classification.

<sup>b</sup> Numbers refer to DNA or tissue collections of Alexei P. Kryukov, Institute of Biology and Soil Science, Russian Academy of Sciences, Vladivostok (AK); Hitoshi Suzuki (HS, HTS); Kimiyuki Tsuchiya, Faculty of Agriculture, Tokyo University of Agriculture, Atsugi (KT); and Tetsuji Hosoda (TH).

<sup>c</sup> New DDBJ/EMBL/GenBank accessions, this study.

<sup>d</sup> Reference: Sato *et al.* (2003).

<sup>e</sup> Introduced.

**Table 2.** Primers used to amplify the RAG1 and IRBP genes

| Primer name | Primer sequence (5' to 3')              | Reference                                     |
|-------------|---|---|
| RAG1F1842   | GCTTTGATGGACATGGAAGAAGACAT              | Teeling <i>et al.</i> (2000, their RAG1F1705) |
| RAG1F1851   | ACATGGAAGAAGACATCTTGAAGG                | this study                                    |
| RAG1F2357   | AGCCTCCCAAATCTTGTCTTCCACTCCA            | this study                                    |
| RAG1R2486   | AATGTCACAGTGAAGGGCATCTATGGAAGG          | this study                                    |
| RAG1R2951   | GAGCCATCCCTCTCAATAATTTTCAGG             | Teeling <i>et al.</i> (2000, their RAG1R2864) |
| +IRBP217    | ATGGCCAAGGTCTCTTGGATAACTACTGCTT         | Stanhope <i>et al.</i> (1992)                 |
| R +IRBP335  | CAGGAAACAGCTATGACCCATCTCAGACCCTCAGACGCT | Serizawa <i>et al.</i> (2000)                 |
| R +IRBP724  | CAGGAAACAGCTATGACCCCTGCACGTGGACACCATCT  | Sato <i>et al.</i> (2003)                     |
| U -IRBP734  | TGTAACACGACGGCCAGTTCTCTGTGGTGGTGTGGAGG  | Serizawa <i>et al.</i> (2000)                 |
| R +IRBP1085 | CAGGAAACAGCTATGACCAGAGAAGGCCCTGGCCATCCT | Suzuki <i>et al.</i> (2000)                   |
| U -IRBP1145 | TGTAACACGACGGCCAGTGCAGTCCACCAGCGTGTAGT  | Sato <i>et al.</i> (2003)                     |
| -IRBP1531   | CGCAGGTCCATGATGAGGTGCTCCGTGTCCTG        | Stanhope <i>et al.</i> (1992)                 |
| U -IRBP1532 | TGTAACACGACGGCCAGTTGATGAGGTGCTCCGTGTCCT | Suzuki <i>et al.</i> (2000)                   |

Numbers in primer names refer to the location of the 3' end of the primer in the human reference sequence (RAG1-Schatz *et al.*, 1989; IRBP-Fong *et al.*, 1990). Prefixes "+" and "-" in IRBP primer names refer to the reading and complementary strands, respectively.

sponding to human sites 337–1317 and 1324–1530 (Fong *et al.*, 1990). Because all examined *Mustela* lacked three IRBP base pairs (corresponding to human sites 1311–1313; Sato *et al.*, 2003), this fragment of the gene was excluded from data analysis.

#### Isolation, amplification, and sequencing of DNA

Total genomic 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<sub>2</sub>, 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 (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: RAG1—a cycle of denaturation at 95°C for 3 min and 30 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and

extension at 72°C for 1 min; IRBP—30 cycles of denaturation at 94°C for 1 min, and annealing and extension at 70°C for 3 min each (Stanhope *et al.*, 1992; Sato *et al.*, 2003). 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<sub>2</sub> (which was 1.875 mM) and the primer pairs. The second PCR was performed under the following conditions: RAG1—a cycle of denaturation at 95°C for 3 min and 30 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 1 min; IRBP—35 cycles of denaturation at 96°C for 30 sec, annealing at 50°C for 30 sec, and extension at 60°C for 30 sec (Sato *et al.*, 2003).

In the first PCR, a 1.1-kb fragment of RAG1 was amplified using primers RAG1F1842 and RAG1R2951, and a 1.3-kb fragment of IRBP was amplified using primers +IRBP217 and -IRBP1531 (Table 2). In the second PCR, two segments of RAG1 and three segments of IRBP were amplified against the respective products of the first PCR. For RAG1, the following two primer sets

**Table 3.** Heterozygosity found in the RAG1 and IRBP fragments among the 28 carnivorans sampled

| Species                   | Nucleotide position <sup>a</sup> |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
|---------------------------|----------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                           | RAG1                             |                  |                  |                  |                  |                  |                  |                  | IRBP             |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
|                           | 1901                             | 1933             | 1984             | 2164             | 2211             | 2239             | 2344             | 2464             | 431              | 453              | 475              | 672              | 772              | 906              | 1161             | 1177             | 1209             | 1275             | 1434             |
| <i>Melogale moschata</i>  | .                                | C/T <sup>b</sup> | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | A/G <sup>c</sup> | .                | .                | C/T <sup>b</sup> | .                | A/C <sup>b</sup> | .                |
| <i>Meles meles</i>        | .                                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | G/C <sup>b</sup> |
| <i>Mephitis mephitis</i>  | .                                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | A/G <sup>b</sup> | .                | .                | .                | .                | .                |
| <i>Martes americana</i>   | A/G <sup>c</sup>                 | C/T <sup>b</sup> | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | G/C <sup>b</sup> | .                | .                |
| <i>Martes martes</i>      | .                                | .                | .                | .                | .                | .                | .                | A/G <sup>b</sup> | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                |
| <i>Martes zibellina</i>   | .                                | .                | .                | C/T <sup>b</sup> | C/T <sup>c</sup> | C/T <sup>b</sup> | C/T <sup>b</sup> | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                |
| <i>Mustela eversmanii</i> | .                                | .                | A/G <sup>b</sup> | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                |
| <i>Leopardus pardalis</i> | .                                | .                | .                | .                | .                | .                | .                | .                | .                | A/C <sup>b</sup> | C/T <sup>b</sup> | C/T <sup>b</sup> | .                | .                | .                | .                | .                | .                | .                |
| <i>Panthera pardus</i>    | .                                | .                | .                | .                | .                | .                | .                | .                | C/T <sup>c</sup> | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                |
| <i>Paguma larvata</i>     | .                                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | .                | C/T <sup>b</sup> | .                | .                | .                | .                |

<sup>a</sup> According to the human reference sequence: RAG1-Schatz *et al.* (1989); IRBP-Fong *et al.* (1990).

<sup>b</sup> Silent substitutions.

<sup>c</sup> Nonsilent substitutions.

**Table 4.** Sequence composition statistics at different codon positions for the RAG1 and IRBP fragments from the 23 arctoids sampled

| Parameter                     | RAG1     |          |            |           | IRBP      |           |            |           |
|-------------------------------|----------|----------|------------|-----------|-----------|-----------|------------|-----------|
|                               | First    | Second   | Third      | Total     | First     | Second    | Third      | Total     |
| Length, base pairs            | 365      | 365      | 365        | 1095      | 395       | 395       | 395        | 1185      |
| Variable sites: number (%)    | 11 (6.5) | 14 (8.3) | 144 (85.2) | 169 (100) | 48 (20.2) | 34 (14.3) | 156 (65.5) | 238 (100) |
| Informative sites: number (%) | 7 (6.4)  | 7 (6.4)  | 95 (87.2)  | 109 (100) | 27 (17.9) | 18 (11.9) | 106 (70.2) | 151 (100) |
| Mean frequency of A, %        | 29.0     | 34.7     | 15.0       | 26.2      | 20.5      | 24.8      | 7.9        | 17.7      |
| Mean frequency of C, %        | 20.9     | 20.6     | 33.9       | 25.1      | 29.1      | 24.4      | 42.3       | 31.9      |
| Mean frequency of G, %        | 31.8     | 16.8     | 31.7       | 26.7      | 38.7      | 19.7      | 37.7       | 32.0      |
| Mean frequency of T, %        | 18.3     | 28.0     | 19.4       | 21.9      | 11.8      | 31.1      | 12.0       | 18.3      |

were used: (1) RAG1F1851 and RAG1R2486, and (2) RAG1F2357 and RAG1R2951. For IRBP, the three primer sets were used: (1) R +IRBP335 and U -IRBP734, (2) R +IRBP724 and U -IRBP1145, and (3) R +IRBP1085 and U -IRBP1532. The sequencing of the products of the second PCR was carried out according to the manufacturer's instructions, using automated sequencing (Big Dye Terminator cycle sequencing kit) on an ABI 310.

#### Data analysis

A  $\chi^2$ -test of homogeneity was applied to test the assumption of base-compositional homogeneity within either single-gene data set. To test significance of incongruence between the two data sets, the partition homogeneity test was performed. The Kimura two-parameter method was used to compare per site substitution rates between the two genes.

Phylogenetic analysis was done on a combined data set (2280 bp) of the two genes by using the maximum-parsimony optimality criteria and equal weighting of nucleotide substitutions. The analysis was conducted using 100 heuristic tree-bisection reconnection searches in which the input order of taxa was randomized. As recommended by Barriel and Tassy (1998), and references therein, more than one outgroup taxon was used. Trees were rooted such that the collective aeluroid outgroup was forced to be monophyletic with respect to the monophyletic arctoid ingroup, in accordance with the current views on carnivoran phylogeny (e.g., Dragoo and Honeycutt, 1997; Flynn and Nedbal, 1998; Bininda-Emonds *et al.*, 1999). To assess statistical support for hypothesized clades, bootstrap analysis was done with 1000 bootstrap replicates sampling 100 replicates of the random stepwise-addition option. In addition, the decay index, representing the number of extra steps required for a clade not to be unequivocally supported, was calculated.

All analyses were performed using PAUP\* version 4.0b10 (Swofford, 1998). In addition, TreeRot version 2b (Sorenson, 1999) was used to calculate the decay index.

## RESULTS

### Nucleotide variation

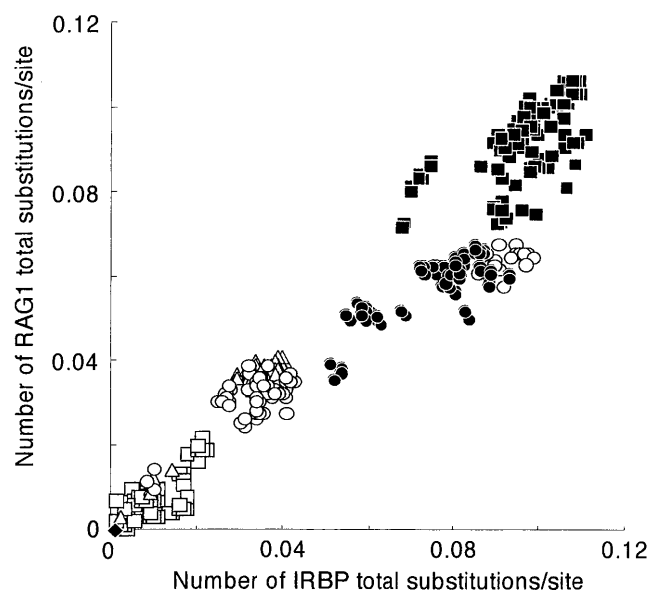
Heterozygosity was found in five mustelid nucleotide sequences of RAG1 and four mustelid, two felid, and one viverrid sequences of IRBP (Table 3). Sequence composition statistics for both gene fragments are listed in Table 4. For either gene, the null hypothesis of homogeneity in base composition across the arctoid taxa was not rejected by the  $\chi^2$ -test ( $P > 0.05$ ).

### Phylogenetic inference

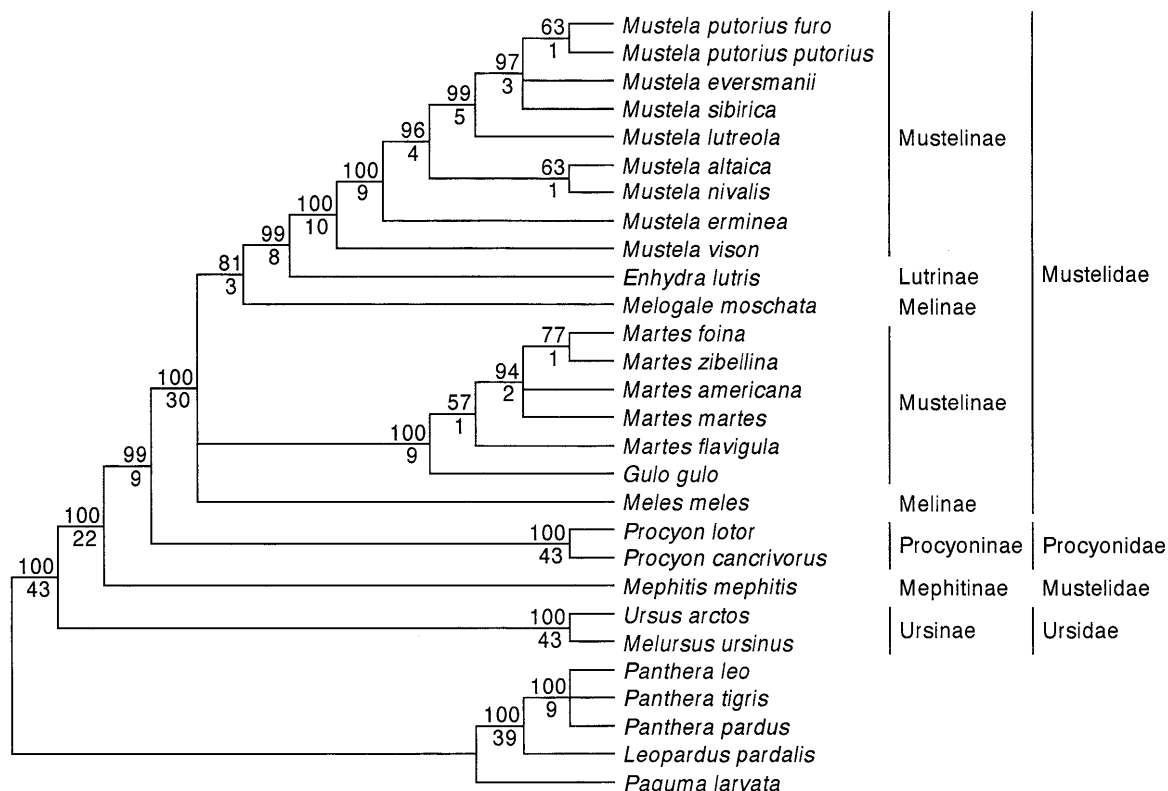
The partition homogeneity test did not reject the null

hypothesis of homogeneity in phylogenetic signal between the RAG1 and IRBP data sets. In addition, a comparison of per site substitution rates between these genes (Fig. 1) indicates similar rates of evolution at least as far back as the divergence between the lineages leading to Arctoidea and Aeluroidea. Therefore, a combined analysis of the two data partitions is justified (Huelsenbeck *et al.*, 1996).

Maximum-parsimony analysis of the combined data set yielded eight shortest trees. The strict consensus of these trees is shown in Fig. 2. There is robust evidence of an out-



**Fig. 1.** Comparison of per site substitution rates between the carnivoran RAG1 and IRBP, as estimated by using the Kimura two-parameter method. The single diamond is for a comparison between the subspecies of *Mustela putorius*. Open squares are for pairwise comparisons among species-group taxa within a genus (*Martes*, *Mustela*, *Procyon*, *Panthera*). Triangles are for pairwise comparisons among species-group taxa of different genera within a subfamily (Mustelinae, Ursinae). Open circles are for pairwise comparisons among species-group taxa of different subfamilies within a family (Mustelidae, Felidae). Filled circles are for pairwise comparisons among species-group taxa of different families within an infraorder (Arctoidea, Aeluroidea). Filled squares are for pairwise comparisons between species-group taxa of Arctoidea and Aeluroidea.



**Fig. 2.** Strict consensus of the eight shortest trees (length, 639 steps; consistency index, 0.70; retention index, 0.89) resulted from maximum-parsimony phylogenetic analysis of concatenated partial nucleotide sequences of RAG1 and IRBP, rooted using aeluroids as outgroups. Numbers above branches are percentage bootstrap values in support of adjacent nodes, and numbers below branches are the decay indices. A traditional circumscription for arctoid families and subfamilies (Wozencraft, 1993) is indicated.

group position of Ursidae with respect to a clade containing Mustelidae and Procyonidae. The family Mustelidae, as traditionally circumscribed, is found to be paraphyletic. The paraphyly is caused by the strongly supported outgroup position of *Mephitis mephitis* (representing Mephitinae) in relation to a clade containing the rest of the mustelids studied and Procyonidae. The subfamilies Melinae and Mustelinae, as traditionally circumscribed, are also found to be nonmonophyletic. While *Melogale moschata* (generally classified as a meline) is relatively strongly supported as basal to the strongly supported clade containing *Mustela* (the name-bearing type of Mustelinae) and *Enhydra* (representing Lutrinae), *Meles meles* (the type species of the type genus of Melinae) is placed in an unresolved trichotomy with the strongly supported clade containing *Martes* and *Gulo* (both usually classified as mustelines) and the *Melogale*-*Mustela*-*Enhydra* clade.

The genus *Martes* is weakly supported as monophyletic, and there is strong evidence for the monophyly of the subgenus *Martes* (*M. martes*, *M. americana*, *M. zibellina*, *M. foina*). Within this subgenus, *Martes foina* and *Martes zibellina* are moderately supported as the closest relatives.

The monophyly of the genus *Mustela* is strongly supported. Strong support, too, is found for *Mustela vison* and *Mustela erminea* as basal and successively more closely

related to a clade encompassing the remainder of the studied species of the genus. Within this clade there exists a relatively well-supported dichotomy between the small-sized (*M. nivalis*, *M. altaica*) and large-sized (*M. lutreola*, *M. sibirica*, *M. eversmannii*, *M. putorius*) species. *Mustela lutreola* is strongly supported as basal to the remaining large-sized species, which, in turn, are placed in an unresolved trichotomy. The conspecificity between *Mustela putorius putorius* and *Mustela putorius furo* is relatively weakly supported.

## DISCUSSION

An outgroup position of Ursidae in relation to a clade containing Mustelidae and Procyonidae, robustly supported by our analysis, is consistent with current views on arctoid phylogeny and contemporary classifications (e.g., Wolsan, 1993; Dragoo and Honeycutt, 1997; McKenna and Bell, 1997; Bininda-Emonds *et al.*, 1999; Flynn *et al.*, 2000). Our results on phylogenetic relationships among mustelids are mostly in agreement with those obtained recently by Sato *et al.* (2003) based on nucleotide sequences from the IRBP and mitochondrial cytochrome *b* genes (see that paper for discussion). Here we discuss the phylogenetic position and taxonomic affiliation of the ferret-badgers and the skunks, which were not included in the study of Sato *et al.* (2003).

### Phylogenetic and taxonomic position of the ferret-badgers

That the ferret-badgers *Melogale moschata*, *Melogale personata* (the name-bearing type of the genus), *Melogale orientalis*, and *Melogale everetti* are each other's closest relatives among extant mustelids is convincingly evidenced by morphological data (e.g., Everts, 1968; Long, 1981; Long and Killingley, 1983; Bininda-Emonds *et al.*, 1999) and has probably never been questioned. The congeneric status of the four species is generally accepted (e.g., Corbet and Hill, 1992; Wozencraft, 1993; Nowak, 1999). Therefore, the use of *Melogale moschata* as a proxy for *Melogale* in exploring the phylogenetic position of this genus is justified. Hence, on the basis of the result of our analysis using *Melogale moschata* (Fig. 2), we put forward the hypothesis that *Melogale* is an outgroup to a clade containing *Mustela* and *Enhydra*, and is more closely related to these genera than it is to *Meles*, *Martes*, or *Gulo*.

Several competing, although less well statistically supported, hypotheses of phylogenetic relationships of *Melogale* have recently been presented. A phylogenetic analysis using 46 morphological characters, carried out by Bryant *et al.* (1993), weakly supported a basal placement of *Melogale* in relation to the remainder of the extant Mustelidae. On the basis of bacular morphology (17 characters), Baryshnikov and Abramov (1997, 1998) proposed placement of *Melogale* in a sister-group position to *Gulo*, also indicating only a distant relationship of *Melogale* with *Meles*. Using morphological data derived from literature sources, Bininda-Emonds *et al.* (1999) placed *Melogale* in a polytomy with a *Meles-Arctonyx-Mydaus* clade, *Taxidea*, a lutrine-mephitine clade, and a clade including the remaining extant mustelids. Finally, on the basis of karyological evidence, Nie *et al.* (2002) suggested an outgroup position of *Melogale* with respect to a clade containing *Meles* and *Mustela*.

Although all these phylogenetic hypotheses, including ours, differ from each other in the placement of *Melogale* within Mustelidae, they consistently indicate that this genus is not part of the monophyletic Melinae. This, together with morphological evidence presented in support of the nonline status for *Melogale* by earlier authors (Schlosser, 1888; Pocock, 1922; Petter, 1971; Rabeder, 1976; Schmidt-Kittler, 1981, 1984; Baryshnikov and Averianov, 1990), justify the exclusion of the ferret-badgers from the monophyletic Melinae. Therefore, we recommend that *Melogale* be classified in a distinct mustelid subfamily, the monotypic Helictidinae, as originally proposed by Gray (1865; his tribe Helictidina, elevated to subfamily rank by Gill [1872]).

### Phylogenetic and taxonomic position of the skunks

While the monophyly of the skunks (including *Mephitis*, *Spilogale*, *Conepatus*, and *Mydaus*) is well supported by evidence from both morphology (e.g., Schmidt-Kittler, 1981; Bryant *et al.*, 1993; Wolsan, 1999) and genetics (e.g., Dragoo and Honeycutt, 1997), the two databases indicate considerably different phylogenetic positions for this clade.

Morphological evidence from both living and fossil taxa (e.g., Wozencraft, 1989; Bryant *et al.*, 1993; Wyss and Flynn, 1993; Baskin, 1998; Wolsan, 1999), as well as some genetic evidence (protein electrophoresis-O'Brien *et al.*, 1989), support the traditional placement of the skunks within the monophyletic Mustelidae. In contrast, genetic evidence from DNA hybridization (Árnason and Widegren, 1986; Wayne *et al.*, 1989; Árnason and Ledje, 1993) and nucleotide sequencing (mitochondrial genes-Vrana *et al.*, 1994; Ledje and Árnason, 1996a, b; Dragoo and Honeycutt, 1997; nuclear genes-this paper; combined mitochondrial and nuclear genes-Flynn *et al.*, 2000), as well as combined evidence from mitochondrial genes and selected morphological characters (Vrana *et al.*, 1994; Dragoo and Honeycutt, 1997), support the skunks as an outgroup to a clade containing the rest of Mustelidae, and also Procyonidae.

Taking account of the persisting conflict between phylogenetic interpretations based on primarily morphological and primarily genetic grounds, we consider the recent elevation of the skunks to the level of family (Ledje and Árnason, 1996a, b; Dragoo and Honeycutt, 1997) as premature, and conservatively recommend that this clade be left at the subfamily level (Mephitinae) within the family Mustelidae, pending further evidence.

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