Evidence from Nuclear DNA Sequences Sheds Light on the Phylogenetic Relationships of Pinnipedia: Single Origin with Affinity to Musteloidea

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ABSTRACT—Considerable long-standing controversy and confusion surround the phylogenetic affinities of pinnipeds, the largely marine group of "fin-footed" members of the placental mammalian order Carnivora. Until most recently, the two major competing hypotheses were that the pinnipeds have a single (monophyletic) origin from a bear-like ancestor, or that they have a dual (diphyletic) origin, with sea lions (Otariidae) derived from a bear-like ancestor, and seals (Phocidae) derived from an otter-, mustelid-, or musteloid-like ancestor. We examined phylogenetic relationships among 29 species of arctoid carnivorans using a concatenated sequence of 3228 bp from three nuclear loci (apolipoprotein B, APOB; interphotoreceptor retinoid-binding protein, IRBP; recombination-activating gene 1, RAG1). The species represented Pinnipedia (Otariidae: *Callorhinus*, *Eumetopias*; Phocidae: *Phoca*), bears (Ursidae: *Ursus*, *Melursus*), and Musteloidea (Mustelidae: *Mustela*, *Enhydra*, *Melogale*, *Martes*, *Gulo*, *Meles*; Procyonidae: *Procyon*; Ailuridae: *Ailurus*; Mephitidae: *Mephitis*). Maximum parsimony, maximum likelihood, and Bayesian inference phylogenetic analyses of separate and combined datasets produced trees with largely congruent topologies. The analyses of the combined dataset resulted in well-resolved and well-supported phylogeny reconstructions. Evidence from nuclear DNA evolution presented here contradicts the two major hypotheses of pinniped relationships and strongly suggests a single origin of the pinnipeds from an arctoid ancestor shared with Musteloidea to the exclusion of Ursidae.

Key words: Arctoidea, Carnivora, nuclear DNA, phylogeny, Pinnipedia

INTRODUCTION

The phylogenetic affinities of pinnipeds, the largely marine group of "fin-footed" members of the placental mammalian order Carnivora, are of considerable long-standing controversy and confusion (e.g., Duffield Kulu, 1972; Flynn *et al.*, 1988; Wozencraft, 1989; Wyss, 1989; Bininda-Emonds, 2000). An impressive bibliography has accumulated relating the enigma of the phylogenetic relationships of pinnipeds to terrestrial carnivorans, including studies based on either morphological or genetic grounds, or integrating morphological and genetic data (morphology—e.g., Mivart, 1885; Weber, 1904; McLaren, 1960; Gambarjan and Karapetjan, 1961; Ling, 1965; Mitchell, 1967; Tedford, 1976; de Muizon, 1982a, b; Ginsburg, 1982; Wiig, 1983; Wyss, 1987, 1988, 1989; Flynn *et al.*, 1988; Wozencraft, 1989; Berta and Ray, 1990; Nojima, 1990; Berta, 1991; Wolsan, 1993; Wyss and Flynn, 1993; Berta and Wyss, 1994; Hunt and Barnes, 1994; Tedford *et al.*, 1994; Bininda-Emonds and Russell, 1996; Kohno, 1996; Flynn and Nedbal, 1998; genetics—e.g., Leone and Wiens, 1956; Pauly and Wolfe, 1957; Fay *et al.*, 1967; Borisov, 1969; Sarich, 1969a, b, 1976; Seal, 1969; Seal *et al.*, 1970, 1971; Duffield Kulu, 1972; Farris, 1972; Árnason, 1974, 1977, 1981; Prager and Wilson, 1978; Romero-Herrera *et al.*, 1978; Anbinder, 1980; de Jong, 1982, 1986; de Jong and Goodman, 1982; Dutrillaux *et al.*, 1982; de Jong *et al.*, 1984, 1993; Árnason and Widegren, 1986; Couturier and Dutrillaux, 1986; Miyamoto and Goodman, 1986; Tagle *et al.*, 1986; Braunitzer and Hofmann, 1987; McKenna, 1987, 1992; Wayne *et al.*, 1989; Czelusniak *et al.*, 1990; Keith *et al.*, 1991; Árnason and Ledje, 1993; Hashimoto *et al.*, 1993; Stanhope *et al.*, 1993; Masuda and Yoshida, 1994; Vrana *et al.*, 1994; Árnason *et al.*, 1995, 2002; Ledje and Árnason, 1995, 1996a, b; Lento *et al.*, 1995; Ikehara *et al.*, 1996; Zhang and Ryder, 1996; Dragoo and Honeycutt, 1997; Byrnes *et al.*, 1998; Flynn and Nedbal, 1998; Schreiber *et al.*, 1998; Emerson *et al.*, 1999; Gatesy *et al.*, 1999; Flynn *et al.*, 2000, 2005; Pecon Slattery *et al.*, 2000; Zehr *et al.*, 2001; Árnason and Janke, 2002; Vassetzky and Kramerov, 2002; Davis *et al.*, 2004; Yu *et al.*, 2004; Delisle and Strobeck, 2005; combined genetics and morphology—Vrana *et al.*, 1994; Dragoo and Honeycutt, 1997; Flynn and Nedbal, 1998; Bininda-Emonds *et al.*, 1999; Bininda-Emonds, 2003).

Despite this extensive interest and substantial accumulation of information, doubts remain and the phylogenetic relationships of pinnipeds have yet to be satisfactorily resolved. Although the arctoid carnivoran nature of the pinnipeds is currently largely accepted (for exceptions, see, e.g., Ginsburg, 1999; Aristov and Baryshnikov, 2001), there remain disagreements over whether the pinnipeds have evolved from two unrelated arctoid ancestors (diphyletic origin) or from a single arctoid ancestor (monophyletic origin), and, in the instance of pinniped monophyly, whether the monophyletic origin was with affinity to bears

(Ursidae) or to weasels, otters, martens, badgers, raccoons, red panda, skunks, and allies (Musteloidea). Until most recently, the two major competing hypotheses were that the pinnipeds have a dual origin, with sea lions (Otariidae) derived from a bear-like ancestor and seals (Phocidae) derived from an otter-, mustelid-, or musteloid-like ancestor, or that they have a single origin from a bear-like ancestor. The dual-origin notion overwhelmingly dominated in the morphological systematic literature over most of the later part of the past century (e.g., McLaren, 1960; Mitchell and Tedford, 1973; Ray, 1976; Repenning, 1976; Tedford, 1976; Savage, 1977; Repenning *et al.*, 1979; de Muizon, 1982a, b; Ginsburg, 1982; Barnes *et al.*, 1985; Barnes, 1989, 1997; Wolsan, 1989, 1991; Wozencraft, 1989; Nojima, 1990) and is still being defended by some systematists (Koretsky and Barnes, 2003; Pavlinov, 2003). The notion of a single origin with affinity to bears has become widely accepted during the last two decades (Flynn, 1988; Flynn *et al.*, 1988; Berta *et al.*, 1989; Berta and Ray, 1990; Berta, 1991; Wyss and Flynn, 1993; Berta and Wyss, 1994; Hunt and Barnes, 1994; Tedford *et al.*, 1994; Vrana *et al.*, 1994; Werdelin, 1996; McKenna and Bell, 1997; Byrnes *et al.*, 1998; Berta and Sumich, 1999; Deméré *et al.*, 2003; Davis *et al.*, 2004; and others).

Recent attention in carnivoran phylogeny reconstruction has centered on DNA sequence data. Using these data, the overwhelming majority of phylogenetic studies on Carnivora in general, and Arctoidea in particular, have analyzed information obtained from mitochondrial loci. However, studies comparing the utility and efficacy of mitochondrial versus nuclear DNA sequences in phylogeny reconstruction indicate that nuclear sequences, especially when combined from various loci, are phylogenetically more informative and more effective in resolving phylogenetic relationships at deeper levels of evolutionary divergence. These studies span a wide range of animal taxa (e.g., Prychitko and Moore, 2000; Baker *et al.*, 2001; Matthee *et al.*, 2001; Springer *et al.*, 2001) and also include Arctoidea (Slade *et al.*, 1994; Koepfli and Wayne, 2003; Sato *et al.*, 2003). In all instances, the low amount of homoplasy exhibited by the nuclear genes is the reason given for the greater utility of the nuclear genes compared with the mitochondrial genes.

In this study of deep-level phylogenetic relationships among arctoids we relied on DNA sequence data obtained from nuclear genes, sampled from all relevant extant clades of Arctoidea and proved informative in arctoid phylogenetic reconstruction (Sato *et al.*, 2003, 2004). Evidence from nuclear DNA evolution presented here contradicts the two major hypotheses of pinniped relationships and strongly suggests a single origin of the pinnipeds from an arctoid ancestor shared with Musteloidea to the exclusion of Ursidae.

MATERIALS AND METHODS

Sampling

A total of 34 species were examined, of which 29 represented all relevant extant clades of the arctoid Carnivora and five represented the aeluroid Carnivora (Table 1). For each of these species, partial nucleotide sequences of three single-copy protein-coding (orthologous) nuclear genes were either newly generated or derived from Sato *et al.* (2003, 2004). The three genes included: the apolipoprotein B (APOB) gene, the gene encoding interphotoreceptor retinoid-binding protein (IRBP), and the recombination-activating gene 1 (RAG1). The studied APOB gene segment consisted of a fragment of exon 26, 963 base pairs (bp) in length, corresponding to human homologous locations 8488–8764 and 9140–9825 in DDBJ/EMBL/GenBank accession M19828 (Ludwig *et al.*, 1987). The studied IRBP gene segment consisted of a fragment of exon 1, 1188 bp in length, corresponding to human homologous locations 337–1317 and 1324–1530 in DDBJ/EMBL/GenBank accession J05253 (Fong *et al.*, 1990). The studied RAG1 segment consisted of a fragment of the exon, 1095 bp in length, corresponding to human homologous locations 1852–2946 in DDBJ/EMBL/GenBank accession M29474 (Schatz *et al.*, 1989).

As all examined species of *Mustela* and *Martes*, as well as *Enhydra lutris*, *Gulo gulo*, *Meles meles*, and *Melogale moschata*, lacked a 15-bp fragment of the APOB gene segment, corresponding to human homologous locations 9593–9607 (this study), and all examined species of *Mustela* also lacked a 3-bp fragment of the IRBP gene segment, corresponding to human homologous locations 1311–1313 (Sato *et al.*, 2003), these gene fragments were excluded from phylogenetic analyses.

DNA extraction, amplification, and sequencing

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 PC 808, ASTEC). In the first PCR, a 1-kb fragment of the APOB gene was amplified using primers APOB-F8487 and APOB-R9826, a 1.3-kb fragment of the IRBP gene was amplified using primers +IRBP217 and –IRBP1531, and a 1.1-kb fragment of RAG1 was amplified using primers RAG1F1842 and RAG1R2951 (Table 2). Each first PCR mix contained 20 mM Tris (pH 8.4), 50 mM KCl, 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 Taq DNA polymerase, recombinant (Invitrogen), 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: a cycle of denaturation at 94°C for 3 min and 30 cycles of denaturation at 95°C for 45 sec, annealing at 55°C for 30 sec,

and extension at 72°C for 90 sec followed by an extension cycle at 72°C for 10 min.

The second PCR was performed under the same thermal cycling conditions as the first PCR. 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 primer pairs. In the second PCR, sets of primer pairs were employed to amplify partially overlapping gene fragments. For the APOB gene, the following two primer sets were used: (1) APOB-F8487 and APOB-R9324, and (2) APOB-F9287 and APOB-R9826 (Table 2). For the IRBP gene, 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. For RAG1, the two primer sets were used: (1) RAG1F1851 and RAG1R2486, and (2) RAG1F2357 and RAG1R2951.

The sequencing of the second PCR products was carried out with the same primers as for the second PCR and the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems), and run on an ABI 310 automated sequencer following the manufacturer's protocol.

Phylogenetic analyses

Phylogenetic analyses were conducted on the following four datasets: (1) 948 bp of the APOB gene, (2) 1185 bp of the IRBP gene, (3) 1095 bp of RAG1, and (4) 3228 bp of the total combined data. Trees were rooted using five aeluroid species (Table 1) as outgroups. All datasets were analyzed using, as optimality criteria, maximum parsimony (Edwards and Cavalli-Sforza, 1964; Camin and Sokal, 1965; Farris, 1970, 1977; Fitch, 1971), maximum likelihood (Edwards and Cavalli-Sforza, 1964; Cavalli-Sforza and Edwards, 1967; Felsenstein, 1981), and Bayesian inference (Rannala and Yang, 1996; Mau and Newton, 1997; Yang and Rannala, 1997; Larget and Simon, 1999; Mau *et al.*, 1999).

Maximum parsimony

Maximum-parsimony analyses were performed with PAUP* version 4.0b10 (Swofford, 2002). Trees were obtained from heuristic searches using 100 replicates of random sequence addition and tree-bisection-reconnection branch swapping. Nucleotide substitutions were equally weighted and treated as unordered. All other settings were set by default.

Robustness of support for inferred clades was evaluated using nonparametric bootstrapping (Efron, 1979; Felsenstein, 1985a) and Bremer (branch) support (Bremer, 1988, 1994), the latter representing the difference in tree length between the most-parsimonious tree and that lacking a particular clade. Bootstrap proportions

were computed with PAUP* 4.0b10 using heuristic searches for 1000 bootstrap replicates, with 100 random sequence additions per replicate and tree-bisection-reconnection branch swapping. Bremer support values were calculated using TreeRot version 2b (Sorenson, 1999). For limitations of the nonparametric bootstrap method and discussion of the interpretation of the bootstrap proportion, see Hedges (1992), Zharkikh and Li (1992a, b, 1995), Felsenstein and Kishino (1993), Hillis and Bull (1993), Li and Zharkikh (1994), Berry and Gascuel (1996), Efron *et al.* (1996), Newton (1996), DeBry and Olmstead (2000), Alfaro *et al.* (2003), Holmes (2003), Huelsenbeck and Rannala (2004), and Yang and Rannala (2005). For limitations of the Bremer support index, see Lee (2000) and DeBry (2001).

Maximum likelihood

Maximum-likelihood analyses were conducted with PAUP* version 4.0b10 using the models and parameters of nucleotide substitution that best fit the data as determined by hierarchical likelihood-ratio tests implemented in Modeltest version 3.06 (Posada and Crandall, 1998). Trees were obtained from heuristic searches using as-is sequence addition and tree-bisection-reconnection branch swapping, with all other settings set by default.

Support for hypothesized clades was assessed by nonparametric bootstrap resampling analysis and likelihood support (Lee and Hugall, 2003), the latter representing the difference in negative log-likelihood between the most-likely tree and that lacking a particular clade. Both analyses were performed using PAUP* 4.0b10. Bootstrap proportions were obtained from heuristic searches for 100 bootstrap replicates, with as-is sequence additions per replicate and tree-bisection-reconnection branch swapping. Likelihood support values were calculated using reverse constraint searches as described by Lee and Hugall (2003).

Bayesian inference

Bayesian-inference analyses were carried out with MrBayes version 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) using best-fitting nucleotide-substitution models inferred via hierarchical likelihood-ratio tests implemented in MrModeltest version 2.2 (Nylander, 2004) for the separate datasets, and a mixed-model approach for the combined dataset. The models applied were as follows: HKY+Γ for the APOB dataset, HKY+I+Γ for the IRBP dataset, and SYM+I+Γ for the RAG1 dataset (HKY, Hasegawa-Kishino-Yano [Hasegawa *et al.*, 1985]; Γ, gamma distribution; I, invariable sites; SYM, symmetrical model [Zharkikh, 1994]). Model parameters were estimated as part of the analyses, and each

gene partition in the combined-data analysis was allowed to have its own estimates. Trees were generated using the Metropolis-coupled Markov-chain Monte-Carlo algorithm (Altekar *et al.*, 2004). The algorithm was run twice for each dataset to assure convergence. Each run consisted of four simultaneous chains, one cold and three incrementally heated, started from a random tree. Chains were run for 1 million generations, and sampled once every 100 generations. For each analysis, the first 1000 trees were discarded as burn-in. The remaining 9000 post-burn-in trees were used to construct a 50% majority-rule consensus tree and to calculate posterior probabilities of inferred clades. For discussion on the Bayesian posterior probability versus the nonparametric-bootstrap proportion as measures of phylogenetic reliability, see Suzuki *et al.* (2002), Wilcox *et al.* (2002), Alfaro *et al.* (2003), Douady *et al.* (2003), Erixon *et al.* (2003), Huelsenbeck and Rannala (2004), Simmons *et al.* (2004), and Yang and Rannala (2005).

Analyses of congruence among gene genealogies

The analyses of partitioned Bremer support (Baker and DeSalle, 1997) and partitioned likelihood support (Lee and Hugall, 2003) were performed not only to explore the effect of different gene partitions on the inferred combined-data phylogenetic hypotheses, but also to evaluate the level of heterogeneity in phylogenetic signal among the partitions. A positive value of the partitioned Bremer support or partitioned likelihood support shows support for a particular clade by a given partition, whereas a negative value indicates that the most-parsimonious or most-likely explanation (respectively) of the data in that partition is not congruent with the combined-data hypothesis. Partitioned Bremer support values were calculated using TreeRot version 2b and, as recommended by Lambkin *et al.* (2002), on each equally most-parsimonious tree separately. Partitioned likelihood support values were computed with PAUP* 4.0b10. Statistical significance of negative partitioned Bremer support values was evaluated using the nonparametric Templeton (Wilcoxon signed ranks) test (Templeton, 1983; Felsenstein, 1985b). The significance of negative partitioned likelihood support values was assessed with the nonparametric Kishino-Hasegawa (Kishino and Hasegawa, 1989) and Shimodaira-Hasegawa (Shimodaira and Hasegawa, 1999) tests.

Phylogenetic incongruence among gene genealogies was additionally assessed using pairwise comparisons between bootstrap proportions or posterior probabilities for the conflicting clades that exclude each other mutually among tree topologies inferred from analyses of single-gene datasets (de Queiroz, 1993). Bootstrap proportions of $\geq 70\%$ and posterior probabilities of ≥ 0.95 were considered corresponding to a probability of ≥ 0.95 that a clade is correct (Hillis and Bull, 1993; Huelsenbeck and Rannala, 2004), and thus

indicative of significant conflict.

Partition homogeneity (incongruence-length difference) tests (Farris *et al.*, 1995a, b) as implemented in PAUP* 4.0b10 were performed as a supplementary measure of phylogenetic discordance among gene genealogies. A number of authors (e.g., Dolphin *et al.*, 2000; Yoder *et al.*, 2001; Barker and Lutzoni, 2002; Darlu and Lecointre, 2002; Dowton and Austin, 2002) have encountered problems with this test that call into question its validity as a criterion for topological congruence between gene genealogies. These studies, however, do not support categorical or unqualified rejection of the test (Hipp *et al.*, 2004).

RESULTS

Heterozygosity

In addition to the heterozygosities reported by Sato *et al.* (2003, 2004), found in five mustelid nucleotide sequences of RAG1 and four mustelid, two felid, and one viverrid sequences of the IRBP gene, there are two heterozygosities among the newly generated sequences of RAG1 (C/T silent substitutions at locations 2092 and 2419 in *Callorhinus ursinus*) and five heterozygosities among the newly generated sequences of the IRBP gene (C/G silent substitution at location 816 in *Phoca vitulina*; C/T silent substitutions at location 642 in *Phoca largha* and locations 375 and 1218 in *Phoca vitulina*; C/T nonsilent substitution at location 1262 in *Phoca vitulina*). Moreover, 10 heterozygosities were found among the nucleotide sequences of the APOB gene, including A/C silent substitution at location 9260 in *Leopardus pardalis*; A/G silent substitutions at locations 9374, 9545, and 9785 in *Melogale moschata*, *Procyon lotor*, and *Leopardus pardalis*, respectively; A/G nonsilent substitution at location 8710 in *Mustela putorius*; A/T and C/G nonsilent substitutions at locations 9506 and 8524, respectively, in *Mustela erminea*; C/T silent substitutions at locations 8741, 9167, and 9557 in *Mustela putorius*, *Leopardus pardalis*, and *Panthera pardus*, respectively.

Sequence characteristics

Sequence-composition statistics for the arctoid gene segments studied are listed in Table 3. The sequence of the IRBP gene is longest and also contains the highest numbers of observed variable sites (41.0%) and parsimony-informative sites (42.9%), whereas the APOB sequence is shortest and contains the smallest numbers of these sites (29.4% and 26.0%, respectively). The majority of observed variable and parsimony-informative sites were found in the third position of codons. For each gene, the null hypothesis of homogeneity in base composition across the arctoid taxa was not rejected by the χ^2-test ($P > 0.05$).

Phylogenetic inference

Tree topologies summarizing the results of maximum parsimony, maximum likelihood, and Bayesian-inference phylogenetic analyses of the separate and combined datasets are shown in Figs. 1–4.

Congruence among gene genealogies

There is a high degree of congruence in the recovered single-gene tree topologies among the maximum parsimony, maximum likelihood, and Bayesian-inference optimality criteria, and less so among the APOB, IRBP, and RAG1 datasets. Of the trees illustrated in Figs. 1–3, those based on the same dataset but different optimality criteria either have identical branching arrangements (Fig. 2B, C) or differ only slightly in resolution. No conflicting mutually-exclusive clades were found between any of these trees (Tables 6, 9). In contrast to this, the majority of the trees that are based on different single-gene datasets are not only different in topological resolution, but also contradict one another in one or more inferred clades. A pairwise comparison of all combinations of these trees revealed nine different pairs of self-contradictory clades, concentrated in four tree regions (Table 6). Three of these pairs, containing clades 10–12 and 21, are associated with three alternative placements of *Meles meles* with respect to *Melogale moschata* and the *Martes*-*Gulo* clade. Three other pairs, containing clades 13–16, are involved in the variable position of *Martes martes*, *Martes americana*, or *Martes foina* relative to *Martes zibellina* and *Martes melampus*. Two pairs that contain clades 18 and 19 are related to alternative placements of *Martes flavigula* and *Gulo gulo* with respect to the rest of the *Martes*-*Gulo* clade (subgenus *Martes*). The four remaining pairs, which contain clades 24–27, are associated with three alternative placements of *Ailurus fulgens* (Ailuridae) relative to the *Procyon* clade (Procyonidae) and *Mephitis mephitis* (Mephitidae).

Pairwise comparisons between support values for the conflicting clades that exclude each other mutually between any of the tree topologies in Figs. 1–3 showed that for the majority of the conflicts, at least one of the self-contradictory clades was supported by a bootstrap proportion of < 70% or posterior probability of < 0.95, indicating insignificant incongruence (Table 9). The only instances where both self-contradictory clades were supported by a bootstrap proportion of ≥ 70% or posterior probability of ≥ 0.95 occurred between the RAG1 tree of Fig. 3C (Bayesian inference) and any of the APOB trees in Fig. 1 (clades 13 versus 15) and between either of the RAG1 trees in Fig. 3B–C (maximum likelihood and Bayesian inference) and any of the IRBP trees in Fig. 2 (clades 24 versus 25). This suggests the presence of significant

disagreement between the inferred RAG1 genealogy and either of the inferred APOB and IRBP genealogies.

However, neither the partitioned Bremer support analysis (Table 7) nor the partitioned likelihood support analysis (Table 8) revealed any significant conflict in phylogenetic signal among the gene partitions in the combined-data tree topologies inferred from maximum parsimony and maximum likelihood analyses. Only 11 (13%) of the 84 partitioned Bremer support values and 17 (19%) of the 90 partitioned likelihood support values were negative. None of these negative values proved significant (all one-tailed *P* values > 0.05) under the Templeton test (partitioned Bremer support) or the Kishino-Hasegawa and Shimodaira-Hasegawa tests (partitioned likelihood support).

The lack of significant phylogenetic incongruence among the gene genealogies was also indicated by partition homogeneity tests, which failed to reject the null hypothesis of homogeneity in phylogenetic signal between any of the single-gene datasets.

Relative phylogenetic contribution of gene partitions

The nuclear gene segments studied exhibit low levels of homoplasy, considerably lower than does the mitochondrial cytochrome *b* gene (Fig. 5). This is also shown by the high values of the consistency and retention indices for the nuclear genes (Table 4). The nuclear genes are also characterized by high decisiveness, as judged by the high values of the index of data decisiveness (Table 4). The APOB gene segment is least homoplastic and most decisive, whereas the IRBP and RAG1 segments display comparable amounts of homoplasy and are similarly decisive (Fig. 5, Table 4).

The IRBP gene showed the best performance for resolving relationships, the APOB gene was less effective, and RAG1 was least efficient, recovering 24–27, 22–24, and 19–23 clades, respectively (Figs. 1–3, Table 6). The low resolution of the single-gene analyses was improved when the sequences were concatenated, yielding nearly completely resolved relationships (28–30 recovered clades; Fig. 4, Table 6).

Tree topologies inferred from the IRBP dataset alone show the largest number of clades (24–25) recovered in agreement with the combined-data topologies based on the same optimality criterion (Table 6). Trees derived from the APOB dataset show 20–24 clades shared with the combined-data topologies, and the RAG1 trees consistently show only 18 shared clades. However, it is the trees based on the APOB dataset that in total exhibit the fewest number of pairwise incongruences with all combined-data topologies. That total number is nine for all APOB trees, ranging from zero to two for an individual APOB tree, whereas for the IRBP and RAG1 datasets it is 18 (spanning from zero to four for an individual tree) and 33 (spanning from

one to six for an individual tree), respectively (Table 9). In addition, the RAG1 dataset is the only partition whose analyses (maximum likelihood and Bayesian inference) resulted in significant pairwise incongruence with the combined-data topologies, as suggested by comparing the strength of bootstrap or posterior probability support between the self-contradictory clades (Table 9).

As indicated by the analyses of partitioned Bremer support (Table 7) and partitioned likelihood support (Table 8), the IRBP partition contributes the most support (38.5–38.7%) to the combined-data topologies derived from maximum parsimony and maximum likelihood analyses. The RAG1 partition contributes 32.3–32.9% of overall support, and the APOB partition contributes the least support (28.5–29.2%). From among the clades recovered by these combined-data analyses, 13 (maximum parsimony) and 15 (maximum likelihood) receive positive support from all three partitions, 10 and 13 (respectively) from two partitions, and five and two (respectively) from only one partition. The numbers of the negative contributions for the maximum parsimony and maximum likelihood combined-data topologies, respectively, are as follows: one and three from the IRBP partition, three and seven from the APOB partition, and seven and seven from the RAG1 partition.

Pinniped relationships

There is robust evidence of the monophyletic Pinnipedia. The species of Phocidae, on the one hand, and the species of Otariidae, on the other, are clustered together in a sister-group relationship in all trees inferred from both the single-gene and combined-data analyses (Figs. 1–4). This relationship was recovered on nearly all of the maximum-parsimony bootstrap-estimated trees (99–100% bootstrap support in single-gene analyses and 100% bootstrap support in the combined-data analysis) and on all maximum-likelihood bootstrap-estimated trees (100% bootstrap support in both single-gene and combined-data analyses), and also consistently supported by a 1.00 posterior probability value in all Bayesian-inference analyses (Table 6). All data partitions positively contributed to the high values of the overall Bremer support (25; Table 7) and likelihood support (63.50; Table 8) for the pinniped clade.

All combined-data analyses and all but two single-gene analyses supported a close relationship between Pinnipedia and Musteloidea, to the exclusion of Ursidae which has a basal position within Arctoidea (Figs. 1–4). The two exceptions are the maximum-parsimony analysis of the APOB dataset (Fig. 1A) and maximum-likelihood analysis of the RAG1 dataset (Fig. 3B), which failed to resolve the relationships among the three clades. The pinniped-musteloid clade was recovered on 95% and 70%, respectively, of the

maximum parsimony and maximum likelihood bootstrap-estimated trees in the combined-data analysis, and also supported by a posterior probability of 0.83 in the Bayesian-inference combined-data analysis (Table 6). Single-gene analyses provided weaker (albeit not very week) support for this clade, with bootstrap proportions of 51%, 65%, and 83% and posterior probabilities of 0.53, 0.67, and 0.71. Even though the Bremer support and likelihood support values for the pinniped-musteloid clade in the combined-data tree topologies are not high (6 and 0.91, respectively), it is noteworthy that this clade received positive support from all data partitions under the maximum-likelihood optimality criterion (Table 8) and all but one partitions under the maximum-parsimony criterion (Table 7). The single, albeit minor, conflicting signal is present from the APOB partition, with a partitioned Bremer support value of –0.5 versus +3.0 and +3.5 from the IRBP and RAG1 partitions, respectively.

DISCUSSION

Pinniped monophyly versus diphyly

While the traditional, long-standing classification of the seals, sea lions, and walruses in a single taxon (Pinnipedia) has increasingly over time implied a single origin for these largely marine carnivores, a double origin for this group has been suggested from time to time to ultimately become the dominant view in the latter half of the past century. Since that time, considerable evidence in favor of pinniped monophyly has been accumulated, while support for pinniped diphyly has eroded. Currently, there appears to be little evidence available to support the dual-origin notion.

Although the hypothesis of a diphyletic origin of the pinnipeds has received some support from morphology (e.g., Mivart, 1885; McLaren, 1960; Tedford, 1976; de Muizon, 1982a, b; Ginsburg, 1982; Wozencraft, 1989; Nojima, 1990), only in few studies (Tedford, 1976; de Muizon, 1982a, b; Ginsburg, 1982; Wozencraft, 1989) is this support provided using cladistic methodology. What is more, Tedford's (1976) phylogenetic hypothesis, historically perhaps the most influential argument in favor of pinniped diphyly, is indeed put forward in conflict with the premises of cladistics (Wiig, 1983). The hypotheses of de Muizon (1982a, b) and Ginsburg (1982) are manually generated cladograms based on characters weighted and ordered subjectively. Moreover, de Muizon's (1982a, b) cladograms include phocids and musteloids only, with no other carnivoran taxa included explicitly in the comparison. In turn, Wozencraft's (1989) result, although inferred from maximum-parsimony analysis done on a large set of data, has not been confirmed by Wyss and Flynn's (1993) maximum-parsimony analysis based on a revised data matrix of Wozencraft (1989)

and using increased taxon sampling.

Wyss and Flynn's (1993) analysis, instead, suggests a single origin of the pinnipeds, a notion also supported by other morphological studies employing cladistic methodology (Wyss, 1987, 1988, 1989; Berta and Ray, 1990; Berta, 1991; Wolsan, 1993; Berta and Wyss, 1994; Bininda-Emonds and Russell, 1996; Werdelin, 1996; Flynn and Nedbal, 1998). Substantial evidence in support of pinniped monophyly has come from genetics, including nuclear DNA sequences (Ledje and Árnason, 1995; Flynn and Nedbal, 1998; Flynn *et al.*, 2000, 2005; Zehr *et al.*, 2001; Yu *et al.*, 2004), mitochondrial DNA sequences (Masuda and Yoshida, 1994; Vrana *et al.*, 1994; Árnason *et al.*, 1995, 2002; Lento *et al.*, 1995; Ledje and Árnason, 1996a, b; Zhang and Ryder, 1996; Dragoo and Honeycutt, 1997; Flynn and Nedbal, 1998; Emerson *et al.*, 1999; Flynn *et al.*, 2000, 2005; Árnason and Janke, 2002; Davis *et al.*, 2004; Delisle and Strobeck, 2005), DNA hybridization (Árnason and Widegren, 1986; Wayne *et al.*, 1989; Árnason and Ledje, 1993; Byrnes *et al.*, 1998), protein sequences (Romero-Herrera *et al.*, 1978; de Jong, 1982, 1986; de Jong and Goodman, 1982; de Jong *et al.*, 1984, 1993; Miyamoto and Goodman, 1986; Tagle *et al.*, 1986; Braunitzer and Hofmann, 1987; McKenna, 1987, 1992; Czelusniak *et al.*, 1990; Stanhope *et al.*, 1993), serum immunology (Borisov, 1969; Sarich, 1969a, b, 1976; Seal *et al.*, 1970, 1971; Farris, 1972; Prager and Wilson, 1978), and karyology (Fay *et al.*, 1967; Seal *et al.*, 1971; Duffield Kulu, 1972; Árnason, 1974, 1977; Anbinder, 1980; Dutrillaux *et al.*, 1982; Couturier and Dutrillaux, 1986). Pinniped monophyly has also consistently been supported by studies integrating genetic and morphological data (Vrana *et al.*, 1994; Dragoo and Honeycutt, 1997; Flynn and Nedbal, 1998; Bininda-Emonds *et al.*, 1999; Bininda-Emonds, 2003).

Our study provides consistent robust support for the monophyletic Pinnipedia from three nuclear loci, with 99–100% bootstrap support and 1.00 Bayesian posterior probabilities from both the single-gene and combined-data analyses. The values of bootstrap proportions reported previously in support of pinniped monophyly range from less than 50% to 100% (Masuda and Yoshida, 1994; Árnason *et al.*, 1995, 2002; Ledje and Árnason, 1995, 1996a, b; Lento *et al.*, 1995; Bininda-Emonds and Russell, 1996; Dragoo and Honeycutt, 1997; Flynn and Nedbal, 1998; Emerson *et al.*, 1999; Flynn *et al.*, 2000, 2005; Zehr *et al.*, 2001; Árnason and Janke, 2002; Davis *et al.*, 2004; Yu *et al.*, 2004; Delisle and Strobeck, 2005). All Bayesian posterior probability values given in the literature for the monophyletic Pinnipedia equal 1.00 (Davis *et al.*, 2004; Delisle and Strobeck, 2005; Flynn *et al.*, 2005). No quantitative clade support has been reported in favor of pinniped diphyly.

Musteloid versus ursid affinities of Pinnipedia

Although the notion of a monophyletic origin of the pinnipeds with affinity to ursids has recently become widely accepted and appears to be currently the prevailing view, a point also reflected by its acceptance in general and influential texts (e.g., McKenna and Bell, 1997; Berta and Sumich, 1999), the actual support for this hypothesis is relatively weak. A close relationship of the pinnipeds to ursids has received some support from morphology (Weber, 1904; Flynn *et al.*, 1988; Berta and Ray, 1990; Berta, 1991; Wyss and Flynn, 1993; Berta and Wyss, 1994; Hunt and Barnes, 1994; Werdelin, 1996; Flynn and Nedbal, 1998), a study combining morphological evidence with mitochondrial DNA sequence data (Vrana *et al.*, 1994), as well as from genetics, including mitochondrial DNA sequences (Vrana *et al.*, 1994; Lento *et al.*, 1995; Ledje and Árnason, 1996a; Davis *et al.*, 2004; Delisle and Strobeck, 2005), DNA hybridization (Byrnes *et al.*, 1998), and serum immunology (Leone and Wiens, 1956; Pauly and Wolfe, 1957). However, the values of quantitative clade support that have been presented for this relationship are low (Wyss and Flynn, 1993; Vrana *et al.*, 1994; Lento *et al.*, 1995; Ledje and Árnason, 1996a; Werdelin, 1996; Flynn and Nedbal, 1998; Davis *et al.*, 2004; Delisle and Strobeck, 2005).

The alternative, and less popular, view that the pinnipeds are derived from an ancestor shared with musteloids, to the exclusion of ursids, has recently been supported by a broad spectrum of data sets. These comprise morphological evidence from skeleton, dentition, and soft anatomy (Wolsan, 1993; Bininda-Emonds and Russell, 1996; Kohno, 1996), combined evidence from morphology and genetics (Dragoo and Honeycutt, 1997; Bininda-Emonds *et al.*, 1999; Bininda-Emonds, 2003), and also genetic evidence. The last is derived from protein sequences (Miyamoto and Goodman, 1986; Ikehara *et al.*, 1996), DNA hybridization (Árnason and Widegren, 1986; Árnason and Ledje, 1993), mitochondrial DNA sequences (Zhang and Ryder, 1996; Dragoo and Honeycutt, 1997; Davis *et al.*, 2004), nuclear DNA sequences (long interspersed nuclear element LINE-1, 741 bp: Ledje and Árnason, 1995; transthyretin [TTR] gene intron 1, 847–851 bp: Flynn and Nedbal, 1998; Zehr *et al.*, 2001; TTR intron 1 + IRBP, 2341 bp: Yu *et al.*, 2004), as well as combined mitochondrial and nuclear DNA sequence data, containing a nuclear sequence of 851 bp from the TTR intron 1 (Flynn and Nedbal, 1998; Flynn *et al.*, 2000) and a concatenated nuclear sequence of 2977 bp from the TTR, IRBP, and thyroxine-binding globulin (TBG) genes (Flynn *et al.*, 2005). Nonetheless, similarly as for the ursid-affinity notion, the musteloid affinity of Pinnipedia has largely received weak quantitative clade support (Bininda-Emonds and Russell, 1996; Zhang and Ryder, 1996; Dragoo and Honeycutt, 1997; Bininda-Emonds *et al.*, 1999; Bininda-Emonds, 2003; Davis *et al.*, 2004; Delisle and Strobeck, 2005). A bootstrap-

estimated confidence ≥ 70% or a Bayesian posterior probability ≥ 0.95 for the pinniped-musteloid clade have only been reported for analyses using nuclear DNA sequences, with the strongest support coming from studies using a concatenated sequence from a group of nuclear genes (Yu *et al.*, 2004; Flynn *et al.*, 2005).

The present study is based on the largest nuclear sequence data set yet employed for reconstructing the phylogenetic relationships of pinnipeds, sampled from a comprehensive taxon set representing all relevant extant arctoid clades. We analyzed a concatenated sequence of 3228 bp from three nuclear loci (APOB, IRBP, RAG1) of 29 arctoid species. Flynn *et al.* (2005: Appendix 1) analyzed a concatenated sequence of 2977 bp from three nuclear loci (IRBP, TBG, TTR) of eight arctoid species, and Yu *et al.* (2004: Table 1) analyzed a concatenated sequence of 2341 bp from two nuclear loci (IRBP, TTR) of 13 arctoid species. The three studies provide independent evidence and strong support for the affinity of Pinnipedia and Musteloidea. Bootstrap proportions and Bayesian posterior probabilities obtained in these studies in support of the pinniped-musteloid clade range from 70% to 99% and from 0.83 to 1.00, respectively. Our study additionally supports this relationship by providing confidence in congruence of phylogenetic signal among three different nuclear genes.

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

Taxon	Organism		Genes		
	Collection number[a]	Source location	APOB	IRBP	RAG1
Arctoidea					
Musteloidea					
Ailuridae					
Ailurus fulgens, red panda	JS191	Asa Zoological Park	AB193430[b]	AB188520[b]	AB188525[b]
Mephitidae					
Mephitis mephitis, striped skunk	HTS3	Obihiro Zoo	AB193406[b]	AB109331[c]	AB109358[c]
Mustelidae					
Enhydra lutris, sea otter	TH257	Alaska, USA	AB193403[b]	AB082978[d]	AB109355[c]
Gulo gulo, wolverine	TH150	Sakhalin, Russia	AB193407[b]	AB082962[d]	AB109340[c]
Martes americana, American marten	HS990	Maine, USA	AB193408[b]	AB082963[d]	AB109341[c]
Martes flavigula, yellow-throated marten	AK11	Primorye, Russia	AB193409[b]	AB082964[d]	AB109342[c]
Martes foina, stone or beech marten	HS1396	Thuringia, Germany	AB193410[b]	AB082965[d]	AB109343[c]
Martes martes, European pine marten	AK702	Moscow, Russia	AB193411[b]	AB082966[d]	AB109344[c]
Martes melampus, Japanese marten	HS517	Wakayama, Honshu, Japan	—	AB082967[d]	—
	HS523	Kumamoto, Honshu, Japan	AB208514[b]	—	AB208515[b]
Martes zibellina, sable	TH47	Hokkaido, Japan	AB193412[b]	AB109329[c]	AB109345[c]
Meles meles, Eurasian badger	TH223	Thuringia, Germany	AB193404[b]	AB082979[d]	AB109356[c]
Melogale moschata, Chinese ferret-badger	AK703	Vietnam	AB193405[b]	AB109330[c]	AB109357[c]
Mustela altaica, mountain weasel	AK805	Altai region, Russia	AB193413[b]	AB082968[d]	AB109346[c]
Mustela erminea, stoat or ermine	TH106	Hokkaido, Japan	AB193414[b]	AB082969[d]	AB109347[c]
Mustela eversmanii, steppe polecat	HS2169	Chita region, Russia	AB193415[b]	AB082970[d]	AB109348[c]

Mustela furo, domestic ferret	TH27	experimental animal	AB193418[b]	AB082974[d]	AB109351[c]
Mustela lutreola, European mink	AK13	Novosibirsk, Russia	AB193416[b]	AB082972[d]	AB109349[c]
Mustela nivalis, least weasel	HS686	Aomori, Honshu, Japan	AB193417[b]	AB082973[d]	AB109350[c]
Mustela putorius, European polecat	AK720	Moscow, Russia	AB193419[b]	AB082975[d]	AB109352[c]
Mustela sibirica, Siberian weasel	TH98	Wakayama, Honshu, Japan	AB193420[b]	AB082976[d]	AB109353[c]
Mustela vison, American mink	TH49	Hokkaido, Japan[e]	AB193421[b]	AB082977[d]	AB109354[c]
Procyonidae					
Procyon cancrivorus, crab-eating raccoon	HS1423	Yokohama City Zoo	AB193426[b]	AB109332[c]	AB109360[c]
Procyon lotor, northern raccoon	KT2994	Miyazaki, Kyushu, Japan[e]	AB193427[b]	AB082981[d]	AB109359[c]
Pinnipedia					
Otariidae					
Callorhinus ursinus, northern fur seal	JS186	Hokkaido, Japan	AB193422[b]	AB188516[b]	AB188521[b]
Eumetopias jubatus, Steller sea lion	NT02-01	Hokkaido, Japan	AB193423[b]	AB188517[b]	AB188522[b]
Phocidae					
Phoca largha, spotted seal	NG02-02	Hokkaido, Japan	AB193424[b]	AB188519[b]	AB188524[b]
Phoca vitulina, harbor seal	NZ02-43	Hokkaido, Japan	AB193425[b]	AB188518[b]	AB188523[b]
Ursidae					
Melursus ursinus, sloth bear	HS1421	Yokohama City Zoo	AB193428[b]	AB109334[c]	AB109362[c]
Ursus arctos, brown bear	HS1420	Yokohama City Zoo	AB193429[b]	AB109333[c]	AB109361[c]
Aeluroidea					
Felidae					
Leopardus pardalis, ocelot	HS1229	Yokohama City Zoo	AB193431[b]	AB109335[c]	AB109363[c]
Panthera leo, lion	HS1205	Yokohama City Zoo	AB193432[b]	AB109336[c]	AB109364[c]
Panthera pardus, leopard	HS1203	Yokohama City Zoo	AB193433[b]	AB109337[c]	AB109365[c]

Panthera tigris, tiger	HS1201	Yokohama City Zoo	AB193434[b]	AB109338[c]	AB109366[c]
Viverridae					
Paguma larvata, masked palm civet	HS1198	Yokohama City Zoo	AB193435[b]	AB109339[c]	AB109367[c]

[a] Numbers refer to DNA or tissue samples stored by Alexei P. Kryukov, Institute of Biology and Soil Science, Russian Academy of Sciences, Vladivostok (AK); Hitoshi Suzuki (HS, HT); Jun J. Sato (JS); Kimiyuki Tsuchiya, Faculty of Agriculture, Tokyo University of Agriculture, Atsugi (KT); Mari Kobayashi (NG, NT, NZ); and Tetsuji Hosoda (TH).

[b] New DDBJ/EMBL/GenBank accessions, this study.

[c] Reference: Sato *et al.* (2004).

[d] Reference: Sato *et al.* (2003).

[e] Introduced.

Table 2. Primers used for DNA amplification and sequencing

Gene	Primer name[a]	Primer sequence (5' to 3')	Reference
APOB	APOB-F8487	GTGCCAGGTTCAATCAGTATAAGT	Amrine-Madsen *et al.* (2003, 187F)
	APOB-F9287	TATAACCAGTCAGATATTGTTGCT	This study
	APOB-R9324	GGTGCCCTCTAATTTGTACTGCAG	This study
	APOB-R9826	CCAGCAAAATTTTCTTTTACTTCAA	Jiang *et al.* (1998), Amrine-Madsen *et al.* (2003, J1R)
IRBP	+IRBP217	ATGGCCAAGGTCCTCTTGGATAACTACTGCTT	Stanhope *et al.* (1992)
	–IRBP1531	CGCAGGTCCATGATGAGGTGCTCCGTGTCCTG	Stanhope *et al.* (1992)
	R +IRBP335	CAGGAAACAGCTATGACCCATCTCAGACCCTCAGACGCT	Serizawa *et al.* (2000)
	R +IRBP724	CAGGAAACAGCTATGACCCCTGCACGTGGACACCATCT	Sato *et al.* (2003)
	R +IRBP1085	CAGGAAACAGCTATGACCAGAGAAGGCCCTGGCCATCCT	Suzuki *et al.* (2000)
	U –IRBP734	TGTAAAACGACGGCCAGTTCTCTGTGGTGGTGTTGGAGG	Serizawa *et al.* (2000)
	U –IRBP1145	TGTAAAACGACGGCCAGTGCGGTCCACCAGCGTGTAGT	Sato *et al.* (2003)
	U –IRBP1532	TGTAAAACGACGGCCAGTTGATGAGGTGCTCCGTGTCCT	Suzuki *et al.* (2000)
RAG1	RAG1-F1842	GCTTTGATGGACATGGAAGAAGACAT	Teeling *et al.* (2000, RAG1F1705)
	RAG1-F1851	ACATGGAAGAAGACATCTTGGAAGG	Sato *et al.* (2004)
	RAG1-F2357	AGCCTCCCAAAATCTTGTCTTCCACTCCA	Sato *et al.* (2004)
	RAG1-R2486	AATGTCACAGTGAAGGGCATCTATGGAAGG	Sato *et al.* (2004)
	RAG1-R2951	GAGCCATCCCTCTCAATAATTTCAGG	Teeling *et al.* (2000, RAG1R2864)

[a] Orientation of the primer is indicated by "F" or "+" (forward) or "R" or "–" (reverse). Numbers refer to the location of the 3' end of the primer in the human reference sequence (APOB: DDBJ/EMBL/GenBank accession M19828 [Ludwig *et al.*, 1987]; IRBP: J05253 [Fong *et al.*, 1990]; RAG1: M29474 [Schatz *et al.*, 1989]).

Table 3. Sequence-composition statistics for the arctoid APOB, IRBP, and RAG1 gene segments

Parameter	APOB				IRBP				RAG1			
	Codon positions			Total	Codon positions			Total	Codon positions			Total
	First	Second	Third		First	Second	Third		First	Second	Third	
Length, base pairs	316	316	316	948	395	395	395	1185	365	365	365	1095
Variable sites: number (%)	49 (26.2)	38 (20.3)	100 (53.5)	187 (100)	54 (20.7)	36 (13.8)	171 (65.5)	261 (100)	15 (7.9)	16 (8.5)	158 (83.6)	189 (100)
Parsimony-informative sites: number (%)	27 (25.5)	25 (23.6)	54 (50.9)	106 (100)	33 (18.9)	23 (13.1)	119 (68.0)	175 (100)	11 (8.7)	9 (7.1)	107 (84.3)	127 (100)
Empirical frequency of A, %	39.4	34.1	24.9	32.8	20.5	24.9	7.9	17.8	29.1	34.7	15.4	26.4
Empirical frequency of C, %	14.2	27.6	23.9	21.9	29.2	24.3	42.4	32.0	20.8	20.6	33.3	24.9
Empirical frequency of G, %	23.8	10.8	19.1	17.9	38.7	19.5	37.9	32.1	31.8	16.7	31.4	26.6
Empirical frequency of T, %	22.6	27.5	32.1	27.4	11.6	31.2	11.8	18.2	18.3	28.0	19.9	22.0

Table 4. Statistics for the strict-consensus trees inferred from maximum-parsimony analyses of the separate and combined datasets

Dataset	Equally most-parsimonious trees	Tree length	CI[a]	RI[b]	DD[c]
APOB	16	239	0.776	0.927	0.917
IRBP	24	429	0.655	0.881	0.860
RAG1	60	334	0.647	0.882	0.864
APOB+IRBP+RAG1	7	1009	0.674	0.891	0.873

[a] Consistency index (Kluge and Farris, 1969) for parsimony-informative substitutions.

[b] Retention index (Archie, 1989, HERM; Farris, 1989).

[c] Data decisiveness (Goloboff, 1991).

Table 5. Negative log-likelihoods (–lnL) of the most-likely tree topologies, the best-fit nucleotide-substitution models, and model parameter values for maximum-likelihood analyses of the separate and combined datasets

Dataset	–lnL	Model[a]	Parameters[b]												
			Nucleotide frequencies				α	I	Ti/Tv	Substitution rates					
			A	C	G	T				A↔C	A↔G	A↔T	C↔G	C↔T	G↔T
APOB	3327.70595	HKY+Γ	0.328	0.218	0.183	0.271	0.835	0.000	3.318	n/a	n/a	n/a	n/a	n/a	n/a
IRBP	4602.52518	HKY+I+Γ	0.192	0.307	0.308	0.193	0.694	0.454	3.286	n/a	n/a	n/a	n/a	n/a	n/a
RAG1	3787.13508	TrNef+I+Γ	0.250	0.250	0.250	0.250	0.730	0.525	n/a	1.000	5.148	1.000	1.000	9.053	1.000
APOB+IRBP+RAG1	11958.40983	TrNef+I+Γ	0.250	0.250	0.250	0.250	0.816	0.417	n/a	1.000	5.831	1.000	1.000	7.101	1.000

[a] HKY, Hasegawa-Kishino-Yano (Hasegawa *et al.*, 1985); Γ, gamma distribution of variable sites; I, proportion of invariable sites; TrNef, Tamura-Nei (Tamura and Nei, 1993) equal frequencies.

[b] α, gamma-distribution shape parameter; I, proportion of invariable sites; Ti/Tv, transition/transversion ratio.

Table 6. Comparison of clade support, topological resolution, and phylogenetic congruence among the trees of Figs. 1–4. Bootstrap proportions (maximum parsimony and maximum likelihood) or posterior probabilities (Bayesian inference) are given for the recovered clades. Dashes indicate that a particular clade was not recovered

Clade		Datasets and optimality criteria[a]												Ref. Nos. of contradictory clades
Ref. No.[b]	Name	APOB			IRBP			RAG1			APOB+IRBP+RAG1			
		MP	ML	BI	MP	ML	BI	MP	ML	BI	MP	ML	BI	
1	*Mustela putorius* + *M. furo*	—	—	0.50	64	65	1.00	—	—	—	63	68	1.00	
2	*Mustela putorius* + *M. furo* + *M. eversmanii*	83	78	1.00	—	—	—	—	—	—	92	95	1.00	
3	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica*	—	—	—	97	95	1.00	—	—	—	98	99	1.00	
4	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola*	—	—	—	74	87	0.98	98	98	1.00	100	100	1.00	
5	*Mustela altaica* + *M. nivalis*	—	—	—	63	63	0.77	—	—	—	58	60	0.79	
6	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola* + *M. altaica* + *M. nivalis*	—	—	—	94	94	1.00	—	—	—	94	97	1.00	
7	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola* + *M. altaica* + *M. nivalis* + *M. erminea*	87	82	0.97	90	95	1.00	100	100	1.00	100	100	1.00	
8	*Mustela*	98	96	1.00	100	100	1.00	95	100	1.00	100	100	1.00	
9	*Mustela* + *Enhydra*	99	95	1.00	99	100	1.00	77	82	0.99	100	100	1.00	
10	*Mustela* + *Enhydra* + *Melogale*	—	—	—	84	84	0.95	—	—	—	79	58	0.92	11
11	*Mustela* + *Enhydra* + *Meles*	—	—	—	—	—	—	—	55	0.89	—	—	—	10, 21
12	*Mustela* + *Enhydra* + *Melogale* + *Meles*	—	—	—	—	60	0.59	—	59	0.82	—	61	—	21
13	*Martes martes* + *M. zibellina* + *M. melampus*	90	83	1.00	—	—	—	—	—	—	52	51	0.96	14, 15
14	*Martes martes* + *M. americana*	—	—	—	—	46	0.60	—	—	—	—	—	—	13, 16
15	*Martes zibellina* + *M. melampus* + *M. foina*	—	—	—	—	—	—	—	65	0.95	—	—	—	13
16	*Martes martes* + *M. zibellina* + *M. melampus* + *M. foina*	—	60	0.90	—	—	—	—	—	—	—	57	0.99	14
17	*Martes martes* + *M. zibellina* + *M. melampus* + *M. americana* + *M. foina*	72	59	0.96	84	85	1.00	67	60	0.77	98	100	1.00	
18	*Martes*	—	—	—	—	57	0.74	—	69	0.82	—	—	—	19

19	*Martes martes* + *M. zibellina* + *M. melampus* + *M. americana* + *M. foina* + *Gulo*	76	88	1.00	—	—	—	—	—	—	—	53	0.96	18
20	*Martes* + *Gulo*	63	72	0.99	85	92	1.00	100	100	1.00	100	100	1.00	
21	*Mustela* + *Enhydra* + *Melogale* + *Martes* + *Gulo*	95	86	0.99	—	—	—	—	—	—	68	—	0.86	11, 12
22	Mustelidae	100	100	1.00	100	100	1.00	100	100	1.00	100	100	1.00	
23	Procyonidae	100	100	1.00	100	100	1.00	100	100	1.00	100	100	1.00	
24	Mustelidae + Procyonidae	55	46	0.51	92	100	1.00	—	—	—	93	93	1.00	25
25	Procyonidae + Ailuridae	—	—	—	—	—	—	67	89	0.99	—	—	—	24
26	Mustelidae + Procyonidae + Ailuridae	—	—	—	60	84	0.98	61	60	0.62	58	90	0.97	27
27	Ailuridae + Mephitidae	69	51	—	—	—	—	—	—	—	—	—	—	26
28	Musteloidea	81	90	1.00	99	100	1.00	87	93	1.00	100	100	1.00	
29	Phocidae	100	100	1.00	100	100	1.00	100	100	1.00	100	100	1.00	
30	Otariidae	100	100	1.00	100	100	1.00	100	100	1.00	100	100	1.00	
31	Pinnipedia	99	100	1.00	100	100	1.00	99	100	1.00	100	100	1.00	
32	Musteloidea + Pinnipedia	—	65	0.71	78	51	0.67	83	—	0.53	95	70	0.83	
33	Ursidae	100	100	1.00	100	100	1.00	100	100	1.00	100	100	1.00	
34	Musteloidea + Pinnipedia + Ursidae	100	100	1.00	100	100	1.00	100	100	1.00	100	100	1.00	
35	*Panthera tigris* + *P. pardus*	64	62	0.93	—	—	—	—	—	—	64	65	0.94	
36	*Panthera*	99	100	1.00	100	98	1.00	95	94	1.00	100	100	1.00	
37	*Panthera* + *Leopardus*	100	100	1.00	100	100	1.00	100	100	1.00	100	100	1.00	
Number of recovered clades		22	24	24	24	27	27	19	22	23	28	30	30	
Number of shared clades[c]		20	22	24	24	25	24	18	18	18	n/a	n/a	n/a	

[a] MP, maximum parsimony; ML, maximum likelihood; BI, Bayesian inference.

[b] Clade reference numbers correspond to those shown in Figs. 1–4.

[c] Number of the recovered clades that are shared with the APOB+IRBP+RAG1 tree inferred under the same optimality criterion.

Table 7. Bremer support and partitioned Bremer support values for clades recovered in the strict-consensus tree inferred from maximum-parsimony analysis of the combined APOB, IRBP, and RAG1 datasets (Fig. 4A)

Clade		Bremer support	Partitioned Bremer support		
Ref. No.[a]	Name		APOB	IRBP	RAG1
1	*Mustela putorius* + *M. furo*	1	+0.0	+1.0	–0.0
2	*Mustela putorius* + *M. furo* + *M. eversmanii*	2	+1.0	+1.0	0
3	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica*	3	0	+3.0	0
4	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola*	5	0	+1.0	+4.0
5	*Mustela altaica* + *M. nivalis*	1	0	+1.0	0
6	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola* + *M. altaica* + *M. nivalis*	4	+0.0	+4.0	–0.0
7	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola* + *M. altaica* + *M. nivalis* + *M. erminea*	10	+1.6	+3.0	+5.4
8	*Mustela*	16	+5.8	+6.0	+4.2
9	*Mustela* + *Enhydra*	14	+7.6	+7.0	–0.6
10	*Mustela* + *Enhydra* + *Melogale*	3	+1.1	+2.5	–0.6
13	*Martes martes* + *M. zibellina* + *M. melampus*	1	+2.3	–0.5	–0.7
17	*Martes martes* + *M. zibellina* + *M. melampus* + *M. americana* + *M. foina*	4	+3.6	0	+0.4
20	*Martes* + *Gulo*	10	+1.6	+2.0	+6.4
21	*Mustela* + *Enhydra* + *Melogale* + *Martes* + *Gulo*	2	+3.0	0	–1.0
22	Mustelidae	40	+8.6	+17.0	+14.4
23	Procyonidae	53	+13.0	+25.0	+15.0
24	Mustelidae + Procyonidae	6	–0.4	+5.0	+1.4
26	Mustelidae + Procyonidae + Ailuridae	1	–2.0	+2.0	+1.0
28	Musteloidea	21	+4.0	+9.0	+8.0
29	Phocidae	30	+10.0	+13.5	+6.5
30	Otariidae	24	+6.0	+8.0	+10.0
31	Pinnipedia	25	+7.0	+11.0	+7.0
32	Musteloidea + Pinnipedia	6	–0.5	+3.0	+3.5
33	Ursidae	60	+17.0	+21.0	+22.0
34	Musteloidea + Pinnipedia + Ursidae	84	+29.0	+21.0	+34.0
35	*Panthera tigris* + *P. pardus*	1	+1.0	0	–0.0
36	*Panthera*	14	+4.6	+5.0	+4.4
37	*Panthera* + *Leopardus*	63	+22.5	+22.5	+18.0
Total		504	+147.4	+194.0	+162.7
Percent of total		100	29.2	38.5	32.3

[a] Clade reference numbers correspond to those shown in Fig. 4A.

Table 8. Likelihood support and partitioned likelihood support values for clades recovered in the most-likely tree inferred from maximum-likelihood analysis of the combined APOB, IRBP, and RAG1 datasets (Fig. 4B)

Clade		Likelihood support	Partitioned likelihood support		
Ref. No.[a]	Name		APOB	IRBP	RAG1
1	*Mustela putorius* + *M. furo*	9.56	–0.99	+8.12	+2.43
2	*Mustela putorius* + *M. furo* + *M. eversmanii*	14.19	+16.42	–2.40	+0.17
3	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica*	8.30	+0.01	+9.56	–1.27
4	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola*	21.98	–0.13	+4.32	+17.79
5	*Mustela altaica* + *M. nivalis*	1.47	+0.18	+1.72	–0.42
6	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola* + *M. altaica* + *M. nivalis*	14.09	+0.31	+14.59	–0.81
7	*Mustela putorius* + *M. furo* + *M. eversmanii* + *M. sibirica* + *M. lutreola* + *M. altaica* + *M. nivalis* + *M. erminea*	26.79	–0.13	+10.63	+16.29
8	*Mustela*	53.83	+22.10	+17.01	+14.73
9	*Mustela* + *Enhydra*	33.91	+14.56	+18.64	+0.71
10	*Mustela* + *Enhydra* + *Melogale*	0.45	+0.04	+3.65	–3.24
12	*Mustela* + *Enhydra* + *Melogale* + *Meles*	0.13	–4.29	+1.66	+2.76
13	*Martes martes* + *M. zibellina* + *M. melampus*	3.11	+8.58	+0.54	–6.02
16	*Martes martes* + *M. zibellina* + *M. melampus* + *M. foina*	3.62	+2.77	–8.25	+9.10
17	*Martes martes* + *M. zibellina* + *M. melampus* + *M. americana* + *M. foina*	17.45	–1.22	+14.08	+4.59
19	*Martes martes* + *M. zibellina* + *M. melampus* + *M. americana* + *M. foina* + *Gulo*	1.44	+5.98	–1.28	–3.25
20	*Martes* + *Gulo*	31.76	+6.75	+6.13	+18.88
22	Mustelidae	117.25	+33.06	+39.01	+45.17
23	Procyonidae	139.71	+42.52	+53.26	+43.93
24	Mustelidae + Procyonidae	9.37	–3.24	+13.23	–0.62
26	Mustelidae + Procyonidae + Ailuridae	5.10	–4.94	+6.01	+4.03
28	Musteloidea	52.26	+13.12	+20.95	+18.20
29	Phocidae	62.39	+27.88	+25.34	+9.17
30	Otariidae	58.07	+17.29	+14.41	+26.37
31	Pinnipedia	63.50	+15.99	+35.12	+12.39
32	Musteloidea + Pinnipedia	0.91	+0.55	+0.20	+0.16
33	Ursidae	138.76	+37.13	+54.12	+47.50
34	Musteloidea + Pinnipedia + Ursidae	178.26	+52.13	+50.74	+75.39
35	*Panthera tigris* + *P. pardus*	5.84	+2.27	+1.89	+1.67
36	*Panthera*	34.59	+6.93	+14.85	+12.80
37	*Panthera* + *Leopardus*	95.72	+31.06	+37.60	+27.06

Total	1203.81	+342.69	+465.45	+395.66
Percent of total	100	28.5	38.7	32.9

[a] Clade reference numbers correspond to those shown in Fig. 4B.

Table 9. Occurrence of conflicting mutually-exclusive clades suggesting significant (above diagonal) and insignificant (below diagonal) phylogenetic incongruences between any of the trees in Figs. 1–4. The significance assessment is based on a comparison of the strength of bootstrap or posterior probability support between the self-contradictory clades. Asterisks indicate clades with a bootstrap proportion of ≥ 70% (MP, ML) or a posterior probability of ≥ 0.95 (BI). Incongruences with both self-contradictory clades designated by an asterisk are considered significant. Clade reference numbers correspond to those given in Figs. 1–4 and Table 6.

Dataset	Optimality criterion[a]	Datasets and optimality criteria[a]											
		APOB			IRBP			RAG1			APOB+IRBP+RAG1		
		MP	ML	BI	MP	ML	BI	MP	ML	BI	MP	ML	BI
APOB	MP	–								13* vs. 15*			
	ML		–							13* vs. 15*			
	BI			–						13* vs. 15*			
IRBP	MP	26 vs. 27	26 vs. 27		–				24* vs. 25*	24* vs. 25*			
	ML	12 vs. 21* 13* vs. 14 18 vs. 19* 26* vs. 27	12 vs. 21* 13* vs. 14 14 vs. 16 18 vs. 19* 26* vs. 27	12 vs. 21* 13* vs. 14 14 vs. 16 18 vs. 19*		–			24* vs. 25*	24* vs. 25*			
	BI	12 vs. 21* 13* vs. 14 18 vs. 19* 26* vs. 27	12 vs. 21* 13* vs. 14 14 vs. 16 18 vs. 19* 26* vs. 27	12 vs. 21* 13* vs. 14 14 vs. 16 18 vs. 19*			–		24* vs. 25*	24* vs. 25*			
RAG1	MP	24 vs. 25 26 vs. 27	24 vs. 25 26 vs. 27	24 vs. 25	24* vs. 25	24* vs. 25	24* vs. 25	–					
	ML	11 vs. 21* 12 vs. 21* 13* vs.15 18 vs. 19* 24 vs. 25* 26 vs. 27	11 vs. 21* 12 vs. 21* 13* vs. 15 18 vs. 19* 24 vs. 25* 26 vs. 27	11 vs. 21* 12 vs. 21* 13* vs. 15 18 vs. 19* 24 vs. 25*	10* vs. 11	10* vs. 11	10* vs. 11		–		24* vs. 25*	24* vs. 25*	24* vs. 25*

	BI	11 vs. 21* 12 vs. 21* 18 vs. 19* 24 vs. 25* 26 vs. 27	11 vs. 21* 12 vs. 21* 18 vs. 19* 24 vs. 25* 26 vs. 27	11 vs. 21* 12 vs. 21* 18 vs. 19* 24 vs. 25*	10* vs. 11	10* vs. 11	10* vs. 11			–	24* vs. 25*	24* vs. 25*	13* vs. 15* 24* vs. 25*	
APOB+IRBP+RAG1	MP	26 vs. 27	26 vs. 27			12 vs. 21 13 vs. 14	12 vs. 21 13 vs. 14	24* vs. 25	10* vs. 11 11 vs. 21 12 vs. 21 13 vs. 15	10* vs. 11 11 vs. 21 12 vs. 21 13 vs. 15*	–			
	ML	12 vs. 21* 26* vs. 27	12 vs. 21* 26* vs. 27	12 vs. 21*		13 vs. 14 14 vs. 16 18 vs. 19	13 vs. 14 14 vs. 16 18 vs. 19	24* vs. 25	10 vs. 11 13 vs. 15 18 vs. 19	10 vs. 11 13 vs. 15* 18 vs. 19	12 vs. 21	–		
	BI	26* vs. 27	26* vs. 27			12 vs. 21 13* vs. 14 14 vs. 16* 18 vs. 19*	12 vs. 21 13* vs. 14 14 vs. 16* 18 vs. 19*	24* vs. 25	10 vs. 11 11 vs. 21 12 vs. 21 13* vs. 15 18 vs. 19*	10 vs. 11 11 vs. 21 12 vs. 21 18 vs. 19*		12 vs. 21	–	

[a] MP, maximum parsimony; ML, maximum likelihood; BI, Bayesian inference.

FIGURE CAPTIONS

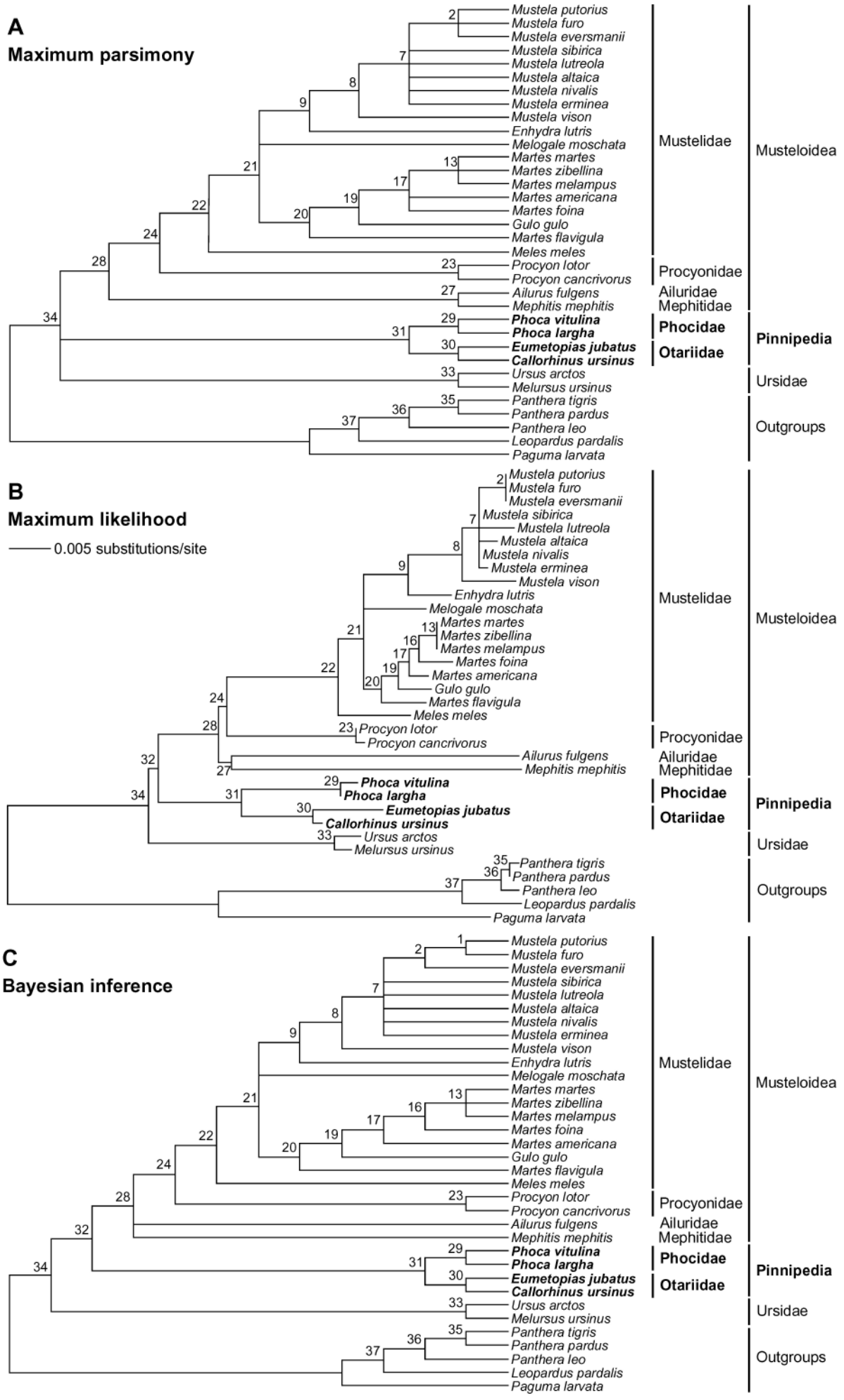
Fig. 1. Phylogenetic position of pinnipeds based on the APOB dataset. **A**, strict consensus tree inferred from maximum-parsimony analysis (for tree statistics, see Table 4). **B**, single most-likely tree inferred from maximum-likelihood analysis (for log-likelihood, substitution model, and model parameters, see Table 5). **C**, 50% majority-rule consensus tree inferred from Bayesian-inference analysis. Numbers at nodes are the reference numbers for the respective clades. Maximum-parsimony and -likelihood bootstrap support and Bayesian-inference posterior-probability values for recovered clades are given in Table 6.

Fig. 2. Phylogenetic position of pinnipeds based on the IRBP dataset. **A**, strict consensus tree inferred from maximum-parsimony analysis (for tree statistics, see Table 4). **B**, single most-likely tree inferred from maximum-likelihood analysis (for log-likelihood, substitution model, and model parameters, see Table 5). **C**, 50% majority-rule consensus tree inferred from Bayesian-inference analysis. Numbers at nodes are the reference numbers for the respective clades. Maximum-parsimony and -likelihood bootstrap support and Bayesian-inference posterior-probability values for recovered clades are given in Table 6.

Fig. 3. Phylogenetic position of pinnipeds based on the RAG1 dataset. **A**, strict consensus tree inferred from maximum-parsimony analysis (for tree statistics, see Table 4). **B**, single most-likely tree inferred from maximum-likelihood analysis (for log-likelihood, substitution model, and model parameters, see Table 5). **C**, 50% majority-rule consensus tree inferred from Bayesian-inference analysis. Numbers at nodes are the reference numbers for the respective clades. Maximum-parsimony and -likelihood bootstrap support and Bayesian-inference posterior-probability values for recovered clades are given in Table 6.

Fig. 4. Phylogenetic position of pinnipeds based on the combined (APOB+IRBP+RAG1) dataset. **A**, strict consensus tree inferred from maximum-parsimony analysis (for tree statistics, see Table 4). **B**, single most-likely tree inferred from maximum-likelihood analysis (for log-likelihood, substitution model, and model parameters, see Table 5). **C**, 50% majority-rule consensus tree inferred from Bayesian-inference analysis. Numbers at nodes are the reference numbers for the respective clades. Maximum-parsimony and -likelihood bootstrap support and Bayesian-inference posterior-probability values for recovered clades are given in Table 6, Bremer-support values in Table 7, and likelihood-support values in Table 8.

Fig. 5. Comparison of the levels of homoplasy among the arctoid nuclear APOB, IRBP, and RAG1 and mitochondrial cytochrome *b* genes, assessed by plotting the pairwise number of observed substitutions against the corresponding pairwise number of inferred substitutions (Hassanin *et al.*, 1998). Solid lines are the linear regressions ($y = ax + b$) delineated with the coefficient of determination (R^2), which are used to evaluate the actual level of homoplasy. Broken lines correspond to a theoretical situation where there is no homoplasy ($y = x$). For the nuclear genes, pairwise comparisons among the sequences from the studied 29 arctoid species were performed. The species and DDBJ/EMBL/GenBank accessions used to calculate the cytochrome *b* scatterplot, and references for these sequences, are as follows: *Ailurus fulgens*, X94919[a]; *Mephitis mephitis*, X94927[a]; *Enhydra lutris*, AB051244[b]; *Gulo gulo*, AB051245[b]; *Martes americana*, AB051234[b]; *Martes flavigula*, AB051235[b]; *Martes foina*, AB051236[b]; *Martes martes*, AB051237[b]; *Martes melampus*, AB051238[b]; *Martes zibellina*, AB012360 (Kurose *et al.*, 1999); *Meles meles*, X94922[a]; *Melogale moschata*, AF498158 (Koepfli and Wayne, 2003); *Mustela altaica*, AB051239[b]; *Mustela erminea*, AB051240[b]; *Mustela eversmanii*, AB026102[c]; *Mustela furo*, AB026103[c]; *Mustela lutreola*, AB026105[c]; *Mustela nivalis*, AB051241[b]; *Mustela putorius*, AB026107[c]; *Mustela sibirica*, AB051242[b]; *Mustela vison*, AF057129 (Koepfli and Wayne, 1998); *Procyon lotor*, X94930[a]; *Eumetopias jubatus*, NC_004030 (Árnason *et al.*, 2002); *Phoca largha*, X82305 (Árnason *et al.*, 1995); *Phoca vitulina*, NC_001325 (Árnason and Johnsson, 1992); *Melursus ursinus*, U23562 (Talbot and Shields, 1996); *Ursus arctos*, NC_003427 (Delisle and Strobeck, 2002). [a] Ledje and Árnason (1996a), [b] Hosoda *et al.* (2000), [c] Kurose *et al.* (2000).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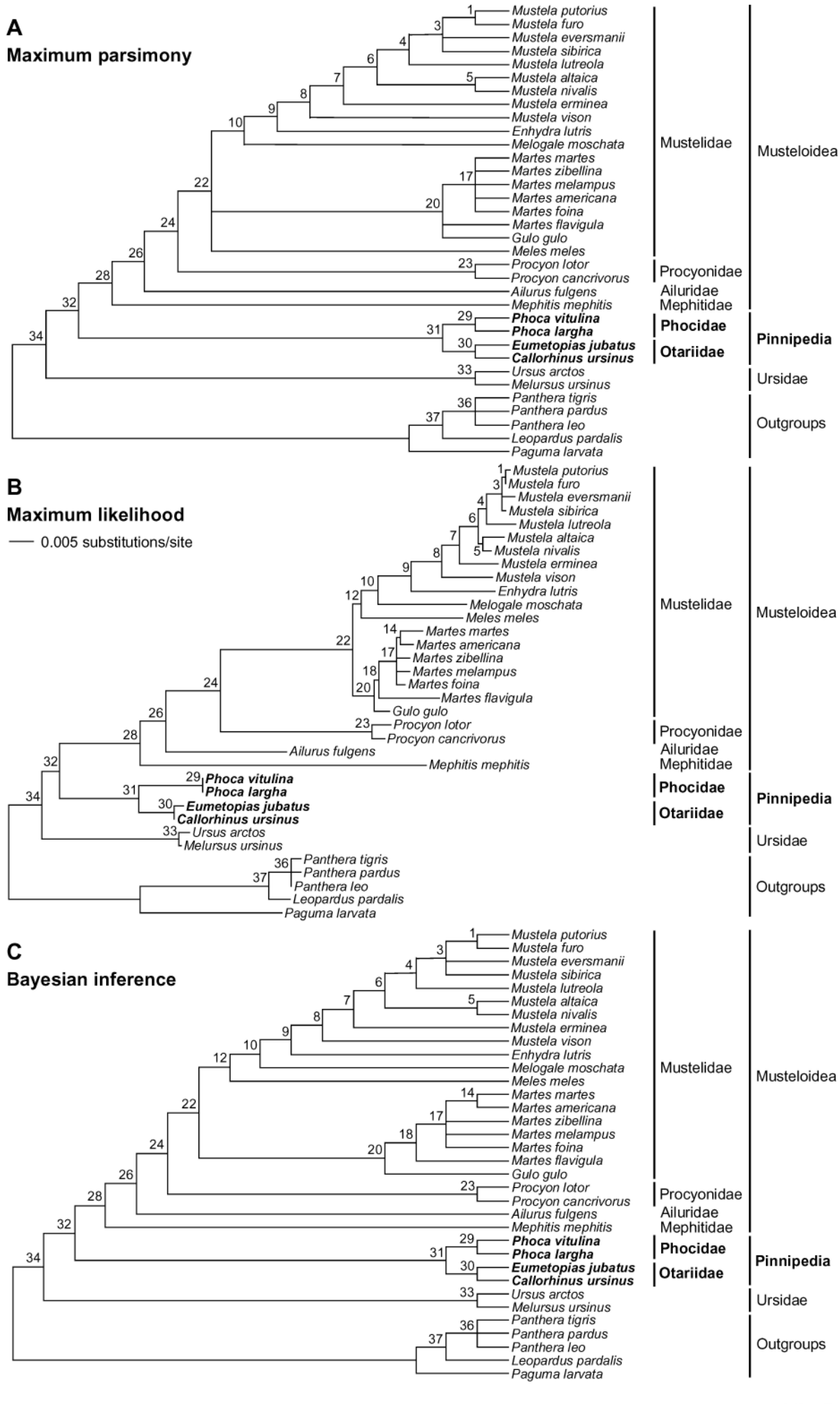
Mustela furo
Mustela eversmanii
Mustela sibirica
Mustela lutreola
Mustela altaica
Mustela nivalis
Mustela erminea
Mustela vison
Enhydra lutris
Melogale moschata
Martes martes
Martes zibellina
Martes melampus
Martes americana
Martes foina
Gulo gulo
Martes flavigula
Meles meles
Procyon lotor
Procyon cancrivorus
Ailurus fulgens
Mephitis mephitis
Phoca vitulina
Phoca largha
Eumetopias jubatus
Callorhinus ursinus
Ursus arctos
Melursus ursinus
Panthera tigris
Panthera pardus
Panthera leo
Leopardus pardalis
Paguma larvata
Mustelidae
Procyonidae
Ailuridae
Mephitidae
Phocidae
Otariidae
Musteloidea
Pinnipedia
Ursidae
Outgroups
B
Maximum likelihood
0.005 substitutions/site
C
Bayesian inference

Fig.1 APOB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Maximum parsimony
Mustela putorius
Mustela furo
Mustela eversmanii
Mustela sibirica
Mustela lutreola
Mustela altaica
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Mustela vison
Enhydra lutris
Melogale moschata
Martes martes
Martes zibellina
Martes melampus
Martes americana
Martes foina
Martes flavigula
Gulo gulo
Meles meles
Procyon lotor
Procyon cancrivorus
Ailurus fulgens
Mephitis mephitis
Phoca vitulina
Phoca largha
Eumetopias jubatus
Callorhinus ursinus
Ursus arctos
Melursus ursinus
Panthera tigris
Panthera pardus
Panthera leo
Leopardus pardalis
Paguma larvata
Mustelidae
Procyonidae
Ailuridae
Mephitidae
Phocidae
Otariidae
Musteloidea
Pinnipedia
Ursidae
Outgroups
B
Maximum likelihood
0.005 substitutions/site
C
Bayesian inference

Fig.2 IRBP

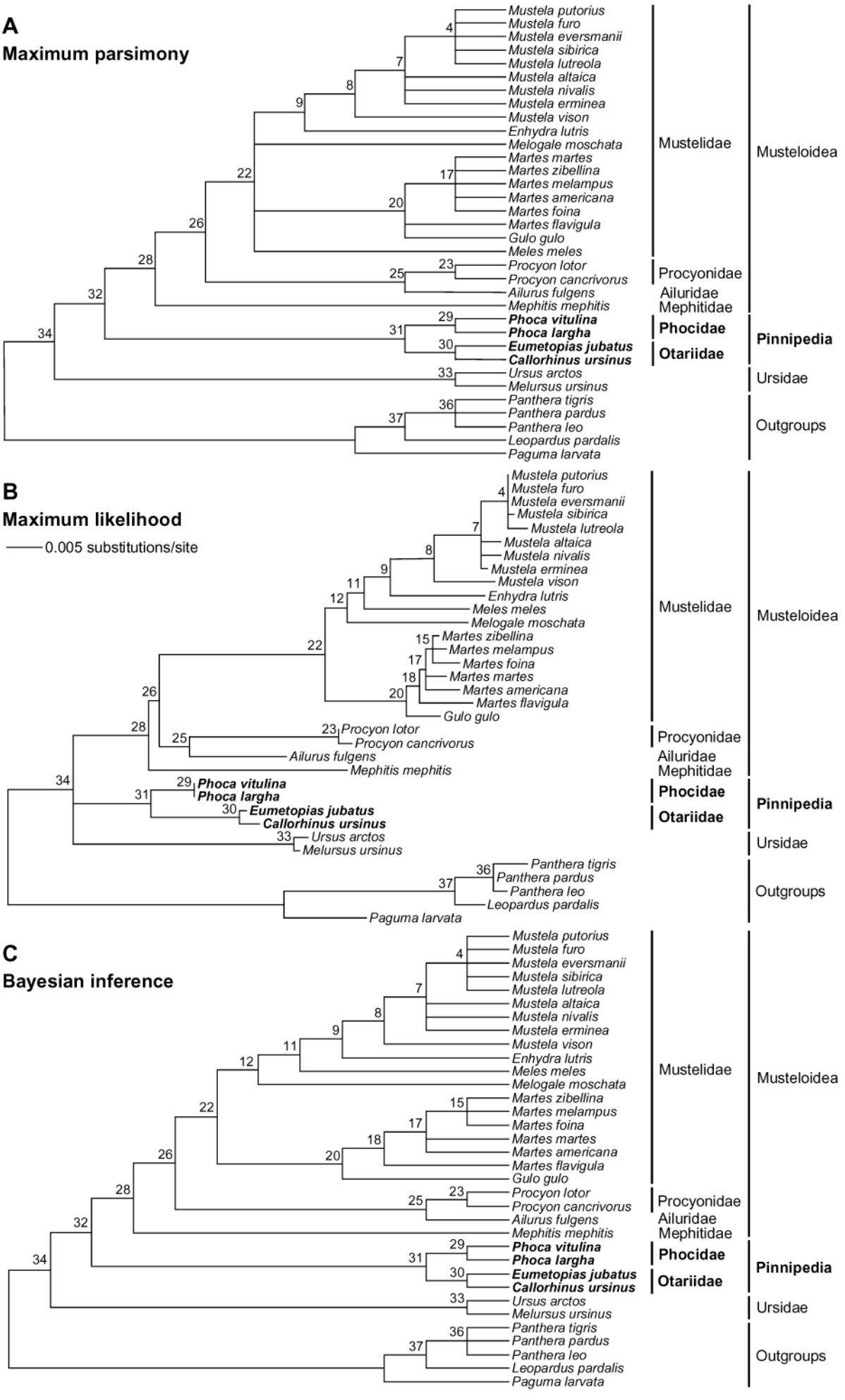
A
Maximum parsimony
Mustela putorius
Mustela furo
Mustela eversmanii
Mustela sibirica
Mustela lutreola
Mustela altaica
Mustela nivalis
Mustela erminea
Mustela vison
Enhydra lutris
Melogale moschata
Martes martes
Martes zibellina
Martes melampus
Martes americana
Martes foina
Martes flavigula
Gulo gulo
Meles meles
Procyon lotor
Procyon cancrivorus
Ailurus fulgens
Mephitis mephitis
Phoca vitulina
Phoca largha
Eumetopias jubatus
Callorhinus ursinus
Ursus arctos
Melursus ursinus
Panthera tigris
Panthera pardus
Panthera leo
Leopardus pardalis
Paguma larvata
Mustelidae
Procyonidae
Ailuridae
Mephitidae
Phocidae
Otariidae
Musteloidea
Pinnipedia
Ursidae
Outgroups
B
Maximum likelihood
0.005 substitutions/site
C
Bayesian inference

Fig.3 RAG1

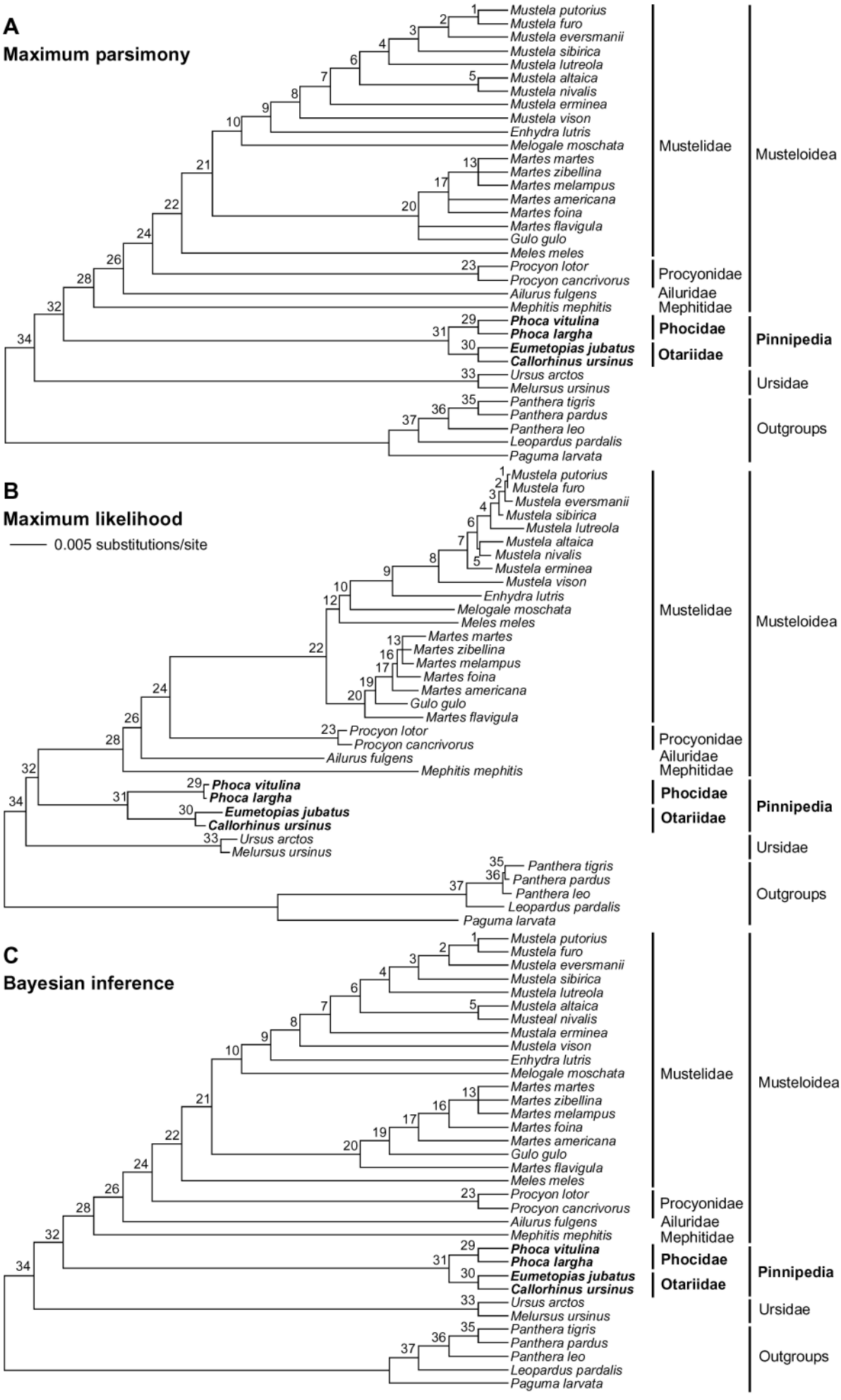

Fig.4 Combined

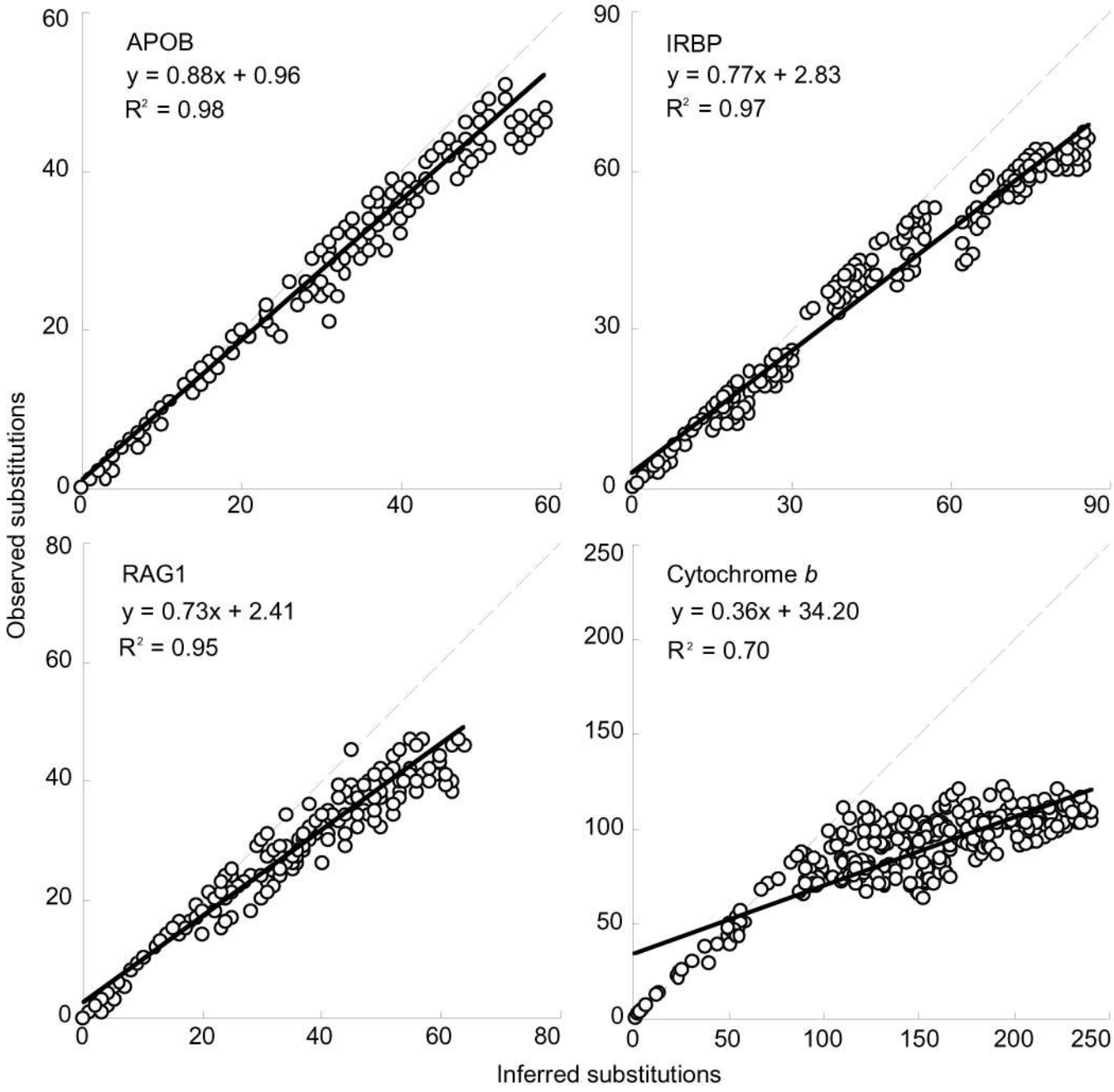
APOB
y = 0.88x + 0.96
R² = 0.98
IRBP
y = 0.77x + 2.83
R² = 0.97
RAG1
y = 0.73x + 2.41
R² = 0.95
Cytochrome b
y = 0.36x + 34.20
R² = 0.70
Observed substitutions
Inferred substitutions

Fig. 5