Mitogenic analyses of caniform relationships

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Received 30 November 2006; revised 1 June 2007; accepted 22 June 2007

Abstract

Extant members of the order Carnivora split into two basal groups, Caniformia (dog-like carnivorans) and Feliformia (cat-like carnivorans). In this study we address phylogenetic relationships within Caniformia applying various methodological approaches to analyses of complete mitochondrial genomes. Pinnipeds are currently well represented with respect to mitogenomic data and here we add seven mt genomes to the non-pinniped caniform collection. The analyses identified a basal caniform divergence between Cynoidea and Arctoidea. Arctoidea split into three primary groups, Ursidae (including the giant panda), Pinnipedia, and a branch, Musteloidea, which encompassed Ailuridae (red panda), Mephitidae (skunks), Procyonidae (raccoons) and Mustelidae (mustelids). The analyses favored a basal arctoid split between Ursidae and a branch containing Pinnipedia and Musteloidea. Within the Musteloidea there was a preference for a basal divergence between Ailuridae and remaining families. Among the latter, the analyses identified a sister group relationship between Mephitidae and a branch that contained Procyonidae and Mustelidae. The mitogenomic distance between the wolf and the dog was shown to be at the same level as that of basal human divergences. The wolf and the dog are commonly considered as separate species in the popular literature. The mitogenomic result is inconsistent with that understanding at the same time as it provides insight into the time of the domestication of the dog relative to basal human mitogenomic divergences.

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Keywords: Molecular phylogeny; Mitogenomics; Carnivora; Cynoidea; Arctoidea; Ursidae; Pinnipedia; Musteloidea; The wolf/dog split and basal human divergences

1. Introduction

Wyss and Flynn (1993) characterized the history of carnivoran classification as having been long and turbulent. When these authors and also Hunt and Tedford (1993) wrote their reviews in the same volume of “Mammal Phylogeny, Placentals”, carnivoran molecular data were still limited with respect to both taxonomic coverage and the amount available for individual taxa. Consequently, the discussion in the two papers was primarily based on classical approaches. The earliest carnivoran molecular study, that of Leone and Wiens (1956), was of a considerably earlier date, however. Among the issues that Leone and Wiens (1956) examined by applying serological approaches was the phylogenetic position of the giant panda, Ailuropoda melanoleuca, which the authors placed as a close relative of the bears among arctoid carnivores by explicitly expressing that “The results of this study definitely indicate that the giant panda belongs in the family Ursidae”. The findings of Leone and Wiens (1956) were corroborated by the likewise non-character state studies of Sarich (1973) and O’Brien et al. (1985) and later also in character state phylogenetic analyses based on complete mitochondrial (mt) genes (Ledje and Arnason, 1996a,b). In a historical perspective it is interesting that the position of the giant panda was essentially never an issue among European phylogeneticists, who traditionally placed it within the Ursidae. Without doubt the German name of the giant panda, Bambusbär, combined with German systematic tradition played a role here.

Early molecular studies of caniform relationships were to a considerable extent concentrated on the pinnipeds. The question whether pinnipeds were mono- or diphylectic...
was for a long time a hotly debated issue as was also their position within the caniforms. Sarich (1969a,b), applying immunological approaches, provided molecular evidence for pinniped monophyly. The results challenged the common morphological view at that time which postulated pinniped diphyly with Phocidae (true seals) originating from the Mustelidae, and Otarioidae (Odobenidae and Otariidae) from the Ursidae (but see Scheffer, 1958, who argued in favor of monophyletic Pinnipedia as a separate order). Pinniped monophyly was further supported in nuclear analyses in the 1980s (de Jong and Goodman, 1982; Arnason and Widegren, 1986; Miyamoto and Goodman, 1986), in the 1990s by analyses of complete mitochondrial (mt) genes (Arnason et al., 1995; Ledje and Arnason, 1996a,b) and more recently in analyses of complete mt genomes (Arnason et al., 2002, 2006; Arnason and Janke, 2002; Davis et al., 2004; Delisle and Strobeck, 2005). The accumulating molecular support for pinniped monophyly gradually led to a revision of the morphological view advocating pinniped diphyly. However, these morphological studies (e.g. Wyss, 1987; Wyss and Flynn, 1993; Berta and Wyss, 1994; Deméré, 1994) generally supported a sister group relationship between Phocidae and Odobenidae (=Phocomorpha) to the exclusion of Otariidae, a hypothesis, that in addition to being incongruent with essentially all molecular findings, had also been shown to be inconsistent with comparative pinniped karyology (Arnason, 1974, 1977).

While basal relationships within recent Ursidae and Pinnipedia can be considered as well established, those within the Musteloidea have been less definite. This has also been the case with respect to the relationship between Ursidae, Pinnipedia and Musteloidea. The immunological work of Sarich (1969a,b) did not allow identification of the closest relative of the pinnipeds among the arctoids. Braunitzer and Hofmann (1987) examined the relationship of the giant and the red panda using hemoglobin data. The authors noted that the position of the Pinnipedia above the bears (Ursinae) in the tree was quite surprising, but did not discuss the topic further. However, the interpretation of the results is complicated by the fact that the giant panda did not group with the Ursinae but rather joined the red panda (Ailurus) on a common branch as the result of the great similarity of the sequences of the two species. The non-character state (DNA-hybridization) study of Arnason and Ledje (1993) showed “that the affinities between the pinnipeds and the mustelids [musteloids] were far greater than those between the pinnipeds and any other group of terrestrial carnivores [carnivorans]”. Although the approach applied by Arnason and Ledje (1993) had drawbacks compared to later character state analyses the findings were probably the first explicit molecular suggestion for a sister group relationship between Pinnipedia and Musteloidea to the exclusion of Ursidae. The recent studies of Delisle and Strobeck (2005), Flynn et al. (2005), Fulton and Strobeck (2006), Sato et al. (2006) and Yu and Zhang (2006) have all favored an arctoid tree with a sister group relationship between Pinnipedia and Musteloidea to the exclusion of Ursidae, even though the support for this relationship was not particularly strong in all instances.

With respect to the Musteloidea the questions of basal relationships have primarily been related to the position of the monotypic Ailuridae (represented by the red panda, Ailurus fulgens) and the relationship between Mephitidae, Mustelidae and Procyonidae. Analyses of complete single mt genes, cyt b (Ledje and Arnason, 1996a) and 12S (Ledje and Arnason, 1996b) and combination of these (Ledje and Arnason, 1996b), did not resolve the position of Ailurus, leaving it in an unresolved polytomy among other basal arctoid splits. Within the remaining Musteloidea the same data sets identified a sister group relationship between Mephitidae (skunks), and a branch encompassing Procyonidae and Mustelidae (sine Mephitidae), thereby making traditional Mustelidae (mustelids and skunks) paraphyletic. The paraphyly of traditional Mustelidae has been corroborated in essentially all subsequent molecular analyses (e.g. Drago and Honeycutt, 1997; and more recently in the studies of Sato et al., 2004, 2006; Delisle and Strobeck, 2005; Flynn et al., 2005; and Fulton and Strobeck, 2006). Unless otherwise stated we will in the following refer to Mustelidae sensu stricto (s.s.), i.e. Mustelidae sine Mephitidae.

As discussed by Flynn et al. (2000) the phylogenetic position of the Ailuridae was for a long time difficult to establish. Analysis of complete mt genomes (Delisle and Strobeck, 2005) placed Ailurus within the Musteloidea as the sister group of the Mephitidae. A position of Ailurus within the Musteloidea was also obtained in the studies of Flynn et al. (2005) based on a combination of mt and nuclear genes, Sato et al. (2006) using three nuclear genes, and by Fulton and Strobeck (2006) in analyses of five nuclear data sets. One of these data sets, IRBP, was common to the three studies. Compared to Delisle and Strobeck (2005) the three studies favored other positions of Ailurus. Thus, Flynn et al. (2005) recovered Ailurus as the sister group of remaining Musteloidea in their Bayesian analyses (the MP results were inconclusive in this respect), while both Sato et al. (2006) and Fulton and Strobeck (2006) favored a basal Musteloidea split between Mephitidae and a branch that contained Ailurus, Procyonidae and Mustelidae. On this branch Procyonidae and Mustelidae joined as sister groups to the exclusion of Ailurus. Also Yu and Zhang (2006) examined various carnivoran molecular relationships. In the absence of mephitid species Ailurus was recovered as sister to Mustelidae/Procyonidae in that study.

The particular aim of the current study was to examine basal mitogenomic relationships within Arctoidea and Musteloidea and to compare the findings with previous studies that have addressed the same divergences. In order to extend the amount of data we have added seven new caniform mt genomes (one cynoid and six arctoid) to the preexisting data set. The arctoid extension includes inter alia the spotted skunk, Spilogale putorius, Ailurus fulgens
and *A. melanoleuca*. The inclusion of *Spilogale* splits the mephitid branch, which was previously represented by a single species. This complementation was considered valuable for stabilizing the position of Mephitidae relative to other Musteloida and for investigating the position of *Ailurus* in the tree. Similarly, the inclusion of *Ailuropoda* splits the ursid branch at deepest possible position, a circumstance that might be of importance for the examination of the relationship between Ursidae, Pinncipedia and Musteloida. In addition to deeper divergences the study also addresses the mitogenomic distance between the wolf and the dog in relation to that between basal human divergences.

The current study has focused on molecular aspects of arctoid relationships. This has been at the expense of a discussion of morphological hypotheses. For an extended account of various morphological issues the reader should consult Bininda-Emonds et al. (1999), Flynn et al. (2005) and Sato et al. (2006) and the comprehensive coverage of references provided in these studies.

### 2. Materials and methods

The 35 mt genomes included in the study are listed in Table 1 together with their accession numbers (when applicable). The accession numbers of the new genomes are shown in bold. The pinniped study of Arnason et al. (2006) included the mt genomes of all species of extant phocids and sea lions, three fur seals and the walrus. In order to avoid unnecessary redundancy only a selection of the pinniped mt genomes was included here. The sequences of the mt genomes of the wolf, the wolverine and the European badger were established using cloned fragments from enriched mtDNA preparations following the procedure described in Arnason et al. (1991). Thus the repetitions in the control regions of these species represent single clones. Remaining genomes were PCR amplified using a selection of conserved primers constructed in our laboratory. The PCR-products were purified by ultra-filtration (Millipore) and sequenced employing an ABI or a LICOR-4000L system. Due to the highly heteroplasmic nature of the repeated parts of the caniform control regions (e.g. Arnason and Johnsson, 1992) the sequences of caniform specimens other than the grey wolf, the wolverine and the European badger represent a majority rule consensus and not a particular clone.

The phylogenetic analyses were carried out on the concatenated sequences of protein-coding genes using the amino acid (aa) and nucleotide (nt) sequences (1st plus 2nd codon positions and 1st, 2nd and 3rd codon positions) of the 12 heavy strand encoded protein-coding genes. The light strand encoded NADH6 gene was not included as it deviates markedly in nt and aa composition from the other protein-coding genes. Sequences were aligned applying Se-Al v 2.0a11 (Rambaut, 1996).

Phylogenetic analyses were performed using the TREE-PUZZLE (Schmidt et al., 2002), PHYLIP (Felsenstein, 1993), MOLPHY (Adachi and Hasegawa, 1996), PAUP* (Swofford, 2002), PHYML (Guindon and Gascuel, 2003)
and MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001) program packages. For aa analyses with MrBayes the mtMAM model of evolution (Cao et al., 1998) was applied under the AIC (Akaike Information Criteria) evaluation (Akaike, 1974) in accordance with Prottest (Abascal et al., 2005). In maximum likelihood using TREE-PUZZLE the mtREV-24 model (Adachi and Hasegawa, 1996) was applied in the absence of mtMAM model in the TREE-PUZZLE package. The GTR model of nt evolution (Lanave et al., 1984) was used for distance and likelihood analyses as suggested by modeltest (Posada and Crandall, 1998). All ML analyses were carried out with and without assuming four classes of gamma distribution (Yang, 1994) and one class of invariable sites.

Divergence times were estimated (aa rate heterogeneity data set) according to Sanderson (2002) using the “r8s” version of the 1.70 program package, applying the non-parametric rate smoothing. The divergence between caniforms and feliforms set at 52 million years ago, MYA, (Flynn and Galiano, 1982) was used as a reference point for calibration. The age of the oldest phocid fossils, ~28 MY (Koretsky and Sanders, 2002), served as an internal arctoid calibration point set at 30 MYA for the divergence between otarioids and phocids (Arnason et al., 2006). Ages of different divergences were also estimated under a Bayesian framework using the program MULTIDIVTIME as implemented in the T3 program package (ftp://abacus.gene.ucl.ac.uk/pub/T3) and applying the same calibration points. 100,000 generations of MCMC (Markov chain Monte Carlo) were sampled after discarding the first 100,000 generations as burnin.

3. Results

3.1. The caniform mitogenomic tree

Fig. 1 shows the best tree identified in maximum likelihood (ML) analysis of the concatenated aa sequences of 12 mt protein-coding genes (3601 aa). Two perissodactyls, the Indian rhino and the donkey, were used as outgroup to root the tree. The choice of outgroup was based on the close ordinal relationship between Perissodactyla and Carnivora as demonstrated by Xu et al. (1996).

The basal carnivore split between Feliformia and Caniformia was conclusively supported, as was also the traditionally recognized basal caniform split between Cynoidea and Arctoidea. The ML aa analyses and ML nt analyses of 1st and 2nd codon positions (1 + 2 CPs) identified a basal arctoid split between Ursidae and a branch containing Pinnipedia and Mustelidae. The support for this topology was not statistically conclusive, however. The three alternative topologies related to this split (see Fig. 2(a)–(c)) were therefore investigated further by applying different approaches and data sets. The results of this extended examination are shown in Table 2. As evident in Table 2 the inclusion of 3rd codon positions led either to reduced support to tree 2(a) or, as in most

Fig. 1. Caniform relationships based on maximum likelihood (ML) analysis of the concatenated sequences (3601 amino acids) of 12 mitochondrial protein-coding genes. The tree was rooted with perissodactyl taxa. Branches receiving 100% ML bootstrap support are marked with *. Within Arctoidea the analyses favored a sister group relationship between Pinnipedia and Musteloidea to the exclusion of Ursidae. The support for this relationship was not conclusive, however (Table 2). Similarly, the Musteloida branch was not conclusively supported. This circumstance is essentially related to the instability of the position of Ailurus (Table 3). Procyonidae (raccoon) was the preferred sister to Mustelidae s.s. (i.e. Mustelidae sive Mephitidae) to the exclusion of Mephitidae, making traditional Mustelidae paraphyletic. Mustelidae s.s. was conclusively supported, but the favored relationships within the group (Table 3) were inconsistent with traditional systematic schemes. Within Ursidae the Malayan sun bear (Helarctos) was sister to Ursus arctos/U. maritimus to the exclusion of U. americanus, making Ursinae paraphyletic. The support for this relationship was not conclusive, however (Table 4).

Fig. 2. The three possible relationships between Ursidae, Pinnipedia and Musteloidea in the rooted Arctoidea tree. The results of maximum likelihood examination of different topologies and data sets are given in Table 2. Topology (a) corresponds to the tree in Fig. 1.

instances, a shift in the favored topology to either topology (b) or (c). The somewhat labile relationship between Ursidae, Pinnipedia and Musteloidea (the basal arctoid node) is
further underlined by the fact that MP and NJ analyses of 1 + 2 + 3 CPs (both GTR and GTR + 4C + I) joined Ailurus and Ursidae on a common branch, i.e. moved Ailurus from its generally obtained position as the sister of remaining Musteloidea.

The position of Ailurus relative to remaining Musteloidea and some other musteloid relationships were analysed in a similar way as the relationships between Ursidae, Pinnipedia and Musteloidea. The seven musteloid trees examined are shown in Fig. 3 (a)–(g) and the results of the examination in Table 3. As evident in Table 3 there was a general support for a sister group relationship between Ailurus and remaining musteloids. The support for this relationship was not conclusive, however, and the grouping of Ailurus and Mephitidae on a common branch as sister to remaining musteloids (tree b) was favored in analyses of 1 + 2 + 3 CPs (both GTR and GTR + 4I + I) and also by MP in one approach. Similarly, tree (c) was favored in one analysis and tree (d) in another.

Both the position of the giant panda within the Ursidae and its distinct position relative to the bears were strongly supported. Ursinae relationships, i.e. the position of Helarctos relative to Ursus were also investigated. The three possible trees are shown in Fig. 4 and the results of the analyses in Table 4. The favored topology (a) joined Ursus

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**Table 2**

ML evaluation of the relative positions of Ursidae, Pinnipedia and Musteloidea

<table>
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<tr>
<th>Tree in Fig. 2</th>
<th>$-\Delta \log L$</th>
<th>S.E.</th>
<th>pSH</th>
<th>$-\Delta \log L$</th>
<th>S.E.</th>
<th>pSH</th>
<th>MrB</th>
<th>MLuni</th>
<th>MLhet</th>
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The three topologies are shown in the simplified trees in Fig. 2. The log $L$ values of the best trees are shown in square brackets. 4I + 1 refers to gamma distribution with four classes of variable sites and one of invariable sites. GTR and mtREV refer to the models of evolution applied. 12 and 123 indicates which codon positions were used. 1leu refers to 1st leucine codon positions (C or T) being treated as Y, and 3r/y that 3rd positions differences were treated as transversions. Empty columns indicate bootstrap support values below 50%. Other relationships are in accord with the tree in Fig. 1.
arctos and *U. maritimus* on a common branch with *Helarctos* as their sister. Thus, there was a basal Ursinae split between *U. americanus* and the branch with the other three species, making genus *Ursus* paraphyletic. The support for this topology was not unequivocal, however, even though monophyletic *Ursus* was in general the least supported alternative (Table 4).

Although there was a general consistency between the trees favored by different analytical approaches and data sets (amino acids or different nt data) the lack of complete congruency demonstrated that the discrepancies among different phylogenetic studies may to some extent be related to the analytical methods applied.

### 3.2. Comparison with previous arctoid molecular studies

The basal caniform split between Cynoidea (represented by a single extant family, Canidae) and Arctoidea (Ursidae, Pinnipedia, Musteloidea) is consistent with recent mitogenomic and nuclear analyses that have addressed this particular relationship using data sets encompassing both canids and the three basal arctoid lineages Ursidae, Pinnipedia and Musteloidea (e.g. Delisle and Strobeck, 2005; Flynn et al., 2005; Fulton and Strobeck, 2006; Sato et al., 2006; Yu and Zhang, 2006). The results concur with a common morphological view of basal caniform relationships (Flynn et al., 1988; Wyss and Flynn, 1993; Flynn and Wesley-Hunt, 2005 and references therein), although it has been argued that this relationship would require parallel evolution of several basicranial characters (Wozencraft, 1989).
As mentioned in the introduction the morphological view of arctoid relationships had postulated pinniped diphyly with sister group relationship between mustelids and phocids, and the corresponding relationship between ursids and otarioids. After the general acceptance of pinniped monophyly the common morphological understanding has been that ursids and pinnipeds share a common ancestry, a notion that also has been supported in some molecular studies (for a comprehensive list of references see Sato et al., 2006). The current mitogenomic findings favoring the sister group relationship between Musteloidea and Pinnipedia are consistent with recent molecular studies (e.g. Delisle and Strobeck, 2005; Flynn et al., 2005; Fulton and Strobeck, 2006; Sato et al., 2006; Yu and Zhang, 2006; see also Braunitzer and Hofmann, 1987, and Arnason and Ledje, 1993). The somewhat inconclusive support for the favored tree (see Table 2) should nevertheless be noted as it may explain why the data sets of single mt genes have in general left unresolved the relationship between Ursidae, Pinnipedia and Musteloidea.

Like the relationship between Ursidae, Pinnipedia and Musteloidea the position of Ailurus among the arctoids had remained unsettled in analyses of single genes (e.g. Ledje and Arnason, 1996a,b). The LINEs hybridizations of Arnason and Ledje (1993) had suggested affinities between Pinnipedia and Ailurus and Procyonidae similar to those between Pinnipedia and Mustelidae but the analyses did not allow delineation of the relationships within Musteloidea itself. Although not explicitly mentioned in the study of Braunitzer and Hofmann (1987) their tree based on hemoglobin data showed a sister group relationship between Pinnipedia and Musteloidea to the exclusion of the bears (Ursinae). The study did not identify monophyletic Ursidae, however, as the giant panda and Ailurus were placed on a common branch that in turn was sister to Procyonidae/Mustelidae. Although karyological relationships may be difficult to monitor it is of interest for the discussion of arctoid relationships that the general cytogenetic similarity between Pinnipedia and Procyonidae has been found to be greater than that between Pinnipedia and other arctoids (Wurster and Benirschke, 1968; Arnason, 1974, 1977). This similarity is also evident in the karyotype of Ailurus (Tian et al., 2002).

The position of Ailurus as sister to remaining musteloids, as favored in the current study, is consistent with the Bayesian results of Flynn et al. (2005) based on a minimum of three nuclear sequences. The problems associated with resolving basal musteloid relationships are evident in Table 3, however. The same difficulties were observed in the maximum parsimony analysis of Flynn et al. (2005), which left these relationships unresolved. Tree (b) in Fig. 3, with a sister group relationship between Ailurus and Mephitidae to the exclusion of remaining musteloids, was favored by Delisle and Strobeck (2005). Although this tree is inferior to the favored topology in most analytical approaches it was the best option in one approach, see Table 3. Tree (c) in Fig. 3 corresponds to the results of Fulton and Strobeck (2006) and Sato et al. (2006). Also this topology was favored in one approach in the current analysis.

The limited resolution in basal parts of the musteloid branch is further underlined by the demonstration that a previously unrecognized topology, Fig. 3 tree (d), has about the same general support as topologies (b) and (c). The studies of Sarich (1973) and O’Brien et al. (1985),

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The topologies of the simplified trees are shown in Fig. 4. The log L values of the best trees are shown in square brackets. Relationships outside Ursidae are as in Fig 1. For abbreviations and analytical details see Table 2.
which corroborated the giant panda results of Leone and Wiens (1956), also included Ailurus and Procyonidae. Due to the unrooted nature of Sarich's (1973) tree he could not express a firm opinion on the relationship between Procyonidae, Ailurus and Ursidae, even though he placed the raccoon as the sister to Ailurus/Ursidea in his Fig. 1. The study of O'Brien et al. (1985) included only ursids and Ailurus and Procyon and could therefore not address basal arctoid or intra-musteloid relationships.

The mitogenomic analyses placed the American badger, *Taxidea taxus*, as the sister to remaining mustelid species. The tree with the two badgers, *T. taxus* and *Meles meles*, joined on the same branch as sister to remaining mustelids, Fig. 3 tree (e) is appreciable worse than the best tree in all analyses (Table 3). The position of *Taxidea* as sister to remaining mustelids is consistent with the recent mitogenomic and nuclear studies (Delisle and Strobeck, 2005; Flynn et al., 2005; Fulton and Strobeck, 2006). The grouping of *Gulo gulo* and *Martes americana*, on a common branch as sister to the branch with *Mustela vison* and *Lontra canadensis*, are consistent with previous and more taxon rich studies that have demonstrated paraphyly of *Martes* (Ledje and Arnason, 1996a,b; Stone and Cook, 2002; Sato et al., 2003, 2004, 2006; Delisle and Strobeck, 2005; Flynn et al., 2005; Fulton and Strobeck, 2006; Yu and Zhang, 2006).

The mitogenomic ursine tree with *Ursus americanus* as sister to a branch containing *Helarctos malayanus* and *U. arctos* and *U. maritimus*, making genus *Ursus* paraphyletic, is consistent with the results of Delisle and Strobeck (2005). The tree, Fig. 4 tree (b), with *U. americanus* and *H. malayanus* on the same branch is the second best topology, while monophyletic *Ursus* was the least probable tree. Yu et al. (2004) examined ursid relationship in a more species rich analysis than those of complete mt genomes. The study was based on complete cytochrome b genes, partial 12S rRNA genes, partial control regions, two mitochondrial tRNA genes and two nuclear sequences, the complete intron 1, \(\approx 1\) kb, of the TTR (transthyretin) gene and the partial exon 1, 1.3 kb, of the IRBP (interphotoreceptor retinoid binding protein) gene. In addition to the four ursine species of the mitogenomic studies the analyses of Yu et al. (2004) included *U. thibetanus*, Asiatic black bear, *Melursus ursinus*, sloth bear, and *Tremarctos ornatus*, spectacled bear. The analysis of mt sequences placed *Tremarctos* as sister to the remaining taxa. Among the latter *Melursus* was sister to remaining taxa, the relationship of which conformed to the mitogenomic tree. Inconsistent with the mt tree the analysis of the nuclear sequences identified monophyletic *Ursus*, while analysis of the concatenated nuclear and mt sequences placed *U. arctos*/*U. maritimus*, *U. americanus*/*U. thibetanus*, and *H. malayanus* in an unresolved trichotomy. It will be of interest to observe whether a more taxon rich mitogenomic sampling or additional nuclear data will provide a more conclusive answer to ursine relationships.

4. Molecular estimates of arctoid divergences

The molecular estimates of divergence times within the current data set are shown in Fig. 5. The estimates are based on the aa data set and the phylogeny shown in Fig. 1. Arnason et al. (2006) provided molecular estimates of pinniped divergences. The same general approach was applied in the current study. A comparison between the two studies shows some differences in the pinniped datings. Although the differences do not fall outside confidence limits the finding draws attention to the potential effects of taxon sampling on the outcome of molecular dating estimates.

The mitogenomic aa estimates placed the split between Cynoidea and Arctoidea at \(\approx 44\) MYA, that between Ursidae and Musteloidea/Pinnipedia at \(\approx 40\) MYA, and that between Musteloidea and Pinnipedia at \(\approx 38\) MYA. These estimates are the automatic function of the age allocated to the two calibration points applied, the divergence between Feliformia and Caniformia set at 52 MYA (F/C-52), and that between Phocidae and Otarioidae set at 30 MYA (P/O-30). The age of F/C-52 is consistent with

![Fig. 5. Arctoid divergence times as estimated from the tree shown in Fig. 1. The calibration points used were a feliform/caniform split set at 52 MYA (Flynn and Galiano, 1982) and a phocid/otarioid split set at 30 MYA (Koretsky and Sanders, 2002; Arnason et al., 2006).](image-url)
the conclusions of Flynn and Galiano (1982) and that of P/O-30 with the fossil age (≈28 MY) of the Oligocene seal (Koretsky and Sanders, 2002). As discussed by Wesley-Hunt and Flynn (2005) the minimum fossil-based age of the divergence between Feliformia and Caniformia is 43 MY, a dating that rests upon the position of the extinct Viverravidae outside rather than within the Feliformia. A F/C calibration point placed more recently than 52 MY would to some degree compress the mitogenomic estimates of caniform divergences. However, an extended treatment of this particular topic must await a more extensive feliform taxon sampling.

Sato et al. (2003) provided estimates of various mustelid divergences applying as a calibration point the split between Procyonidae and Mustelidae set at 28.5 MYA. This calibration point rests upon the age, ≈28 MY, of the oldest procyonid fossil, Pseudobassaris, described (Wolsan, 1993; Wolsan and Lange-Badré, 1996). It is of interest that the two calibration points included in the current study placed the Procyonidae/Mustelidae split at the paleontological age of Pseudobassaris.

The general depth of the divergences shown in Fig. 5 may look unexpected. This is, however, the direct effect of the current sampling. Thus, in the pinniped study of Arnason et al. (2006), which included all extant phocids, several divergences were dated to the Pliocene. In comparison the current study included only two such divergences, those between the brown and polar bears and the wolf and the dog. The molecular estimate placed the divergence between the two bears at ≈2.7 MYA. Although there might not be a causal connection it is noteworthy that this dating coincides with the geologically determined age of the marked expansion of large ice-sheets in the Northern Hemisphere 2.74 MYA (Jansen et al., 2000).

4.1. The wolf/dog split in relation to basal human divergences

Despite the fact that they interbreed freely, the wolf and the dog are commonly referred to as two species in the popular and semi-scientific literature. The species distinction in systematic nomenclature between Canis lupus and Canis familiaris goes back to Linnaeus (e.g. Linnaeus, 1758). The molecular difference between the wolf and the dog amounted to ≈0.6% in a comparison of complete mt genomes. The difference is the same as that between the original non-chimeric Caucasian mt sequence (Arnason et al., 1996a) and the two newly sequenced African sequences (Kung and Mbuti) included. The Caucasian sequence and the two African sequences represent the deepest mt split among recent humans. They are therefore of interest to the examination of the split between the wolf and the dog as this split may have coincided with the domestication of the latter.

The basal human mt split (“mitochondrial Eve”) has traditionally been placed at ≈170,000 YA (Vigilant et al., 1991), a notion supported in analyses of complete mt genomes (Ingman et al., 2000). In both these studies the time of the basal human split was calculated by applying as a calibration point the divergence between Pan and Homo set at 5 MYA (Sarich and Wilson, 1967). It has been argued, however, that the Pan/Homo split took place much earlier (Arnason et al., 1996b, 1998, 2000) and the 5 MYA dating has subsequently been paleontologically invalidated by the age of the “Millennium man”, Orrorin tugenensis (Senut et al., 2001). A conservative paleontological and molecular estimate of the Pan/Homo divergence may place it at ≈8 MYA and consequently the age of the basal human mt diversification at ≈270,000 YA. This could also be the approximate time of the wolf/dog split.

5. Conclusions

The current study has corroborated the gradually established molecular understanding (Arnason and Ledje, 1993; Delisle and Strobeck, 2005; Flynn et al., 2005; Fulton and Strobeck, 2006; Sato et al., 2006; Yu and Zhang, 2006) of an arctoid sister group relationship between Musteloidea and Pinnipedia to the exclusion of Ursidae. The molecular results are inconsistent with the great majority of morphological studies of this particular relationship, which have rather advocated a sister group relationship between Ursidae and Pinnipedia to the exclusion of Musteloidea. However, although not formally published, Kohno (1993) suggested a sister group relationship between Pinnipedia and Musteloidea and the same relationship was recorded by Wolsan (1993), Bininda-Emonds and Russell (1996) and Bininda-Emonds et al. (1999).

Procyonidae and Mustelidae (sine Mephitidae) joined on a common branch within the Musteloidea, with Mephitidae as the sister of Procyonidae/Mustelidae. Also this relationship, initially suggested by Ledje and Arnason, 1996a,b, is inconsistent with the traditional morphological view of mustelid relationships (e.g. Wozenacraft, 1989; Bryant et al., 1993; Wyss and Flynn, 1993; Baskin, 1998; Wolsan, 1999). The current study favored Ailurus as sister to (Mephitidae, (Procyonidae, Mustelidae)). The position of Ailurus was somewhat instable, however, probably as the result of the Ailuridae branch being represented by a single taxon. The position of Ailurus is consistent with that obtained by Flynn et al. (2005) in their analyses of combined mt and nuclear data. The composite tree compiled by Bininda-Emonds et al. (1999) had Ailurus as sister to Procyonidae and Mustelidae (actually Mephitidae and Mustelidae that were joined on a common branch). Bininda-Emonds et al. (1999) cite Braunitzer and Hofmann (1987) for this position of Ailurus. This phylogenetic weight of this conclusion is problematic, however, because Braunitzer and Hofmann (1987) did not recover Ailurus as the sole sister to Mustelidae/Procyonidae, but rather a branch that contained both Ailurus and the giant panda. Considering the mitogenomic distance between Ailurus and the giant panda the hemoglobin similarity between the two species reported by Braunitzer and Hofmann (1987) is surprising.

The pinniped findings are consistent with what can now be considered as the common molecular view of pinniped
relationships, i.e. a basal split between Phocidae and Otarioidae (Odobenidae + Otariidae) (e.g. Arnason et al., 2006). Arnason et al. (2006) in their study argued in favor of pinniped origin in N. America. Provided that hypothesis is correct the temporal closeness of basal arctoid diversifications suggests that the basal arctoid diversification also took place on this continent.

The mitogenomic results corroborated the original molecular view of Leone and Wiens (1956) that the giant panda definitely belongs to the Ursidae. The ursine results did not support monophyly of Ursus, but a more extensive mitogenomic sampling of the Ursinae will be needed for establishing the ursine mitogenomic tree.

McKenna and Bell (1997) in the voluminous work “Classification of Mammals” provided a systematic scheme of caniform relationships. The somewhat limited concurrence between this classification and current molecular interpretations of various arctoid relationships is noticeable, however.

The molecular estimates placed the divergence between the brown and polar bears at ≈2.7 MYA, a dating that corresponds to the Pliocene/Pleistocene transition and the extension of extensive ice-sheets in the N. Atlantic. Provided the molecular estimate of the divergence is reasonably accurate it is evident that the ancestors of the polar bear have survived extended periods of climate considerably warmer than that of today.

The similar mt difference between the wolf and the dog and basal human divergences is incompatible with the maintenance of the wolf and the dog as distinct species. This similarity may also suggest that the wolf/dog split occurred at a time close to the basal diversification of recent humans. This could also be the time of the domestication of the dog, provided the wolf/dog mt split is diagnostic for that event. To align the domestication of the dog to a particular human lineage would be pure speculation, however.

Acknowledgments

We express our gratitude to Dr. Mieczyslaw Wolsan for comments on the manuscript and to persons and institutes that provided us with samples (see Table 1). The study was supported by the Swedish Research Council, The Nilsson-Ehle Foundation and the TMR program of the European Commission.

References


