CHAPTER 2

The evolutionary history and molecular systematics of the Musteloidea

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Artist reconstruction of *Martinogale faulli* Wang et al. 2005b, looking at its own fossilized skull (LACM 56230, holotype). Fossils of *Martinogale* represent one of the earliest and most primitive skunks in the New World. Reproduced with permission from Julie Selan.

Introduction

The superfamily Musteloidea (musteloids) is comprised of four families of ecologically diverse species: Mustelidae, (badgers, martens, weasels, and otters), Mephitidae (skunks), Ailuridae (red pandas), and Procyonidae (coatis, olingos, raccoons, ringtails, and the kinkajou). Although musteloids belong to the mammalian order Carnivora, they exhibit a range of diets, from species eating mostly fruits (kinkajou; Brooks and Kays, Chapter 26, this volume) or plant matter (red panda; Hu et al., Chapter 29, this volume), to those that are omnivorous (raccoons and skunks; Zeveloff, Chapter 27 and Hass and Dragoo, and Gompper, Chapters 24 and 25, respectively, this volume) or hypercarnivorous (e.g., weasels; Lambin, and King et al., Chapters 4 and 10, this volume, and wolverine; Copeland et al., Chapter 18, this volume). Musteloid species are also found in an array of habitat types, such as tundras, forests, grasslands, and boreal and tropical forests (Macdonald et al., Chapter 1, this volume). The adaptations that evolved to allow musteloids to exploit different dietary and habitat niches have resulted in a morphologically diverse group with body size ranging from the diminutive least weasel (*Mustela nivalis*), the smallest species of the Carnivora, weighing as little as 20–30 grams, to the 40 kg sea otter (*Enhydra lutris*) and the 2 m-long giant otter (*Pteronura brasiliensis*; Chapter 1, this volume, see also Estes et al., and Groenendijk et al., Chapters 23 and 22, respectively).

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The disparate and diverse physical and ecological traits exhibited by musteloids (Kitchener et al., and Macdonald and Newman, Chapters 3 and 6, this volume) have, however, made it challenging to decipher their evolutionary history based on morphological characters, such as those of the skull and teeth. Different musteloid lineages, both at and below the family level, provide examples of morphological specialization (e.g. red pandas, sea otters), generalization (e.g., raccoons, skunks, and badgers), convergent or parallel evolution (e.g., kinkajous and olingos), and homoplasy that have often blurred the lines of ancestry. Certainly, convergence rather than ancestral synapomorphy is one of the primary difficulties for classifying the musteloids. Once amino acid and DNA sequences began to be used to analyse musteloid phylogenetic histories, it became apparent that although hypotheses based on morphological data had grouped many lineages together in ways consistent with molecular data, many other relationships were overturned. This has led to broad taxonomic revisions and a new understanding of the relationships within and between groups comprising the Musteloidea. Indeed, the story of how molecular data has transformed our understanding of musteloid phylogeny is one filled with surprising revelations that have enriched our appreciation of evolutionary processes; knowledge that has expanded on how DNA sequences have uprooted our interpretation of the classical phylogenetic relationships among placental mammal lineages generally (Madsen et al. 2001; Murphy et al. 2001; Foley et al. 2016). Nevertheless, morphology still has value for deciphering musteloid evolutionary history because it is the only data that can be used to define fossil species, which help establish the links and temporal context between extinct and living taxa (Chapter 6, this volume).

In this chapter, we summarize our current understanding of evolutionary history and phylogenetic relationships among the four families of which the superfamily Musteloidea is comprised. Much current understanding has been informed by studies employing DNA sequences, on which we focus our discussion. We also highlight important fossil discoveries made during the last several decades that have provided important insights into the temporal history and phylogeny of musteloids. We conclude by drawing attention to several remaining areas of phylogenetic uncertainty, which continue to challenge research on this endlessly fascinating group of mammals.

Origins and relationships among the lineages within the Musteloidea

The order Carnivora is defined by the possession of a pair of carnassial teeth made up of the fourth premolar in the upper jaw and first molar in the lower jaw, which act together to shear and shred flesh and other food items during consumption. The Carnivora includes 16 living families and eight extinct families divided into two suborders, the Feliformia (the cat-like forms) and the Caniformia (the dog-like forms), to which the musteloids belong along with the Canidae (wolves and foxes), Ursidae (bears), and the three Pinnipedia families—Odobenidae (walruses), Otariidae (fur seals and sea lions), and Phocidae (true seals). Within the Caniformia, one further division is recognized on the basis of the structure of the auditory bulla (a hollow bony structure on the ventral, posterior portion of the skull that encloses parts of the middle and inner ear), distinguishing the Cynoidea, which includes the Canidae, and the Arctoidea, which includes the Ursidae, the Pinnipedia, and the Musteloidea (Figure 2.1).

Evidence from multiple studies based on mitochondrial and nuclear DNA sequences indicate that the Musteloidea and Pinnipedia are sister groups within the Arctoidea (Flynn and Nedbal 1998; Flynn et al. 2000, 2005; Yu et al. 2004; Fulton and Strobeck 2006; Sato et al. 2006, 2009; Eizirik et al. 2010; Figure 2.1). This relationship is also supported by super-tree analyses of all species within the Carnivora (Nyakatura and Bininda-Emonds 2012). Other molecular systematic studies have, however, identified alternative relationships, with either Musteloidea and Ursidae joined as sister groups to the exclusion of Pinnipedia, or Pinnipedia and Ursidae as sister groups to the exclusion of Musteloidea (Vrana et al. 1994; Delisle and Strobeck 2005; Yu and Zhang 2006; Meredith et al. 2011). These discrepancies in topological relationships among arctoid lineages may in part be due to differences in the number of species included in phylogenetic trees and the number and types of gene sequences employed between different studies (Flynn et al. 2005; Fulton and Strobeck 2006). The branch leading to the arctoid lineages is also short (only about 2 million years in duration; Eizirik et al. 2010), which is expected to result in incongruence among trees derived from unlinked DNA sequences due to incomplete lineage sorting. Indeed, Doronina et al. (2015) provided clear evidence of this phenomenon across arctoids by analysing the pattern of insertion of hundreds of retroposon elements, which are fragments of DNA inserted into the

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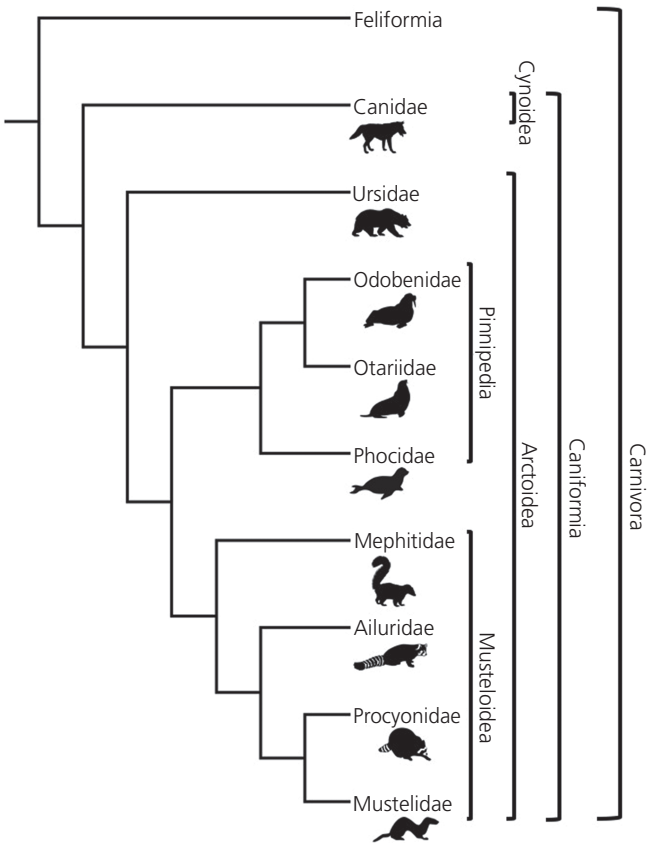


Figure 2.1 Phylogenetic tree depicting the relationships among the four families of the Musteloidea and the position of the Musteloidea within the Caniformia. Other major clades are also indicated. The tree topology is based on the studies of Sato et al. (2009), Eizirik et al. (2010) and Nyakatura and Bininda-Emonds (2012). The phylogenetic tree was generated using Mesquite (Maddison and Maddison 2016).

genome following reverse transcription from an RNA molecule. Retroposons are highly informative with regards to inferring phylogenetic history because their insertion into the genome is largely random, such that the probability of an element being inserted into the same location with the genomes of two or more species is very low. Doronina et al. (2015) scanned the genomes of representative arctoid species (including representatives from all four musteloid families) for retroposed elements. They detected 192 elements that supported the grouping of Pinnipedia + Musteloidea to the exclusion of the Ursidae, although they also found 74 elements that supported a grouping of Musteloidea + Ursidae, and 60 elements that grouped together Pinnipedia + Ursidae. These results expose a conflicting phylogenetic signal among arctoid lineages, as with studies based on DNA sequences, although predominantly a shared common ancestry between Musteloidea and Pinnipedia seems most likely (e.g. Finarelli 2008).

Turning to complementary palaeontological evidence, the earliest true carnivores first appeared during the Late Paleocene/Early Eocene (Fox and Youzwyshyn 1994; Baskin 1998). These carnivores were small, arboreal, viverrid-like (or weasel-like) forms belonging to the extinct Viverravidae and Miacidae (Spaulding and Flynn 2012). During the Late Eocene and into the Oligocene (*c*.40–35 million years ago, Mya) open-land communities were becoming prevalent. Many regions in North America were composed of arid-climate biota, and early into the Miocene there was evidence of wide-spread tropical savannas (Martin 1989; see Chapter 6, this volume). This period was marked by the first occurrence of composite flowers, grasses, grazing ungulates with high-crowned teeth, and rodents. The appearance of the first mustelid-like carnivores was coincident with the increase of rodent diversity (Martin, 1989; Chapter 6, this volume).

Fossil data, based on characters of the basicranium (Baskin 1982; Wang and Tedford 1994), indicates that the caniform families became distinct and diversified during this period of major ecosystem change, 38 to 28.1 Mya (Baskin and Tedford 1996; Wesley-Hunt and

Flynn 2005; Finarelli and Flynn 2006). The extinct species *Mustelavus priscus* from Late Eocene (*c*.38–33.94 Mya) deposits in North America is thought to represent the earliest musteloid record (Scott and Jepson 1936; Baskin and Tedford 1996; Wang et al. 2005a; Sato et al. 2009), although many early taxa that were originally classified as Mustelidae have subsequently been re-assigned as procyonids (Anderson 1989; Baskin 1982). Musteloids make their first appearance in Europe soon thereafter in the early Oligocene (*c*.33 Mya), almost immediately after the Eocene–Oligocene extinction event around 33.5 Mya (also known as the Grande Coupure), which resulted in a major turnover in a large number of mammalian lineages (Baskin and Tedford 1996; Wang et al. 2005a; Chapter 6, this volume). Interestingly, divergence time estimates from molecular clock analyses of nuclear gene data sets are largely concordant with the fossil record, placing the timescale of musteloid diversification during the Late Eocene and Early Oligocene (Sato et al. 2009; Eizirik et al. 2010; Meredith et al. 2011). Numerous other fossil species have been associated with the origins of musteloids or their associated families but uncertainty about the phylogenetic placement of some of these species has precluded a clearer understanding of the early evolutionary history of musteloids. Nonetheless, Finarelli and Flynn (2006) and Finarelli (2008) showed that rather than analysing extant and fossil caniform taxa separately (including those assigned to Musteloidea), combining them together in a total evidence analysis can resolve various phylogenetic discrepancies, likely because ancestral character states can be inferred more accurately. Analyses with similar study designs will likely help further clarify the phylogenetic and temporal links between extinct and modern musteloid lineages in the future.

There has also been controversy concerning the relationships among the four families that constitute the Musteloidea (e.g. Doronina et al. 2015). It is important to emphasize that superfamily Musteloidea originally referred to the grouping of Mustelidae + Procyonidae; where morphological and early molecular studies suggested that these two groups shared a common ancestry. This definition of Musteloidea was subsequently revised once DNA studies revealed that skunks, along with stink badgers (*Mydaus* spp.), both originally placed within the Mustelidae, comprised a distinct family (Mephitidae) highly divergent from both mustelids and procyonids (see below). The definition was expanded again when more comprehensive phylogenetic studies of both mitochondrial and nuclear DNA sequences showed that red pandas (Ailuridae) were also allied to the Musteloidea, and not with the Ursidae (Vrana et al. 1994) or Procyonidae (O'Brien et al. 1985; Slattery and O'Brien 1995). Numerous amino acid and DNA sequence substitutions from the mitochondrial genome, and exon and intron loci of the nuclear genome, strongly support the monophyly of the expanded Musteloidea (Yu et al. 2004; Delisle and Strobeck 2005; Fulton and Strobeck 2006; Sato et al. 2009; Eizirik et al. 2010) (Figure 2.1). Significantly, whereas other caniform families possess a single copy of the *RNASE1* gene, the four musteloid families each have different multiple copies of this gene (two to five copies), suggesting gene expansion of *RNASE1* via gene duplication in the ancestor of musteloids (Liu et al. 2014).

Given that ailurids and mephitids are now recognized as distinct families within the Musteloidea, their phylogenetic relationship to one another and the clade composed of Mustelidae + Procyonidae has proven controversial. Some studies suggest Ailuridae to be the earliest branching musteloid lineage, followed by Mephitidae and Mustelidae + Procyonidae (Flynn et al. 2000, 2005; Árnason et al. 2007; Fulton and Strobeck 2007; Yonezawa et al. 2007; Finarelli 2008). Conversely, other studies have shown Mephitidae to be the earliest branching lineage, followed by Ailuridae and Mustelidae + Procyonidae (Sato et al. 2009; Eizirik et al. 2010). Yet other studies have suggested that Ailuridae and Mephitidae are sister groups (Flynn et al. 2000; Delisle and Strobeck 2005; Árnason et al. 2007; Fulton and Strobeck 2007; Peng et al. 2007; Yonezawa et al. 2007; Yu et al. 2008). It is the second of these phylogenetic hypotheses, however, that generally receives the strongest statistical support in phylogenetic analyses. For example, in an attempt to directly address the inconsistencies among the different phylogenetic hypotheses generated by previous studies, Sato et al. (2009) used ~5,500 nucleotides from five nuclear genes to examine relationships within the Musteloidea, particularly the placement of the red panda, and conducted extensive analyses evaluating all possible alternative topologies among the four musteloid families. They found overwhelming and decisive support for a phylogenetic tree topology in which Mephitidae was the earliest branching lineage and Ailuridae was closely affiliated with Mustelidae + Procyonidae. This topology was later corroborated in a phylogenetic study of all families of the Carnivora based on sampling 7,765 nucleotides collected from 14 nuclear gene segments from 50 representative genera

(Eizirik et al. 2010) as well as in an updated supertree analysis of the Carnivora (Nyakatura and Bininda-Emonds 2012).

We next examine each musteloid family in turn, with reference to historical revisions to taxonomic status, ecological distinctiveness and the bases of current phylogenetic placement.

Family Mustelidae (Fischer 1817)

Depending on the classification applied, the Mustelidae can include as many as 65 living species (see Chapter 1, this volume), making it not only the largest family within the Musteloidea, but also the largest family within the entire order Carnivora. The earliest fossil Mustelidae remains are found in Oligocene (33.9–23 Mya) deposits from Eurasia, including representatives such as the extinct genus *Plesictis* (Wolsan 1993; Tedford et al. 2004; Chapter 6, this volume). Compared to other families of the Carnivora, mustelids display an unparalleled range of behavioural and morphological diversity (see Chapters 3 and 6, this volume). The family includes species with lifestyles adapted for digging (the fossorial badgers; Zhou et al., and Weir et al., Chapters 13 and 19, this volume), climbing in trees (the semi-arboreal martens; Cushman and Wasserman, Chapter 12, this volume), swimming (the amphibious otters; Chapters 22 and 23, this volume), and hunting rodent prey in their burrows (the small and long-bodied weasels and ferrets; Biggins and Eads, Chapter 15, this volume). Moreover, members of the family occupy a wide range of habitats across Africa, Eurasia, and North and South America, including tundra, boreal forests, deserts, grasslands, and tropical forests, making mustelids the most widely distributed musteloid family. Adaptation to these different lifestyles and habitats is reflected in the shape and structure of the skeleton and teeth of different mustelid species, as well as in the composition and coloration of the fur (see Chapter 3, this volume). The most common dental formula among mustelids is 3.1.3.1/3.1.3.2 for the number of incisors, canines, premolars, and molars in the upper and lower jaws, respectively. Because mustelids display such substantial ecomorphological diversity within one family, they provide a striking example of an adaptive radiation; that is, the rapid diversification of species into different ecological niches from a common ancestor (Koepfli et al. 2008a).

Living mustelid species are classified into 22 different genera. Given their varied morphologies, understanding the evolutionary relationships among these genera, and hence the evolutionary history of the family, has proven challenging. Since the early part of the twentieth century, taxonomic arrangements based on morphological criteria have sorted mustelid genera into various subfamilies (Pocock 1921a, b, c; Simpson 1945; McKenna and Bell 1997; Wozencraft 2005), using similarities among species and genera to determine subfamily boundaries. Thus, for example, the renowned evolutionary biologist and palaeontologist, George Gaylord Simpson (1945), identified five subfamilies according to their 'similarity in adaptiveness': Lutrinae (otters), Melinae (badgers), Mellivorinae (honey badger), Mephitinae (skunks), and Mustelinae (martens and weasels). Although this taxonomic scheme was not necessarily intended to reflect phylogeny, it was nonetheless used as a framework for interpreting mustelid biology and evolution for nearly six decades. The first formal appraisal of Simpson's five-subfamily scheme using 30 morphological characters and modern phylogenetic methods found that Lutrinae was monophyletic, as was Mephitinae, which included the stink badgers, *Mydaus* (Bryant et al. 1993). Melinae, Mellivorinae, and Mustelinae did not, however, constitute natural groups, and the resulting phylogeny was therefore largely inconsistent with Simpson's classification. Moreover, the genus *Melogale* (the ferret badgers) was found to be the earliest branching lineage in this phylogeny (Bryant et al. 1993). Combined with the eventual elevation of skunks and stink badgers into their own distinct family (see Family Mephitidae, below), this caused mustelids to be re-classified into two subfamilies, Lutrinae (otters) and Mustelinae (badgers, martens, weasels, and other species) (Wozencraft 2005); despite cladistic analyses of morphological characters clearly showing that Mustelinae was likely not a monophyletic group (Bryant et al. 1993).

As with other musteloid families, when studies on mustelid phylogeny employing DNA sequences began to be published, the resulting molecular phylogenetic trees challenged the pattern of evolutionary relationships among species, genera, and subfamilies inferred from morphology alone, and transformed our understanding of mustelid evolutionary history. The first molecular studies on mustelids tended to focus on relationships within particular groups or subfamilies such as otters (Koepfli and Wayne 1998), martens (Stone and Cook 2002), and weasels (Hosoda et al. 2000). Phylogenetic studies encompassing a larger sample of species from across the Mustelidae then began to appear, based either exclusively on mitochondrial DNA sequences

or incorporating a combination of mitochondrial and nuclear DNA sequences (Koepfli and Wayne 2003; Sato et al. 2003, 2004; Marmi et al. 2004; Yonezawa et al. 2007). Despite these studies often yielding trees with conflicting topologies, likely because of differences in the composition of the sequences and species used for the phylogenetic analyses, several consistent patterns of relationship were nonetheless recovered. For example, weasels (*Mustela*) and the martens and wolverine (*Martes* and *Gulo*) were defined as distinct clades not closely related to each other. Moreover, different genera of badgers (e.g., *Arctonyx*, *Meles*, *Melogale*, and *Taxidea*), were found to be distantly related to one another, and most forming distinct and early branching lineages within the mustelid phylogeny (Koepfli and Wayne 2003; Marmi et al. 2004; Sato et al. 2003, 2004; Yonezawa et al. 2007).

A more complete picture of mustelid phylogeny finally emerged with molecular studies that included a large number of species representing all or nearly all mustelid genera, and more importantly, that employed an extensive set of DNA sequences (thousands or tens of thousands of nucleotides in length) so as to ensure the recovery of well resolved and robust phylogenetic trees (Koepfli et al 2008a; Sato et al. 2012). Together, these more recent studies have confirmed and expanded phylogenetic results based on previous studies that employed fewer DNA sequences and species (Koepfli and Wayne 2003; Sato et al. 2004, 2006, 2009; Fulton and Strobeck 2006; Koepfli et al., 2008b; Wolsan and Sato 2010). A consensus phylogeny synthesizing the findings of these and several other studies is presented in Figure 2.2.

Notably, multiple methods of phylogenetic analysis have all resolved the 22 mustelid genera into eight primary lineages (or clades) defining eight subfamilies: Lutrinae (*Aonyx, Enhydra, Hydrictis, Lontra, Lutrogale, Lutra, Pteronura*), Mustelinae (*Mustela* and *Neovison*), Ictonychinae (*Galictis, Ictonyx, Lyncodon, Poecilogale*, and *Vormela*), Helictidinae (*Melogale*), Guloninae (*Eira, Gulo, Martes*), Melinae (*Arctonyx, Meles*), Mellivorinae (*Mellivora*), and Taxidiinae (*Taxidea*). The most basal, and therefore the earliest, branching lineages within the mustelid family tree consists of the three subfamilies of badgers (Melinae, Mellivorinae, and Taxidiinae). This shows that adaptation for a fossorial lifestyle evolved independently multiple times. The Asian ferret badgers (*Melogale*, Helictidinae) branched off later, and have been resolved as a monotypic lineage that is sister to a clade that includes Lutrinae + Mustelinae + Ictonychinae.

The topology of the Guloninae shows that the tayra (*Eira barbara*), fisher (*Martes pennanti*), and wolverine (*Gulo gulo*) occur as successive ancient splits at the base of this clade, with the wolverine as the sister group to the remaining species in the genus *Martes* and thereby rendering *Martes* paraphyletic. These results concord with those from previous analyses (Hosada et al. 2000; Stone and Cook 2002) and suggest that the fisher should be placed in a separate genus (*Pekania*) and hence recognized as *Pekania pennanti* (Koepfli et al. 2008a; Sato et al. 2012; B. Li et al. 2014; see Powell et al., Chapter 11, this volume). This suggestion accords well with the ancient divergence of this lineage based on recently described fossils (Samuels and Cavin 2013; X. -M. Wang et al. 2012).

The Mustelinae has been re-defined as containing only the true weasels of the genus *Mustela* and the American mink, *Neovison vison;* this is in contrast to the broader taxonomic definition of this subfamily suggested by Simpson (1945) and then Wozencraft (2005) that rendered this subfamily as polyphyletic. One of the most remarkable findings was the clade Ictonychinae, composed of various genera and monotypic lineages of weasel or polecat-like species, which had all originally been allied to various degrees with the true weasels (*Mustela*) and consequently classified within the subfamily Mustelinae (Simpson 1945; Wozencraft 2005). Ictonychinae includes species with distributions spread across three continents: Africa (Saharan striped polecat or Libyan striped weasel, *Ictonyx libyca*, striped polecat or zorilla, *Ictonyx striatus*; African striped weasel, *Poecilogale albinucha*), Eurasia (marbled polecat, *Vormela peregusna*) and South America (grisons, *Galictis cuja, G. vittata*; Patagonian weasel, *Lyncodon patagonicus*). Interestingly, species within this clade are united by possession of aposematic fur coloration (usually distinctive black and white patterns) and enlarged anal scent glands that are used in defensive threat displays (see Buesching and Stankowich, Chapter 5, this volume).

Due to the inclusion of the African striped weasel (*Poecilogale albinucha*), the genus *Ictonyx* is paraphyletic, and consequently Koepfli et al. (2008a) suggest that this latter species be incorporated within *Ictonyx* (although see Sato et al. 2012 for an alternative proposal). In agreement with previous studies (Koepfli and Wayne 1998; Koepfli et al. 2008b), Koepfli et al. (2008a) also split otters (Lutrinae) into three major lineages, one clade containing the Amazonian giant otter (*Pteronura brasiliensis*) as the earliest branching lineage, a clade composed of freshwater otters endemic to

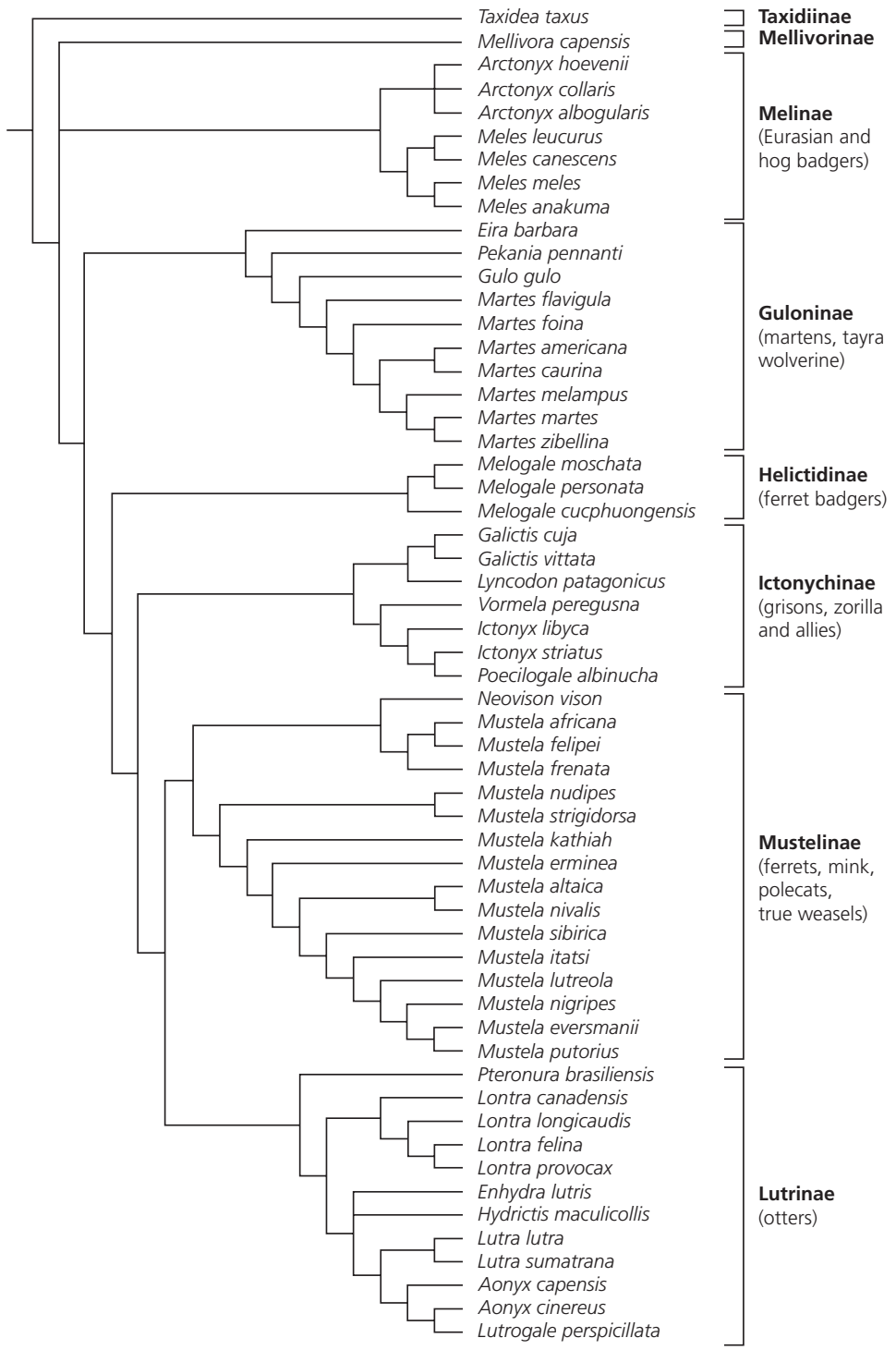


Figure 2.2 Phylogenetic tree showing the pattern of relationships among all extant genera and 57 of the 65 proposed, or recognized, species within the family Mustelidae. Bracketed clades or lineages define the eight recognized subfamilies. The following six species are not depicted in the tree due to absence of formal phylogenetic studies (but see Nyakatura and Bininda-Emonds 2012): *Melogale everetti, Melogale orientalis* (Melinae); *Martes gwatkinsii* (Guloninae); *Mustela lutreolina, Mustela subpalmata* (Mustelinae); and *Aonyx congicus* (Lutrinae). The tree shown is a consensus tree assembled from the following studies: Koepfli and Wayne. (2003); Koepfli et al. (2008a, b); Sato et al. (2009); Harding and Smith (2009); Del Cerro et al. (2010); Nadler et al. (2011); Yu et al. (2011b); Sato et al. (2012); Dawson and Cook (2012); B. Li et al. (2014); and Malyarchuk et al. (2015). The phylogenetic tree was generated in Mesquite (Maddison and Maddison 2016).

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North and South America (genus *Lontra*), and a third clade comprising the sea otter (*Enhydra*) and freshwater otters found in Africa and Eurasia (*Aonyx, Hydrictis, Lutra*, and *Lutrogale*). As *Aonyx* was found to be paraphyletic with respect to *Lutrogale*, revisions to the nomenclature of the latter genus are warranted (see Koepfli et al. 2008b for discussion).

Another interesting feature of mustelid molecular phylogenies, as highlighted by Koepfli et al. (2008a) and Sato et al. (2012), is the short internal branches that characterize relationships among the eight subfamily clades. This suggests that these clades diversified over a relatively short period of evolutionary time. This can confound phylogenetic tree topologies because the phylogenetic signal from different DNA sequences is attenuated and often conflicting across such short branches. Indeed, despite well resolved topologies, several topological conflicts are apparent between the studies by Koepfli et al. (2008a) and Sato et al. (2012). For example, Mustelinae was recovered as the sister group to the Lutrinae by Koepfli et al. (2008a), whereas Sato et al. (2012) consider Ictonychinae and Lutrinae to be joined as sister groups. Similarly, Melinae and Guloninae were linked as sister groups in Koepfli et al. (2008a), albeit with weak statistical support. In contrast, Sato et al. (2012) found that Melinae was either sister to Mellivorinae or constituted a distinct lineage. Clearly, molecular data from additional DNA sequences is needed to help resolve these remaining areas of topological incongruence within the mustelid phylogeny.

These short internal branches do, however, indicate that mustelids underwent a rapid diversification, with six of the eight subfamily clades splitting from one another within a span of about four million years, during the Middle to Late Miocene (Koepfli et al. 2008a; Eizirik et al. 2010; Yu et al. 2011b; Sato et al. 2012). Molecular analyses have also uncovered a second burst of diversification, dated to the Pliocene, during which as many as 20 different lineages representing modern mustelid genera and species originated within a period of less than four million years. Such periods of rapid lineage splitting are one of the hallmarks of an adaptive radiation. For mustelids, these bursts of diversification may have been facilitated by the major palaeo-environmental changes that began during the Middle Miocene and continued through to the Pliocene and Pleistocene, marked by the cooling of global temperatures, the restructuring and redistribution of biomes, and major faunal turnovers across different continents (Koepfli et al. 2008a; Chapter 6, this volume; see also Newman et al., Chapter 21, this volume). These changes may have fostered mustelid diversification by providing opportunities for dispersing, or expanding, into new ecological niches.

The timing of diversification of mustelid lineages implied by molecular dating analyses generally accords well with the known fossil record of the family. For example, the American badger (*Taxidea taxus*) is the earliest branching lineage in the mustelid molecular phylogeny, with an estimated time of divergence in the Early Miocene (23 to 16 Mya) (Koepfli et al. 2008a; Yu et al. 2011b; Sato et al. 2012). Although the earliest remains of *Taxidea* are of Pliocene age, relatives of the American badger from the extinct genera *Pliotaxidea* and *Chamitataxus* have been described from the Late Miocene (Becker and McDonald 1998; Owen 2006). Furthermore, the Miocene age of other fossils such as those linked to the Guloninae (e.g. *Promartes*) and *Plesiogale* is concordant with the diversification of the mustelid subfamilies during the Miocene (Anderson 1989; Wang et al. 2005a; Hughes 2012; see also Rybczynski et al. 2009).

The comprehensive phylogenies recovered by Koepfli et al. (2008a) and Sato et al. (2012) have also been used to reconstruct the biogeographic history of mustelid lineages and species so as to understand the evolution and assembly of mustelid communities across different continents. The most striking finding was that the clades defining the subfamilies Lutrinae, Mustelinae, Ictonychinae, and Guloninae each revealed a deep split that separated a sub-clade composed of New World species (North and/or South America) from a sub-clade largely composed of Old World species (Africa and/or Eurasia). Thus, for example, the grisons (*Galictis*) and Patagonian weasel (*Lyncodon*) from South America were joined together in a sub-clade that was sister to a sub-clade containing the four species of Old World ictonychins. Similarly, within the Mustelinae, two North American endemics, the long-tailed weasel (*Mustela frenata*) and the American mink (*Neovison vison*), along with two South American endemics, the Colombian weasel (*Mustela felipei*) and the Amazon weasel (*Mustela africana*) define a paraphyletic clade that is sister to a clade largely composed of the ferrets, polecats and weasels distributed throughout Eurasia (Harding and Smith 2009). Since *N. vison*, *M. frenata*, *M. africana*, and *M. felipei* were joined together into a clade that radiated exclusively within the Americas apart from the Eurasian and Holarctic species of Mustela, Harding and Smith (2009) suggested that these four species be placed in the genus Vison (Gray 1843),

in recognition of the unique phylogenetic and biogeographic heritage of this lineage.

The analyses of historical biogeographic reconstruction unambiguously identified that many of the modern mustelid lineages originated in and dispersed from Eurasia (Koepfli et al. 2008a), or more specifically, Asia (Sato et al. 2012); a finding consistent with the fact that the earliest fossil specimens that are linked to, or possibly ancestral to, modern mustelid lineages are found in Eurasian deposits (Hunt 1996). These results clearly show that intercontinental dispersal, likely across various land bridges that once connected now separated landmasses, has been a dominant feature of mustelid evolutionary history. Moreover, they also show that much of the mustelid biodiversity present in Africa, North America, and South America is the result of repeated colonizations of lineages that originated outside of these continents, which has important implications for understanding the assembly and structure of mustelid communities where multiple species overlap geographically (Koepfli et al. 2008a).

Our understanding of mustelid evolutionary history has also been significantly enhanced by numerous molecular and/or morphometric studies that have revealed multiple species hidden within lineages, which had long been classified as single species. The most dramatic examples of this come from the two genera that constitute the Melinae (*Arctonyx* and *Meles*; Koepfli et al. 2008a; Sato et al. 2012; see Chapter 13, this volume). Using a combination of mitochondrial and nuclear DNA sequences sampled from 117 (formerly) Eurasian badgers (*Meles meles*) collected across 18 countries encompassing the wide distribution of this species, Del Cerro et al. (2010) showed convincingly that *Meles* divides into four genetically differentiated and allopatrically distributed clades that essentially define four species: *Meles meles* in Europe, *Meles leucurus* in continental Asia, *Meles anakuma* in the Japanese archipelago, and *Meles canescens* in southwestern Asia. Likewise, based on a re-evaluation of museum specimens collected throughout the range of the hog badger (*Arctonyx collaris*), Helgen et al. (2008a) demonstrated that this lineage comprises three species of disparate body size and morphology: the greater hog badger (*Arctonyx collaris*), which represents the world's largest extant badger, the Northern hog badger (*Arctonyx albogularis*) and the much smaller Sumatran hog badger (*Arctonyx hoevenii*). As a result of the studies by Helgen et al. (2008a) and Del Cerro et al. (2010), diversity increased from two recognized species to seven; nearly a four-fold difference! The enigmatic ferret badgers of southeastern Asia also likely provide fertile ground for taxonomic revision. Indeed, molecular and morphometric analyses have been used to recognize a new species of ferret badger from northern Vietnam, the Vietnam ferret badger (*Melogale cucphuongensis*) (Nadler et al. 2011). Molecular dating analysis indicated this species diverged from other ferret badgers about 3.5 Mya. Genetic and morphological evidence also strongly suggest that two species of martens occur in North America: the American marten (*Martes americana*) and the Pacific marten (*Martes caurina*) of the Pacific Northwest (Carr and Hicks 1997; Dawson and Cook 2012). Furthermore, molecular and/or morphological studies have established that mustelid species with disjunct population distributions endemic to the Japanese archipelago merit recognition as distinct species. Along with the Japanese badger, *Meles anakuma* (see above), other examples include the Japanese weasel (*Mustela itatsi*), which had been formerly classified as a subspecies of the Siberian weasel (*Mustela sibirica*) (Abramov 2000a, b; Kurose et al. 2000; Masuda et al. 2012); and the now extinct Japanese otter (*Lutra nippon*), which formerly had been allied with the Eurasian otter (*Lutra lutra*) (Suzuki et al. 1996; Waku et al. 2016). These recognized species join the Japanese marten (*Martes melampus*) as being part of an endemic mustelid fauna, which together demonstrate that the Japanese archipelago has been colonized independently on multiple occasions from ancestral populations on mainland Asia (Sato 2017). Many other mustelid species, encompassing wide distributions with numerous subspecies (e.g. *Lutra lutra* and *Mustela nivalis*), await further scrutiny using molecular and morphological approaches (for example, see Harding and Dragoo 2012). Similarly, the phylogenetic relationships of a number of enigmatic and poorly known or resolved species also require further study, such as the Nilgiri marten (*Martes gwatkinsii*) (Rozhnov 1995) and the Congo clawless otter (*Aonyx congicus*) (Jacques et al. 2009). Only then can we begin to achieve a complete understanding of the extant species diversity contained within the Mustelidae.

Family Mephitidae (Bonaparte 1845)

A skunk by any other name—Historically (at least since the early 1800s), skunks were classified as weasels in the family Mustelidae; however, Bonaparte (1845) recognized that skunks were not like other weasels. He referred to them as Mephitina, although he provided no justification for why they were different. While Gill (1872) is credited with the first use of the subfamily

Mephitinae, it was Rhoads (1894) who first proposed the Mephitidae warranted full family status.

Revisions to the taxonomic status of skunks and relationships to other members of the Mustelidae have been based on the loss of the carnassial notch on the upper fourth premolar, the loss of the upper second molar, and enlargement of anal glands (Bryant et al. 1993; Martin 1989; Qiu and Schmidt-Kittler 1982). Skunks have taken this enlargement to an extreme, with papillae or nipples associated with their anal glands (see Chapters 3 and 5, this volume), rather than a simple duct as seen in mustelids, indicating that the evolution of this character (nipples) is uniquely derived.

Based on genetic data, Dragoo and Honeycutt (1997) along with Flynn et al. (2000) concluded that skunks are not mustelids. This position was corroborated by Eizirik et al. (2010) who found that skunks were at the base of a group of carnivores called the Mustelida which originated between 40–32 Mya and consists of four families: Mustelidae, Mephitidae, Ailuridae, and Procyonidae (as applied in this volume). Thus these earliest 'mustelids' may actually have been more closely related to modern skunks than modern weasels. In recognition of the basal group status of skunks, strictly the term Mephitida should supersede Mustelida for this group of carnivores (see Wolsan 1993; Eizirik et al. 2010). [Note: Eizirik et al. (2010) apply Mustelida to the more inclusive group of Mephitidae + Ailuridae + Mustelidae + Procyonidae, applying Musteloidea to just Mustelidae + Procyonidae, although most authorities tend to apply Musteloidea to the broader taxonomic superfamily, as followed in this volume]. A consensus phylogeny summarizing our current understanding of the relationships within mephitids is shown in Figure 2.3.

The relationship of skunk species within the Mephitidae have also been controversial, although the enlargement of the mastoid sinus in the basicranium suggests the family is monophyletic (Schmidt-Kittler 1981; Wolsan 1999; Wang et al. 2005b). The close relationship of the North and South American skunks is well supported (Anderson 1989; Dragoo 1993; Dragoo et al. 1993; Eizirik et al. 2010; X. Wang et al. 2014), with three living genera recognized: *Mephitis, Spilogale*, and *Conepatus*. Other taxa that have been included with skunks at various junctures include two Old World genera, *Ictonyx* (African zorilla) and *Mydaus* (Asian stink badgers).

The African zorilla has a colour pattern that converges with that of the North American skunks, and

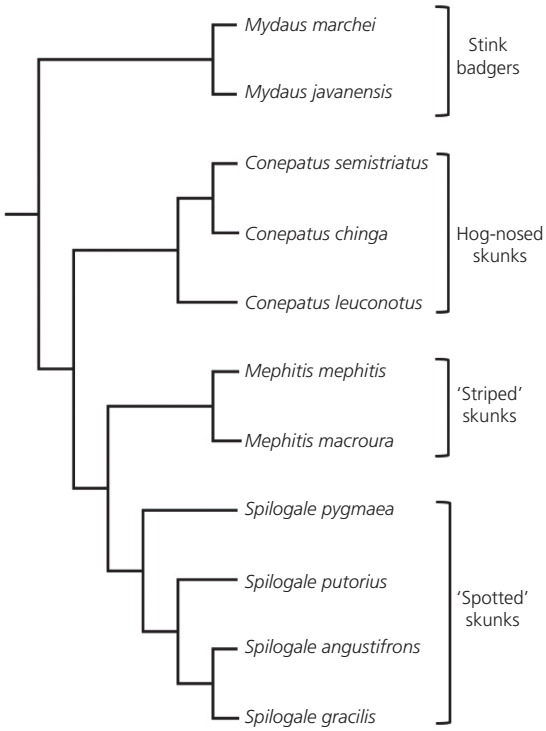


Figure 2.3 Consensus phylogenetic tree showing the relationships among all the extant species of the Mephitidae. The relationships among genera and species are based on a synthesis of the following molecular studies: Dragoo et al. (1993); Dragoo and Honeycutt (1997); Dragoo et al. (2003); Eizirik et al. (2010); Ferguson et al. (2017) and Schiaffini et al. (2013b). The phylogenetic tree was generated in Mesquite (Maddison and Maddison 2016).

is very similar in appearance to the spotted skunks. Many early naturalists confused the two genera (Nowak 1999) and Coues (1877) thought the African Zorillinae to be the Old World representative nearest to the skunks—indeed the zorilla can spray anal gland defences in a very similar way to skunks (see Chapter 5, this volume). O'Brien et al. (1989), in their protein electrophoresis study of black-footed ferrets and other weasels in the genus *Mustela*, included the zorilla with the skunks, although it has subsequently been established as a member of the Mustelidae (see Family Mustelidae above).

Prior to the application of molecular genetic techniques, a sister group relationship was inferred between the skunks and the badgers (Petter 1971; Pocock 1921c; Simpson 1945). This was based on similar cranial characters shared by stink badgers and the skunks.

Indeed, Gray (1869) listed skunks as a distinct tribe in the 'badger family', Melinidae. Radinsky (1973) argued that the fossil record provided support for the skunks and stink badgers being a natural group, with fossil skunks (dating back to the Miocene and Pliocene) occurring in the Old World. Based on inner ear structure (Schmidt-Kittler 1981) and on cranial, post-cranial, and soft anatomy (Bryant et al. 1993), morphological traits have also resolved upon a close relationship between stink badgers and skunks. Newer phylogenetic tree evidence has, however, refuted this traditional placement of stink badgers within the badger subfamily Melinae (Koepfli et al. 2008a), where the traditionally-defined Melinae has become increasingly recognized as polyphyletic (see Family Mustelidae above). Indeed, the application of molecular genetic techniques has incrementally resolved the relationship of the mephitids within the Musteloidea. For instance, early chromosomal data studied by Wurster and Benirschke (1968) suggested that skunks are apomorphic (a novel evolutionary trait unique to a particular species and all its descendants) relative to other mustelids. Subsequently, Perelman et al. (2008) supported these findings, using a multi-directional chromosome painting analysis.

The independent family status of the Mephitidae within the Musteloidea was strongly supported by Dragoo (1993), examining mitochondrial DNA sequences from genera of North American skunks and other carnivores. Ledje and Árnason (1996a, b) drew similar conclusions, as did a more complete study of the Mephitidae by Dragoo and Honeycutt (1997), which included members of all three American (North, Central, and South) genera, in addition to a Sunda stink badger or teledu (*Mydaus javanensis*) and the African zorilla.

With regard to the fossil record, Wayne et al. (1989) and Eizirik et al. (2010) suggest that the origin of the skunk lineage may have occurred in the Eocene approximately 40 Mya (about 30 million years before the appearance of the first recognized skunk fossil), which is prior to the musteloid (*sensu stricto*) stem group that gave rise to Procyonidae and Mustelidae. Interestingly, from craniometry, Radinsky (1973) proposed that the brains of many of the earliest known mustelids, dating back 25–20 Mya, likely had various features more advanced than those of modern skunks and stink badgers.

Two of the earliest known proto-skunks are *Promephitis pristinidens*, which dates back to around 11 Mya (Wang and Qiu 2004) and *Palaeomephitis steinheimensis*, from fossils found at Steinheim am Albuch in southern Germany, dating from the mid-Miocene about 13.5–11 Mya (Wolsan 1999). These two genera may share a lineage with modern stink badgers (*Mydaus*); although the well-preserved teeth of the *Palaeomephitis* specimen, with multiple rootlets on P4 and m1 and distinctive crown morphology, cast doubt on its strict affinity to the Mephitidae, and further fossil evidence is needed to substantiate this taxonomic affiliation (Wang et al. 2005b).

The arrival of skunks in the New World was likely a result of a single invasion across the Bering Land Bridge, with one of the oldest North American skunks, *Martinogale faulli*, found in California, dating back to about 9 Mya (Wang et al. 2005b). This species transitioned into ancestral to the genus *Buisnictis*, which gave rise to the radiation of extant skunks, as well as several extinct genera (X. Wang et al. 2014). One of the oldest fossil specimens of *Conepatus* (*C. sanmiguelensis*), found in Central Mexico, has been dated about 3 Mya, providing a strong indication that hog-nosed skunks were among the first carnivore species to cross into South America during the Great American Biotic Interchange (X. Wang and Carranza-Castaneda 2008).

Four genera and 11 species of extant skunks are currently recognized (Dragoo 2009a; Dragoo and Honeycutt 1997; Dragoo et al. 2003; Schiaffini et al. 2013b; Wozencraft 2005): *Mephitis*, striped and hooded skunks; *Spilogale,* eastern, western, southern, and pygmy spotted skunks; *Conepatus,* American or white-backed, striped or Amazonian, and Molina's hog-nosed skunks; and *Mydaus*, the Palawan and Sunda or Indonesian stink badgers (see Chapter 1, this volume).

Family Ailuridae (Gray 1843)

While, in actuality, the red panda has a relatively straightforward evolutionary history, understanding of that history has been more turbulent, with taxonomists having red pandas swing from branch to branch on the phylogenetic tree. Although it was Hardwicke who first described and named the red panda (as 'Wah') to the western world in a presentation to the Zoological Society of London in 1821, he was unable to publish his work until 1827 (Hardwicke 1827); consequently the Society (with the blessing of Hardwicke) deferred to an earlier published description by Cuvier (1825), crediting him with naming this species. While Hardwicke noted characteristics that were common among red pandas and procyonids, Cuvier (1825) coined the genus *Ailurus* because, due to a reduced number of molars

and a shortened rostrum, he thought red pandas (*Ailurus fulgens*) most resembled cats, and considered them the link between bears and civets. Hardwicke (1827), however, pointed out characteristics that were common among red pandas and procyonids; noting that at the time procyonids were generally classified within the Ursidae (e.g. Gray 1864; see below).

Over time, red pandas were increasingly recognized to belong to their own subfamily or family, based on either shared dental characteristics (Turner 1848) or uniquely derived characters such as the auditory bulla (Flower 1869; Pocock 1921a). Indeed, Flower (1870) took issue with Cuvier's name for the red panda:

"M. Cuvier uses the word 'Panda' as the trivial name, and proposes the generic term Ailurus 'à cause de sa ressemblance extérieure avec le Chat.' This was not a very happy choice, as in all structural characters indicative of true affinity it is almost as widely removed from the true Cats as any member of the group of terrestrial Carnivora."

When it was first suggested that the giant panda (*Ailuropoda melanoleuca;* David 1869) should be classified with the Ursidae, this caused more confusion for the placement of the red panda, leading authors to align the two as somewhat closely related, based on feeding behaviour, dentition, skull characteristics, genitalia, and opposable 'thumbs' (radial sesamoid bone of the hand; see Chapter 3, this volume).

More recently, phylogenetic analyses have seen the red panda classified as a procyonid (Dragoo and Honeycutt 1997; Slattery and O'Brien 1995; Wang 1997), or a bear (Wozencraft 1989; Wyss and Flynn 1993), and positioned variously at the base of the radiation of bears and pinnipeds (Vrana et al. 1994), at the base of a clade comprised of ursids and procyonids (Zhang and Ryder 1993), as sister to the canids (Agnarsson et al. 2010), and as a separate family of uncertain relation (Ledje and Árnason 1996a, b); but most reliably as representing a unique family, the Ailuridae, within the Musteloidea (Eizirik et al. 2010; Flynn et al. 2000), as depicted in Figure 2.1. Whole genome sequencing of the red panda provides support for this position of the Ailuridae (Hu et al. 2017).

Although the Ailuridae is represented by just a single extant species, several taxa occur in the fossil record, and thus family members were more numerous and diverse in the past. For example, *Simocyon* had been a difficult taxon to place within the arctoid carnivores, due to primitive molar characteristics mixed with derived premolar characteristics. Like the red panda, it had vacillated between associations with other arctoid taxa. Additional fossil data has, however, revealed that *Simocyon* was actually most closely related to the red panda, based on cranial features (Wang 1997), and on its radial sesamoid bone or false thumb (Salesa et al. 2006). *Simocyon* was more carnivorous than *A. fulgens* and likely developed its extra 'thumb' to enable it to climb and evade predators (Salesa et al. 2008). Other fossil ailurids include *Pristinailurus* from the Late Miocene or Early Pliocene of eastern North America (Wallace and Wang 2004), and *Parailurus*, which had a Holarctic distribution and very similar tooth morphology to the extant red panda (Tedford and Gustafson 1977). Given the primitive state of many characteristics of this family, it is possible that many more fossil forms may have existed early during the evolutionary history of this group.

Family Procyonidae (Gray 1825)

Linnaeus first classified the raccoon as a bear (*Ursus lotor*, Linnaeus 1758), and raccoons were give the generic name, *Procyon* by Storr (1780). Subsequently, they acquired distinct 'tribe' status within the Ursidae (Gray 1825). It was Turner (1848) who elevated this group to subfamily status, including the genera *Procyon, Nasua* (coatis), *Cercoleptes* (= *Potos*; kinkajou); but again still within the Ursidae. Turner's (1848) classification was based on whether an alisphenoid canal (a channel carrying the carotid artery through the sphenoid skull bone) was present, and on the number of true molars. Two years later Bonaparte (1850) recognized the Procyonidae as a distinct separate family. Mivart (1885) further supported the classification by examining the shape, size, and shear of molars and carnassials.

Turner (1848), and later Flower (1869), were convinced that the ringtail (*Bassariscus*) was also a procyonid; it previously being described as a taxon between genets and coatis (Lichtenstein 1830) and as a viverrid Gray (1843). Turner proposed that teeth alone were not sufficient to address taxonomic relationships and therefore included characteristics of the basicranial region of the skull. However, Gill (1872) classified *Bassariscus* as a separate family as did Hollister (1915) who argued that while there were several characteristics that allied them with the Procyonidae, they were different enough in dental characteristics to warrant being in a separate family. McGrew (1938) suggested that *Bassariscus* had changed little since the early Miocene and was a living fossil, but still a procyonid.

From these early systematic relationships of procyonids the contemporary status of the Procyonidae as a distinct family emerged (see O'Brien et al. 1985, Goldman et al. 1989; Hashimoto et al. 1993; Slattery and O'Brien 1995). The extant Procyonidae includes 14 extant species, classified into six genera, adapted to terrestrial and arboreal habitats (see Chapter 1, this volume). All species, with the exception of the coatis (*Nasua*) are primarily or strictly nocturnal. The dietary ecology of the family ranges from frugivory (kinkajou, *Potos flavus* and olingos, *Bassaricyon*), to carnivory (ringtails, *Bassariscus*) to omnivory (coatis, *Nasua, Nasuella*, and raccoons, *Procyon*). Consequently, most species possess a hypocarnivorous dentition, with a dental formula of 3.1.4.2/3.1.4.2, hence 40 teeth total; except the kinkajou, with 36 teeth (dental formula = 3.1.3.2/3.1.3.2; see Chapter 3, this volume). While all procyonids can climb somewhat, the kinkajou and olingos are especially well adapted to their arboreal lifestyles (Fabre et al. 2013a, b; Chapter 3, this volume), with the kinkajou being one of the only two species in the order Carnivora to possess a prehensile tail (the other being the binturong, *Arctictis binturong*, a southeast Asian viverrid). The similarity in diets and habits exhibited by kinkajous (*Potos*) and olingos (*Bassariscus*) on the one hand, and among coatis (*Nasua* and mountain coatis, *Nasuella*), raccoons (*Procyon*), and ringtails (*Bassaricyon*) on the other, has resulted in each of these groups sharing numerous cranial, dental, and post-cranial skeletal features. Indeed, phylogenetic analyses, based primarily on dental (Baskin 1982, 2004), cranial, and post-cranial (Decker and Wozencraft 1991) morphological characters sampled from fossil and living genera, have divided the Procyonidae into two primary clades: one consisting of *Bassariscus, Nasua, Nasuella*, and *Procyon*, and the other containing *Bassaricyon* and *Potos* (Baskin 1982, 2004; Decker and Wozencraft 1991). Baskin (1982) provided a phylogenetic analysis of the procyonids based on morphological characters and included several fossil taxa. He found that *Procyon* and *Nasua* were sister taxa, sharing a common ancestor to the exclusion of *Bassaricyon*. Subsequent phylogenetic analyses supported *Procyon* + *Nasua*, but in addition showed that this grouping was followed by *Bassariscus* and then a clade of *Bassaricyon* + *Potos*, with numerous fossil taxa in between (Baskin 1989, 2003, 2004).

Decker and Wozencraft (1991) were concerned about the polarity and independence of characters applied by Baskin and undertook phylogenetic analyses of 69 cranial, 28 dental, 12 post-cranial, and 20 soft morphological characters sampled from the living genera. They concluded that *Procyon*, along with a clade containing *Nasua* and *Nasuella*, were sister taxa, followed by *Bassariscus*, with a further clade containing *Bassaricyon* and *Potos* at the base of the procyonid radiation (Figure 2.4).

Ahrens (2012) further corroborates these clades, using a larger sample of morphological characters (78), including novel characters detected using high resolution X-ray computed tomography (CT scans). This approach supported the grouping of *Potos* + *Bassaricyon* and *Nasua* + *Procyon*, but also found that *Ailurus* formed a clade with *Potos* + *Bassaricyon* and that *Bassariscus* was the earliest branching lineage within the Procyonidae. Subsequently Ahrens (2014) has provided additional support for these relationships, based on 21 landmarks relating to brain shape morphology derived from endocasts of species representing all procyonid genera.

Molecular studies of phylogenetic relationships among the procyonids (Zhang and Ryder 1993; Ledje and Árnason 1996a, b; Dragoo and Honeycutt 1997; Flynn and Nedbal 1998; Flynn et al. 2000, 2005; Yu et al. 2004; Delisle and Strobeck 2005) have tended to revolve around higher relationships between the Procyonidae and other carnivore families, and have included only a limited number of select procyonid taxa. Koepfli et al. (2007) provide one of the most complete molecular analyses of the procyonids, with the exception of *Nasuella* (see also Fulton and Strobeck 2007), which indicated a different pattern of relationships between genera than that found by studies based on morphological characters. Specifically, *Procyon* + *Bassariscus* and *Nasua* + *Bassaricyon* each defined a clade joined together as sister lineages. The kinkajou was found to occupy a basal position as the earliest extant lineage within the procyonid tree. These relationships were robustly supported in both studies by numerous informative nucleotide changes in each of the gene segments used in the analyses as well as by phylogenetically informative insertion and deletion events detected in particular gene segments (Fulton and Strobeck 2007; Koepfli et al. 2007). A molecular study analysing relationships among Carnivora families and employing a data set composed of 14 nuclear gene segments from 51 species, including representatives of most procyonid genera, recovered the same topology as found by Fulton and Strobeck (2007) and Koepfli et al. (2007), further confirming the robustness of these relationships (Eizirik et al. 2010). Significantly, one outcome of mapping morphology based classifications (Decker and Wozencraft 1991; Baskin 2004) onto the molecular topology is that

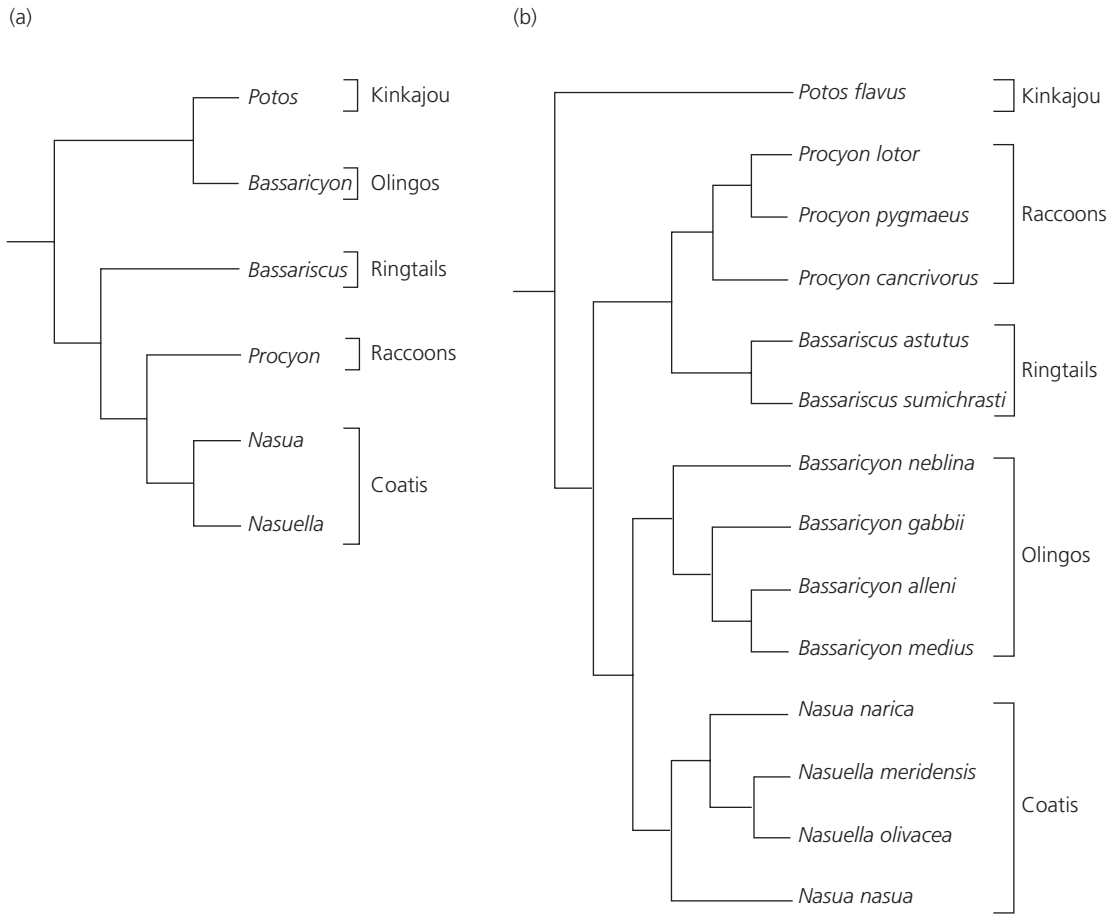


Figure 2.4 (a) Phylogenetic tree showing relationships among the genera of the Procyonidae based on morphological characters (Decker and Wozencraft 1991; Baskin 2004). Also see Ahrens (2012) for an alternative topology based on many more additional characters. (b) Consensus phylogenetic tree displaying the relationships among the 14 recognized species within the Procyonidae. The tree topology is based on a synthesis of the following studies: Fulton and Strobeck (2007); Koepfli et al. (2007); Helgen et al. (2009); Eizirik et al. (2010); and Helgen et al. (2013). The phylogenetic tree was generated using Mesquite (Maddison and Maddison 2016).

many of the morphological similarities among procyonid genera are revealed to result from convergent or parallel evolution, rather than common ancestry (Koepfli et al. 2007).

While the number of extant species included in *Bassariscus* (2), *Nasua* (2), *Potos* (1), and *Procyon* (3) is known, there has long been uncertainty about the precise phylogenetic relationships defining extant species among *Bassaricyon* (olingos) and *Nasuella* (mountain coatis). This taxonomy was clarified only recently by Helgen et al. (2009) in a study integrating molecular and morphometric data along with geographic range modelling. They proposed that *Nasuella* is comprised of two deeply divergent species, the Eastern mountain coati (*Nasuella meridensis*) and the Western mountain coati (*Nasuella olivacea*) (Helgen et al. 2009). These species appear to be geographically isolated along different sections of the Andes Mountains in northern South America. Remarkably, phylogenetic analyses indicated that these two *Nasuella* species were joined as the sister group to the white-nosed coati or coatimundi (*Nasua narica*), rendering the genus *Nasua* (which also includes the South American coati or brown-nosed coati, *Nasua nasua*) non-monophyletic (Helgen et al. 2009). These results support a taxonomic revision in which mountain coatis should be re-classified in the genus *Nasua*.

With regards to *Bassaricyon*, a systematic revision based on anatomical, molecular, morphometric, and

geographic range modelling data has demonstrated that four species of olingos should be recognized: the Eastern lowland olingo (*Bassaricyon alleni*), the Western lowland olingo (*Bassaricyon medius*), the northern olingo (*Bassaricyon gabbii*) and the newly discovered olinguito (*Bassaricyon neblina*) (Helgen et al. 2013). The olinguito is endemic to the cloud forests of the northern Andes Mountains and, according to phylogenetic analysis of mitochondrial and nuclear DNA sequences, is the earliest branching lineage within *Bassaricyon*, diverging approximately 3.5 Mya. The remaining three species diverged less than 2 Mya, with the northern olingo splitting off first, followed by the Eastern lowland and Western lowland olingos joined as sister species (Helgen et al. 2013). Given the timing of the divergence events and reconstruction of historical biogeography, Helgen and colleagues suggested that olingos diversified in response to the formation of the Panamanian Isthmus linking the North and South American continents. The elegant studies on *Nasuella* and *Bassaricyon* highlight our incomplete knowledge about the taxonomy of musteloids in particular and Carnivora in general.

Contemporary procyonids are found only in the Americas, with the exception of the common raccoon (*Procyon lotor*) introduced into Europe and Japan. Fossils, such as *Pseudobassaris* (Pohle 1917; Wolsan 1993; Wolsan and Lange-Badré 1996) dating from the Late Eocene or Oligocene, however, indicate that the first procyonids originated in Western Eurasia. Additional fossils assigned to Procyonidae are also known from European localities, but palaeontologists differ in their opinions over the taxonomic affinities of these (e.g. Wolsan 1993; Baskin 2004); for example, Wolsan (1993) and Wang (1997) proposed that *Ailurus* and *Simocyon* are not members of a monophyletic Procyonidae.

According to the fossil record, procyonids did not arrive in North America until the Early Miocene (Hunt 1996; Baskin 1998, 2004). It is hypothesized, based on dental and cranial characters, that the European taxon *Broiliana*, from the Early Miocene, probably shared a common ancestor with the stem taxon of the North American radiation (Baskin 1998, 2004). Fossils for *Bassariscus* first appear in the Middle Miocene, in the Early Pliocene for *Nasua*, and the Late Miocene for *Procyon* (Baskin 1982, 1998, 2004). *Nasuella, Bassaricyon*, and *Potos* lack a convincing fossil record.

Molecular clock analyses have also revealed several surprising findings regarding the tempo of diversification of procyonid lineages (Koepfli et al. 2007; Eizirik et al. 2010), with substantial discordance from the discovered fossil record. Both studies found that the branch leading to the kinkajou split off first, around the Late Oligocene or Early Miocene, *c*.23 Mya, with other procyonid genera all diversifying during the Miocene (23–5.3 Mya). Subsequent splits between *Bassaricyon, Bassariscus, Nasua*, and *Procyon* occurred from 5.7 to 2.5 Mya (Late Miocene to Late Pliocene) (Koepfli et al. 2007). Furthermore, Koepfli et al. (2007) found that the divergence between coatis and olingos and the divergence between raccoons and ringtails both occurred simultaneously during the Middle Miocene (12–11 Mya), suggesting the sudden alleviation of some as yet unknown major palaeo-environmental barrier or stimulus event on the diversification of procyonids.

These divergence times, inferred from molecular dating, indicate that there must be a large gap in the fossil histories of the kinkajou and olingos, for which only recent fossils are known. Fossil taxa linked to coatis and raccoons are recorded from Late Miocene deposits, but the ages derived from molecular studies still suggest a substantial gap in support from the fossil record. In contrast, the age for the appearance of the ringtail lineage (*Bassariscus*), based on fossils from *c*.15.9 Mya (Middle Miocene) is somewhat older, yet still remarkably consistent with that inferred from molecular studies (Koepfli et al. 2007; Eizirik et al. 2010). Collectively, these analyses suggest that a substantial portion of the procyonid fossil record may yet be uncovered.

These molecular branching dates also have significant bearing on the biogeographic history of procyonids in relation to the Great American Biotic Interchange (Eizirik 2012). Besides being among the first members of the Carnivora to colonize South America via North or Central American ancestors, fossil evidence suggests that procyonids colonized the continent in two independent waves, first during the Late Miocene (11.6–5.3 Mya) and second during the Late Pliocene (3.6–2.9 Mya) (Simpson 1980; Baskin 2004; Woodburne 2010; Forasiepi et al. 2014). The first colonization included the extinct taxa *Cyonasua* and *Chapalmalania* that each diversified into multiple species, found in Late Miocene and Late Pliocene deposits from Argentina and Venezuela, respectively (Forasiepi et al. 2014). This colonization was prior to the closure of the Panamanian Isthmus (circa 3.5 Mya), which established a land-bridge corridor that facilitated the movement of various mammalian lineages between North and South America (Woodburne 2010). The second colonization event occurred after the land bridge was established, and included members of the living procyonid

genera, which are thought to have diversified initially in North and/or Central America (Baskin 2004); Marshall et al. (1984) reports *Nasua* and *Procyon* fossils found from South America in Early to Late Pleistocene deposits. Interestingly, the divergence times inferred from the molecular studies (Koepfli et al. 2007; Eizirik et al. 2010) suggest that living procyonid genera actually diversified around the same time as *Cyonasua* and *Chapalmalania* and well before the closure of the Panamanian land bridge. Indeed, a recent analysis of fossil and molecular data across many taxa suggests that dispersal between North and South America began as early as the Oligocene–Miocene transition, with significant peaks of dispersal taking place around 20 and 6 Mya (Bacon et al. 2015; but see Marko et al. 2015; O'Dea et al. 2016). These results, along with geological data from central Panama (Montes et al. 2012), suggest the presence of a terrestrial dispersal corridor much earlier than has been assumed. Combined with the Miocene divergence times suggested by molecular studies, this raises the important question of whether the lineages representing the extant procyonid genera evolved and diversified first in North America, South America, or some combination of the two.

Conclusions and future directions

In this chapter, we have provided an up-to-date summary of knowledge on the phylogenetic position of the superfamily Musteloidea within the Caniformia, and on the relationships and evolutionary history linking four constituent musteloid families, the Mustelidae, Mephitidae, Ailuridae, and Procyonidae, which together contain approximately 30% of the extant Carnivora species (Sato et al. 2012). One point that clearly stands out is how molecular studies, largely based on multilocus DNA sequences, have unquestionably enriched and transformed our understanding of the evolution of musteloid biodiversity and driven a much more informed re-organization of the musteloid super-family tree. Notably, they have exposed the family-level distinction of the skunks and stink badgers (Mephitidae) from the Mustelidae. Molecular data have also revealed that the red panda is nested within the Musteloidea, and that it represents the sole surviving member of a distinct family of carnivorans (Ailuridae). The tempo and pattern of evolution within the Procyonidae and Mustelidae have also been illuminated, revealing entirely novel relationships. Molecular data, often in combination with morphological data, has been important in accurately delimiting species within certain musteloid genera such as *Conepatus, Bassaricyon, Nasuella*, and *Meles*, leading to some significant re-classifications and new independent species assignments, including an entirely new species, the olinguito.

Despite the abundant advances made during the last several decades, a number of controversies remain and we conclude by highlighting two areas we think are significant and worthy of further study. First, phylogenetic trees reconstructed from molecular or morphological data yield various conflicting hypotheses of relationships both above and below the family level. There is no better example of this than in the entirely incongruent trees depicting the relationships of the procyonid genera. Total evidence analyses, in which molecular and morphological data from both fossil and living lineages are analysed together (e.g. Finarelli 2008; Slater and Harmon 2013), offer a possible solution in reconciling the conflicting signals arising from these data sources. Additionally, newer technologies and methods make it possible to mine musteloid anatomy for new morphological characters or revise previous ones (e.g. Ahrens 2012, 2014). Second, as the examples from *Bassaricyon* (Helgen et al. 2013), *Arctonyx* (Helgen et al. 2008a), and *Meles* (Del Cerro et al. 2010) demonstrate, uncertainty remains about the exact number of extant species found in the Musteloidea (and the Carnivora more generally). Other musteloid genera or species, encompassing wide distributions across diverse habitats, may harbour cryptic or undescribed species. The integration of ecological and morphological data and genome-scale (phylogenomic) molecular data sets, combined with robust species delimitation methods, will help uncover this hidden biodiversity. For example, the four musteloid families are currently being targeted for whole genome sequencing, and the genome assemblies of the domestic ferret (*Mustela putorius furo*) and red panda have already been published (Peng et al. 2014; Hu et al. 2017). Ultimately, an accurate and precise understanding of the biodiversity of musteloid families, both in terms of species numbers and the age and relationships among lineages within these families, will provide a strong framework that can be used to inform the conservation of this remarkable group of animals.

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