

## MOLECULAR SYSTEMATICS OF THE CANIDAE

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**Abstract.**—Despite numerous systematic studies, the relationships among many species within the dog family, Canidae, remain unresolved. Two problems of broad evolutionary significance are the origins of the taxonomically rich canid fauna of South America and the development in three species of the trenchant heel, a unique meat-cutting blade on the lower first molar. The first problem is of interest because the fossil record provides little evidence for the origins of divergent South American species such as the maned wolf and the bush dog. The second issue is problematic because the trenchant heel, although complex in form, may have evolved independently to assist in the processing of meat. We attempted to resolve these two issues and five other specific taxonomic controversies by phylogenetic analysis of 2,001 base pairs of mitochondrial DNA (mtDNA) sequence data from 23 canid species. The mtDNA tree topology, coupled with data from the fossil record, and estimates of rates of DNA sequence divergence suggest at least three and possibly four North American invasions of South America. This result implies that an important chapter in the evolution of modern canids remains to be discovered in the fossil record and that the South American canid endemism is as much the result of extinction outside of South America as it is due to speciation within South America. The origin of the trenchant heel is not well resolved by our data, although the maximum parsimony tree is weakly consistent with a single origin followed by multiple losses of the character in several extant species. A combined analysis of the mtDNA data and published morphological data provides unexpected support for a monophyletic South American canid clade. However, the homogeneity partition tests indicate significant heterogeneity between the two data sets. [Canidae; combined analysis; mtDNA; phylogeny; South America; trenchant heel.]

The Canidae is a diverse group of wolf-, jackal-, and foxlike carnivores that includes about 36 extant species (Nowak, 1991), 23 of which were included in the present study (Table 1). Despite numerous systematic studies, the relationships among many canid species and genera remain unresolved (Langguth, 1969; Clutton-Brock et al., 1976; Nowak, 1979; Berta, 1987, 1988; Wayne and O'Brien, 1987; Wayne et al., 1987a, 1987b, 1989, 1990a, 1990b; Tedford et al., 1995). Two especially problematic systematic issues have broader evolutionary significance. The first concerns the monophyly of South American canids. The nine extant species (Table 1) are classified into as many as seven genera and represent the most taxonomically rich canid fauna in the world. These taxa are morphologically very diverse (Langguth, 1969; Clutton-Brock et al., 1976; Wayne, 1986a, 1986b; Berta, 1987) and include three unusual monotypic genera: the long-legged

*Chrysocyon brachyurus* (maned wolf); the nearly extinct *Atelocynus microtis* (small-eared dog); and the diminutive *Speothos venaticus* (bush dog). The remaining taxa are dominantly foxlike, although the six species of *Pseudalopex*, *Lycalopex*, and *Cerdocyon* range in size from that of a kit fox (e.g., *Pseudalopex griseus*) to that of a coyote (e.g., *Pseudalopex culpaeus*) (Wayne et al., 1989). Until recently, a wolf-size canid, *Dusicyon australis*, was found on the Falkland Islands, off the coast of southern Argentina. The first appearance of South American canids followed the immigration of North American mammals into South America during the early Pleistocene after the geologic emergence of the Isthmus of Panama (Marshall, 1985; Webb, 1985). Just prior to that time, the large carnivorous fauna in South America was limited and included only a few didelphid species and a single phorusrhachid bird (Patterson and Pascual, 1972; Marshall, 1977). Conse-

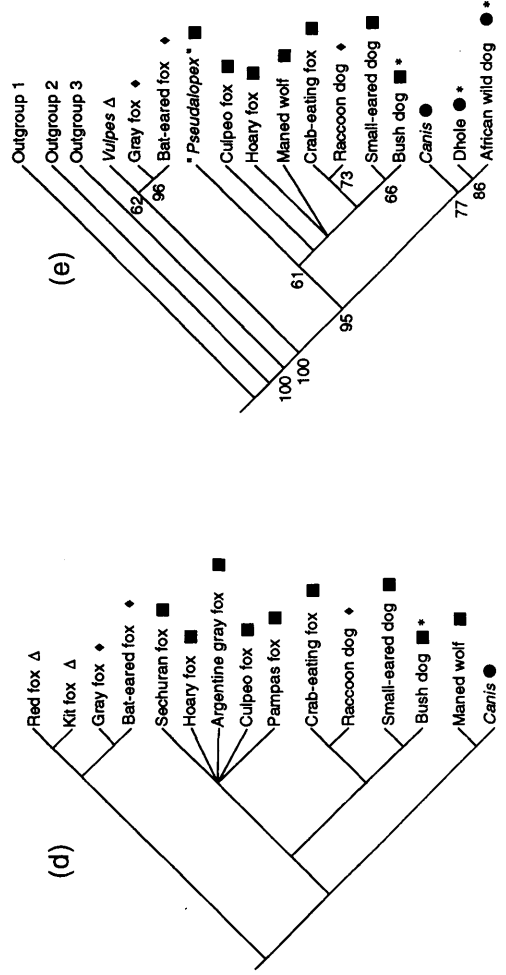
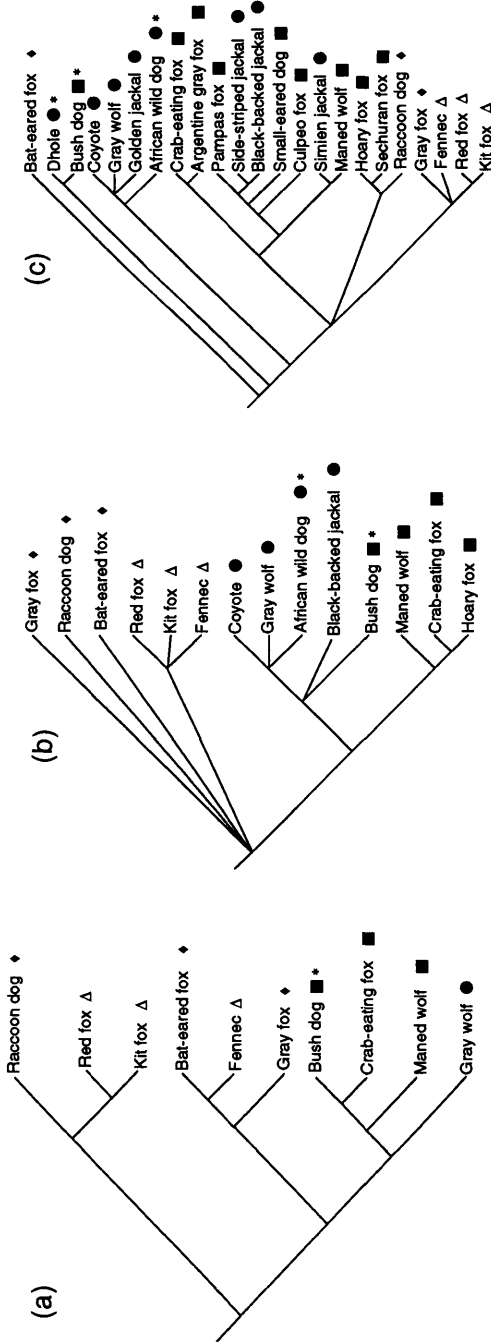
TABLE 1. Natural history and cytogenetic characteristics of canid species included in this study.<sup>a</sup>

Species	Abbreviation	Common name	Geographic range	Habitat	Chromosomes	
					Total	Acrocentric: metacentric
<b>Wolflike</b>						
Small (5–10 kg)						
<i>Canis aureus</i>	CAU	golden jackal	Old World	varied	78	76:0
<i>Canis adustus</i>	CAD	side-striped jackal	sub-Saharan Africa	forest		
<i>Canis mesomelas</i>	CME	black-backed jackal	sub-Saharan Africa	savanna woodland	78	76:0
Large (12–30 kg)						
<i>Canis simensis</i>	CSI	Simien jackal	Ethiopia	alpine grasslands		
<i>Canis lupus</i>	CLU	gray wolf	Holarctic	varied	78	76:0
<i>Canis latrans</i>	CLA	coyote	North America	varied	78	76:0
<b>Trenchant heel</b>						
<i>Canis alpinus</i>	CAL	dhole	Asia	woodland	78	76:0
<i>Lycan pictus</i>	LPI	African wild dog	sub-Saharan Africa	savanna woodland	78	76:0
<i>Speothos venaticus</i> <sup>b</sup>	SVE	bush dog	NE S. America	rainforest	74	72:0
<b>South American</b>						
<b>Enigmatic</b>						
<i>Chrysocyon brachyurus</i>	CBR	maned wolf	NE S. America	high grass plains	76	74:0
<i>Speothos venaticus</i> <sup>b</sup>	SVE	bush dog	NE S. America	rainforest	74	72:0
<b>Foxes</b>						
<i>Lycalopex vetulus</i>	LVE	hoary fox	NE S. America	varied	74	72:0
<i>Cerdocyon thous</i>	CTH	crab-eating fox	NE S. America	varied	74	38:34
<i>Aelocyon microtis</i>	AMI	small-eared dog	NE S. America	varied	74	72:0
<i>Pseudalopex griseus</i>	PGR	Argentine gray fox	Andes	varied	74	72:0
<i>Pseudalopex culpaeus</i>	PCU	culpeo fox	Andes	varied	74	72:0
<i>Pseudalopex gymnocercus</i>	PGY	pampas fox	eastern S. America	varied	74	72:0
<i>Pseudalopex sechurae</i>	PSE	sechuran fox	NW S. America	varied	74	72:0
<b>Red-fox-like</b>						
<i>Vulpes macrotis</i>	VMA	kit fox	western USA	arid lands	50	0:48
<i>Vulpes vulpes</i>	VVU	red fox	Old and New Worlds	varied	34–38	0:32
<i>Fennecus zerda</i>	FZE	fennec fox	Sahara	desert	64	58:4
<b>Others</b>						
<i>Otocyon megalotis</i>	OME	bat-eared fox	sub-Saharan Africa	savanna woodland	72	66:4
<i>Urocyon cinereoargenteus</i>	UCI	gray fox	North America	forest	66	62:2
<i>Nyctereutes procyonoides</i>	NPR	raccoon dog	Japan, China	forest	42+ <sup>c</sup>	14:26

<sup>a</sup> From Wayne et al., 1987a, 1987b, 1989; Wayne, 1993; and references therein.

<sup>b</sup> The bush dog appears twice because it is a South American canid and it has a trenchant heel.

<sup>c</sup> Chromosome number is variable.



quently, an interesting evolutionary question is whether the extant endemic South American canids trace their origin to a single North American lineage or whether several evolutionarily distinct lineages invaded South America. If the latter hypothesis is verified, it would suggest the presence of undiscovered fossils closely allied to the Recent South American canids in Central and North America. The resolution of this question may provide important insights into constraints on morphological evolution in carnivores: a single origin implies rapid morphological change from the common ancestor to produce the diversity seen today; multiple origins would suggest a less dramatic burst of innovation (see Wayne, 1986a, 1986b; Wayne et al., 1989; Van Valkenburgh, 1991).

A second problematic issue concerns the origin of a complex modification of the meat-processing tooth, the carnassial blade. In three canid species, the Asiatic dhole (*Cuon alpinus*), the African wild dog (*Lycyon pictus*), and the bush dog, the lower carnassial molar has a unicuspid talonid (trenchant heel); Simpson (1945) used this character to place these three species in a separate subfamily. However, previous allozyme and morphological phylogenetic hypotheses suggest that the character may have evolved more than once (Fig. 1; Clutton-Brock et al., 1976; Wayne and O'Brien, 1987; Tedford et al., 1995). The trenchant heel increases the length of the cutting blade of the carnassial molar (Van Valkenburgh, 1990) and represents an adaptation for increased carnivory (most canids are omnivores). The three species with trenchant-heeled carnassial teeth are considered the most highly carnivorous of the Canidae (Ewer, 1973; Van Valkenburgh, 1990; Van Valkenburgh and Koepfli, 1993). How-

ever, the trenchant heel shows an iterative pattern of evolution among the extinct taxa of the Canidae and in other carnivore groups (Van Valkenburgh, 1991). Therefore, the trenchant heel, although an elaborate morphological character, may have evolved independently in each of the three extant lineages that possess the character as a selective response to multiple origins of a meat-eating habit.

Although systematic treatments of the Canidae have used a wide variety of morphological, karyological, and molecular genetic techniques (Fig. 1), several specific taxonomic issues remain unresolved (Table 2). For example, morphological and molecular data conflict strongly over the relationships of the bush dog (Fig. 1). Phylogenetic analysis of discrete morphological character data indicates that the bush dog's nearest relative outside South America is the raccoon dog (*Nycterueta procyonoides*), a small omnivorous canid with native populations now found only in southern China and Japan (Figs. 1d, 1e; Berta, 1987; Tedford et al., 1995). However, the diploid number (74) and characteristic acrocentric morphology of the bush dog's karyotype are very similar to those of wolves and jackals and the other South American canids (Table 1; Wayne et al., 1987a). In contrast, the raccoon dog has a predominantly metacentric karyotype that appears plesiomorphic (Table 1; Wayne et al., 1987b). Bush dogs also have allozyme allele frequencies that are more similar to those of *Canis* than to those of the raccoon dog (Fig. 1b; Wayne and O'Brien, 1987).

The two canid phylogenies based on discrete morphological characters are also in conflict. For example, Berta (1987) allied the maned wolf with *Canis* (Fig. 1d), whereas Tedford et al. (1995) placed the

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FIGURE 1. Relationships of canid species. \* = trenchant heel; ■ = South American; Δ = red-fox-like; ● = wolflike. (a) Analysis of G-banded chromosomes (Wayne et al., 1987a, 1987b). (b) Analysis of allozyme genetic distance (Wayne and O'Brien, 1987). (c) Analysis of morphological similarity (Clutton-Brock et al., 1976). (d) Cladistic analyses of morphological characters (Berta, 1987). (e) Cladistic analyses of morphological characters (Tedford et al., 1995). The numbers are the percentage of 1,000 bootstrap runs performed using branch and bound search in PAUP 3.1.1 (Swofford, 1993). Only values >50% are reported. "*Pseudalopex*" includes *P. griseus* (Argentine gray fox), *P. gymnocercus* (pampas fox), and *P. sechurae* (sechuran fox).

TABLE 2. Phylogenetic hypotheses and questions tested in this study (see Fig. 1).

Question	Hypotheses	
	Morphology <sup>a</sup>	Allozymes <sup>b</sup>
1. Relationships of raccoon dog, gray fox, and bat-eared fox	raccoon dog and crab-eating fox are sister taxa (B, T); gray fox is the sister taxon to the bat-eared fox (B, T)	raccoon dog, gray fox, and bat-eared fox each diverged early and none are closely related to any other living canid
2. Monophyly of the South American foxes	inclusion of the raccoon dog (B, T), bush dog (B, T), and maned wolf (T) renders the S.A. foxes paraphyletic	the two species studied, the crab-eating fox and hoary fox, are monophyletic
3. Relationships of the maned wolf and bush dog	bush dog is a sister taxon to the small-eared dog (B, T); maned wolf is a sister taxon to <i>Canis</i> (B) or lies deep within the S.A. fox clade (T)	maned wolf is the sister group to the two S.A. foxes analyzed; bush dog is basal or nearly basal to the African wild dog and <i>Canis</i> species analyzed
4. Evolution of the trenchant heel	independent evolution of trenchant heel in bush dog lineage and in African wild dog/dhole clade (T)	trenchant heel evolved twice or once with one or more reversals (depending on resolution of trichotomies); dhole not included
5. Monophyly of the wolflike canids	not analyzed	wolves, coyotes, and African wild dog are monophyletic; black-backed jackal in trichotomy with these and the bush dog
6. Status of the jackals	not analyzed	not analyzed
7. Relationships of the fennec fox	not analyzed	associated with <i>Vulpes</i>

<sup>a</sup> B = Berta, 1987; T = Tedford et al., 1995.

<sup>b</sup> Wayne and O'Brien, 1987.

maned wolf near the base of a clade consisting predominantly of the South American foxes and the bush dog and raccoon dog (Fig. 1e). Chromosomal and allozyme studies support an affinity of the maned wolf with the South American foxes (Figs. 1a, 1b; Wayne and O'Brien, 1987; Wayne et al., 1987a). Similar disparities among morphological, karyological, and molecular data sets also are apparent in the relationships of the wolflike canids (gray wolves, coyotes, jackals, the Asiatic dhole and the African wild dog) (Fig. 1). The reasons for these disparities are not clear.

In this phylogenetic study, we analyzed 2,001 base pairs (bp) of mitochondrial DNA (mtDNA) sequence to address these broad evolutionary issues and a variety of more specific taxonomic problems (Table 2). To determine whether the South American canids are monophyletic, we analyzed representatives of all the living genera. To determine how many lineages invaded South America, we estimated their diver-

gence times to assess whether these lineages diverged before or after the formation of the Panamanian Isthmus. The estimated divergence times were based on the fossil record directly and on a fossil record-calibrated molecular clock for the Canidae. Similarly, we used the molecular phylogeny to determine whether the trenchant heel evolved multiple times in the Canidae. Finally, we used the homogeneity partition test (Farris et al., 1995) to assess the congruence between our mtDNA data and the only published morphological character matrix for the Canidae (Tedford et al., 1995). We also present a phylogenetic analysis of the combined morphological and mtDNA data.

## MATERIALS AND METHODS

### *Sample Collection and Localities*

We isolated high molecular weight DNA from 23 canid species (Table 1) according to standard methods (Sambrook et al.,

1989). We used samples from two black-backed jackals, one each from the two groups shown to have sufficiently large mitochondrial sequence divergences (>8% in cytochrome *b*) as to suggest the presence of two species (Wayne et al., 1990b). Tissue or blood samples from living or recently deceased individuals were obtained from both wild and captive-bred individuals. The following collection methods were used: blood sampled from an immobilized individual (Wayne et al., 1989), skin sampled using a biopsy dart followed by fibroblast culture, or tissue taken from a recently deceased individual that died through natural causes.

#### DNA Sequencing

We amplified and sequenced a total of 2,001 bp from three protein coding genes, cytochrome *b* (729 bp), cytochrome *c* oxidase I (COI, 588 bp), and cytochrome *c* oxidase II (COII, 684 bp) from 23 species of canids (Table 1). Sequence data from a 1959 tissue sample of the last *Atelocynus microtis* in captivity was less complete than that from other species because the template DNA was highly degraded and difficult to amplify and sequence (Appendix 1). Primer sets for these regions were based on universal polymerase chain reaction (PCR) primers and include cytochrome *b*: H15149 (5'-AAACTGCAGCCC CTCAGAATGATATTTGTCCTCA-3') (Kocher et al., 1989), L14724 (5'-CGAAGCTT GATATGAAAACCATCGTTG-3'), L15513 (5'-CTAGGAGACCCTGACA ACTA-3'), and H15915 (5'-AACTGCAGTCATCTCCG GTTTACAAGAC-3') (Irwin et al., 1991); COI: L6569 (5'-CCTGCAGGAGGAGGAGA TCC-3') and H7227 (5'-AGTATAAGCGTC TGGGTAGTC-3') (Palumbi et al., 1991); and COII: L7552 (5'-AACCATTTCATAACT TGTC AA-3') and H8321 (5'-CTCTTAAT CTTTAACTTAAAG-3') (Ruvolo et al., 1991). Each PCR reaction mixture contained approximately 100 ng of genomic DNA with a reaction buffer of 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl (pH 8.8), 1 mM dNTP mix, and 2–2.5 units of *Taq* DNA polymerase (Promega) in a volume of 50 µl. We used 25 pmoles of each primer

and a Perkin-Elmer Cetus DNA thermocycler programmed for 35 amplification cycles with denaturation at 94°C for 45 sec, annealing at 50°C for 30 sec, and extension at 72°C for 45 sec. Double-stranded reaction products were fractionated by electrophoresis using 2% Nusieve agarose (FMC Corp., Rockland, MD). The appropriate size band was excised, purified with a GeneClean kit (BIO 101, La Jolla, CA), and sequenced using a Sequenase kit (US Biochemical). Except for *Atelocynus microtis*, at least two individuals from each species were sequenced. To confirm sequence information, generally both heavy and light strands were sequenced and compared, and all individuals were sequenced more than two times. The lengths of the sequences were sufficiently well conserved among species that an unambiguous alignment was achieved by eye. There were no insertions or deletions in the entire data set (Appendix 1). Sequences were deposited in Genbank (accession numbers AF028135–AF028230).

#### Phylogenetic Analyses

*Outgroup*.—None of the taxa sequenced can be unequivocally designated as an outgroup to the rest of the species (see Fig. 1), hence the tree had to be rooted with a non-canid taxon. Although the first canids appeared approximately 40 million years ago (MYA), the extant (crown group) canids may have had their origin as recently as 12 MYA (Wayne et al., 1991). Thus, all non-canid outgroups are, unfortunately, likely to be quite distant from the extant canids (e.g., Wayne et al., 1989). Given the limited mitochondrial data for carnivores for the regions of mtDNA we sequenced, we were restricted to using the harbor seal, *Phoca vitulina* (Arnason and Johnsson, 1992), as the outgroup for our study of the Canidae (Wayne et al., 1989; Wyss and Flynn, 1993; Vrana et al., 1994).

*Tree recovery algorithms*.—We used three standard phylogenetic methods, maximum parsimony, maximum likelihood, and neighbor joining, because no single approach has been shown to be always superior for finding the correct tree (Hillis

and Huelsenbeck, 1992; Huelsenbeck and Hillis, 1993; Hillis et al., 1994; Hillis, 1995; Huelsenbeck, 1995). Spectral analysis was also performed on a subset of the data (Penny et al., 1993).

PAUP 3.1.1 (Swofford, 1993) was used to determine the most-parsimonious tree(s) using a heuristic search (with 10 random additions of taxa and TBR branch swapping) on the unweighted sequence data. We also evaluated the effects of codon position and transversion/transition bias by constructing trees based on first and second positions only, transversions in third positions only, and fourfold degenerate sites only (e.g., Wu and Li, 1985; Martin et al., 1990, 1992; Miyamoto et al., 1990). Support for nodes found on the shortest tree (derived from all characters, unweighted) was assessed by bootstrap analysis (Felsenstein, 1985), and 1,000 pseudoreplicates were run using the heuristic search employing TBR branch swapping. MacClade 3.05 (Maddison and Maddison, 1992) was also used to explore the properties of the data set.

The maximum likelihood analyses were run using DNAML in PHYLIP 3.5c (Felsenstein, 1993). This approach allows for unequal expected frequencies of the four nucleotides and unequal transition/transversion ratios. We used the empirically determined frequency of nucleotides and a Kimura two-parameter model with a transition/transversion ratio of 6 to correct for multiple substitutions. This ratio was the average of the pairwise comparisons among ingroup taxa. We also conducted analyses with transition/transversion ratios of 10 and 2 and with fourfold degenerate sites only (with the observed transition/transversion ratio of 2.63). Global rearrangement and jumble options were used to increase the probability that the tree with the greatest likelihood was revealed.

Neighbor-joining analysis with the computer program MEGA (Saitou and Nei, 1987; Kumar et al., 1993) was also performed on the pairwise distances corrected for multiple substitutions using the Kimura two-parameter correction.

Support for the clades in the neighbor-joining tree was assessed using confidence probabilities (CP) and bootstrap values (based on 1,000 pseudoreplicates). Confidence probabilities may be a better measure of statistical confidence than bootstrap values given that the theoretical expectations of the statistic are better defined and that computer simulations suggest it is more reliable than bootstrapping (Zharkikh and Li, 1992a, 1992b; Sitnikova et al., 1995). Distances were also computed using the paralinear/LogDet transformation (Lake, 1994; Lockhart et al., 1994; Steel, 1994) using PAUP\* test version 4.0.0d38 (provided by D. L. Swofford). This method of correcting for multiple substitutions uses a 12-parameter correction and is robust under changing base composition (Swofford et al., 1996). Neighbor joining was used to construct a best tree from the paralinear/LogDet distances, also using PAUP\* 4.0.0d38.

Spectral analysis was used to quantify the degree of support and conflict for each bipartition, or split, in a reduced data set of 20 taxa (the maximum allowed by the program) (e.g., Penny et al., 1993; Lento et al., 1995). We used the Prepare and Hadtree programs (Penny et al., 1993) with two colors and with the Jukes-Cantor correction for multiple substitutions to analyze the relationships among the 20 taxa. Spectral analysis can only use those positions where all taxa have unambiguously identified nucleotides, hence 1,964 bp of the original 2,001-bp data set were used in the spectral analysis. An unrooted tree was constructed using the closest tree criterion (Hendy and Penny, 1993; Swofford et al., 1996). Spectral analysis provides, after a correction for multiple substitutions, an estimate of the number of sites that support each possible bipartition. Different trees consist of different subsets of all possible bipartitions. The closest tree method finds the tree with the bipartitions that minimizes the value of the following:  $(\text{support for the bipartitions not in the tree})^2 + (\text{the discrepancy between the entire signal in the data set and the support for the bipartitions in the tree})^2 / (\text{the number of branches})$

+ 1). The outgroup, the harbor seal, was not included in the spectral analysis. However, given that in all other analyses the most basal canid was always the gray fox (UCI), we rooted the closest tree on this taxon.

*Combined analysis.*—The homogeneity partition test (Farris et al., 1995) as implemented in PAUP\* 4.0.0d49 (provided by D. L. Swofford) was used to assess the congruence between our mtDNA data and Tedford et al.'s (1995) 57 morphological characters (Appendix 2). The 14 extant taxa analyzed by Tedford et al. are present in our mtDNA data matrix, and all were included in the analysis. Because our study was a molecular analysis, it was conducted at (or below) the species level. However, Tedford et al. used the genera *Vulpes* and *Canis* in their analysis; in the combined analysis we selected the red fox (*Vulpes vulpes*) and the gray wolf (*Canis lupus*) sequences to represent these genera. Ten thousand random partitions of the combined data were used in the test of congruence.

## RESULTS

### *Dynamics of Sequence Evolution*

Two most-parsimonious trees of 2,670 steps were found (Fig. 2a; consistency index [CI] = 0.376, CI excluding uninformative characters = 0.329, retention index = 0.476, homoplasy index = 0.624). Of the inferred changes, 2,273 steps were due to changes in the third position, 61 from changes at the second position, and 336 from changes at the first position. The paucity of first and particularly second position changes is reflected in the high degree of conservation of the mtDNA sequences at the amino acid level. The maximum number of inferred amino acid replacements observed between any two ingroup species is 31 of the 667 codons sequenced, and the average pairwise difference is just 16 replacements.

The relationship between the number of first and third position changes between pairs of genes is approximately linear (Fig. 3). The average number of third position

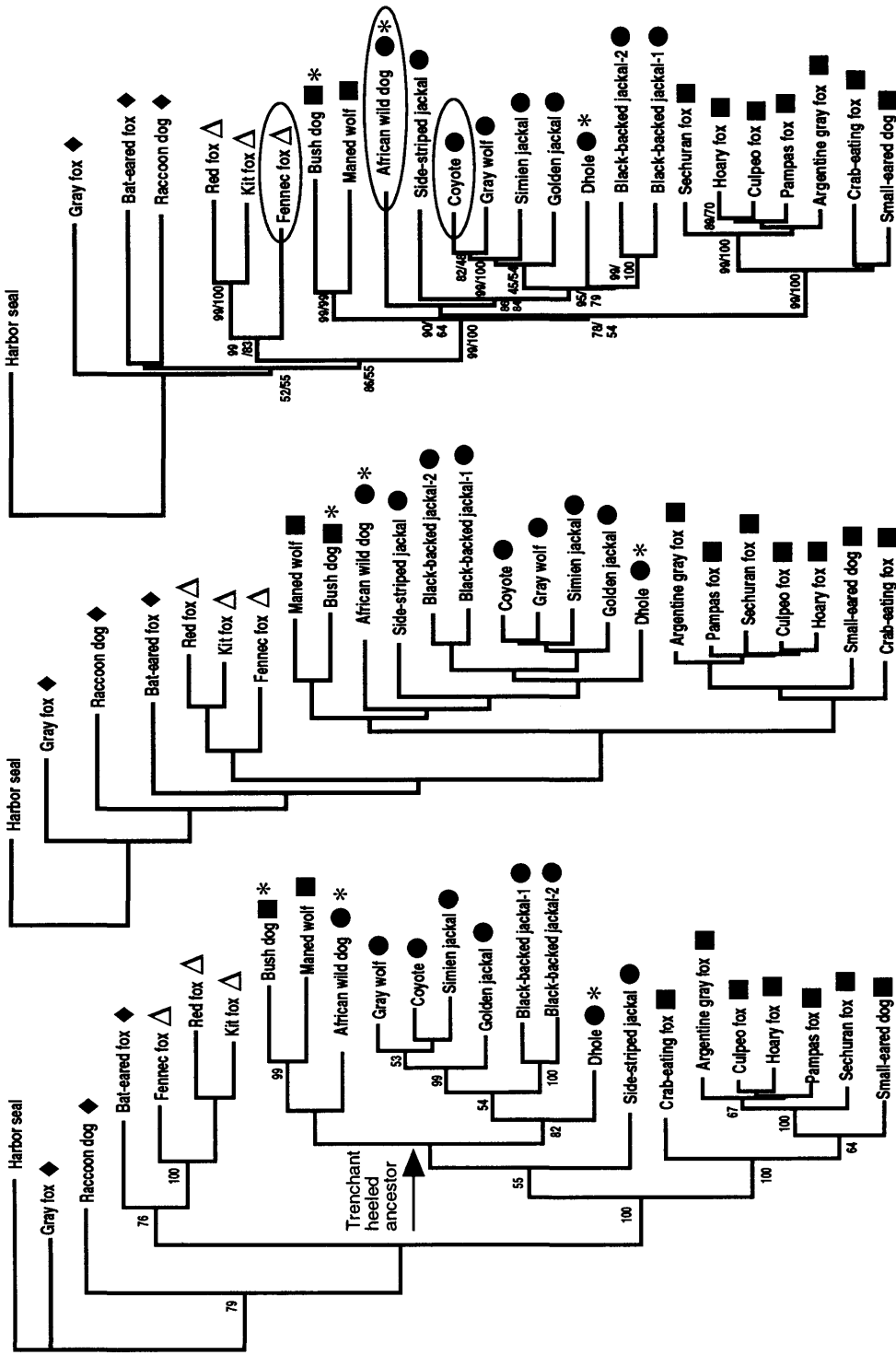
differences in cytochrome *b* between species pairs was 30.2%, just less than the average in COI of 32.7% but more than the average in COII of 26.9%. Too few second position changes have occurred in each gene to make similar comparisons meaningful. For example, in COI the number of changes in the ingroup taxa ranged from 0 to 7. For codon position 1, the average number of differences was 16.2 (6.7%), 3.8 (1.9%), and 10.2 (4.5%) for cytochrome *b*, COI, and COII, respectively.

Excluding outgroup comparisons, there is a highly significant and linear relationship between the number of observed transition and transversion changes and the total number of observed changes (Mantel's test:  $P < 0.01$ ) (Fig. 4). The average transition/transversion ratio, excluding the outgroup, is 5.9 in cytochrome *b*, 13 in COI, and 7 in COII. Few amino acid changes have occurred in the canid sequences. The average ratio of synonymous to nonsynonymous changes in pairwise comparisons of ingroup taxa is 13.5 and is not correlated with the level of sequence divergence ( $r = 0.5$ , Mantel's test:  $P = 0.29$ ).

### *General Outline of Canid Phylogeny*

The phylogenetic approaches used produced remarkably similar topologies (Figs. 2, 5). The following characteristics are common to the four figured trees and the trees produced in the other analyses (with minor exceptions). First, the most basal sequences are those from the raccoon dog, gray fox, and bat-eared fox. The sequence divergence between these taxa and other canids has a narrow range, from about 15% to 19% (Table 3). Second, following these early divergences, two primary monophyletic groupings, supported by high bootstrap values and confidence percentages, were found: (1) the fennec fox, kit fox, and red fox (the red-fox-like canids, sensu Wayne and O'Brien, 1987); the bat-eared fox joined this group in the parsimony analyses of all positions and with first and second positions only and in the spectral analyses; and (2) the wolflike canids (sensu Wayne and O'Brien, 1987) and the South American canids. Within the second group-





(c)

(b)

(a)

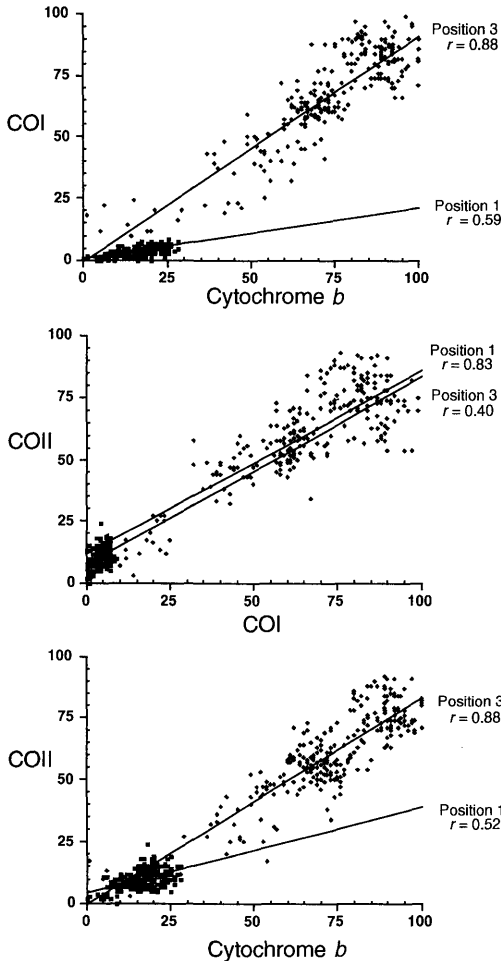


FIGURE 3. The number of third and first position differences (changes) in pairwise comparisons of nucleotide sequences of all 23 ingroup canid taxa (not normalized for the length of sequence). All the relationships are significantly correlated (Mantel's test:  $P \leq 0.001$ ).

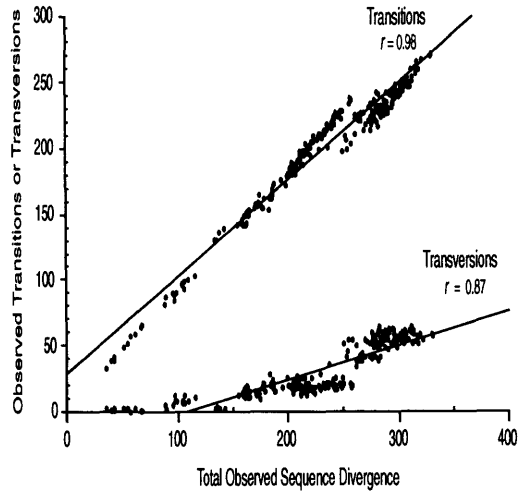


FIGURE 4. The number of transition and transversion changes versus total changes in pairwise comparisons of nucleotide sequences of all 23 ingroup canid taxa. All the relationships are significantly correlated (Mantel's test:  $P < 0.001$ ).

ing, the South American foxes form a very well-supported clade, with nucleotide distances of  $<7.6\%$  and bootstrap values of 100% (maximum parsimony) and CP values of 99% (neighbor joining). The maned wolf and bush dog are also a very well-supported grouping, with a sequence divergence of 11.6%, and the black-backed, Simien, and golden jackals, dhole, gray wolf, and coyote form a well-supported group (bootstrap = 82% [maximum parsimony], CP = 95% [neighbor joining]), with divergence values ranging from 4.6% to 9.3%. The side-striped jackal appears immediately basal to these canids (maximum likelihood with transition/transver-

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FIGURE 2. Maximum parsimony, maximum likelihood and neighbor-joining trees based on 2,001 bp of canid mtDNA sequence. \* = trenchant heel; ■ = South American;  $\Delta$  = red-fox-like; ● = wolflike. (a) Parsimony tree is one of two shortest trees resulting from the heuristic search. The only difference in the tree not shown is that the pampas fox and Argentine gray fox switch positions. Numbers at internodes refer to the percentage of 1,000 bootstrap trees having the indicated groupings (if  $>50\%$ ). (b) Maximum likelihood tree assumes a transition/transversion ratio of 6, the average of all pairwise comparisons between taxa. Ln likelihood ratio = -15063. All branch lengths are significant at the 0.01 level with the exception of the pampas fox/sechuran fox node, which is significant at the 0.05 level. (c) Neighbor-joining tree is based on a Kimura two-parameter model of sequence divergence with a transition/transversion ratio of 6. Numbers at internodes left of the slash are confidence percentages (Rzhetsky and Nei, 1993) and those right of the slash are bootstrap values (Felsenstein, 1985). The internodes immediately below the three circled taxa are those that were dated using the fossil record for computation of rates of mtDNA divergence.

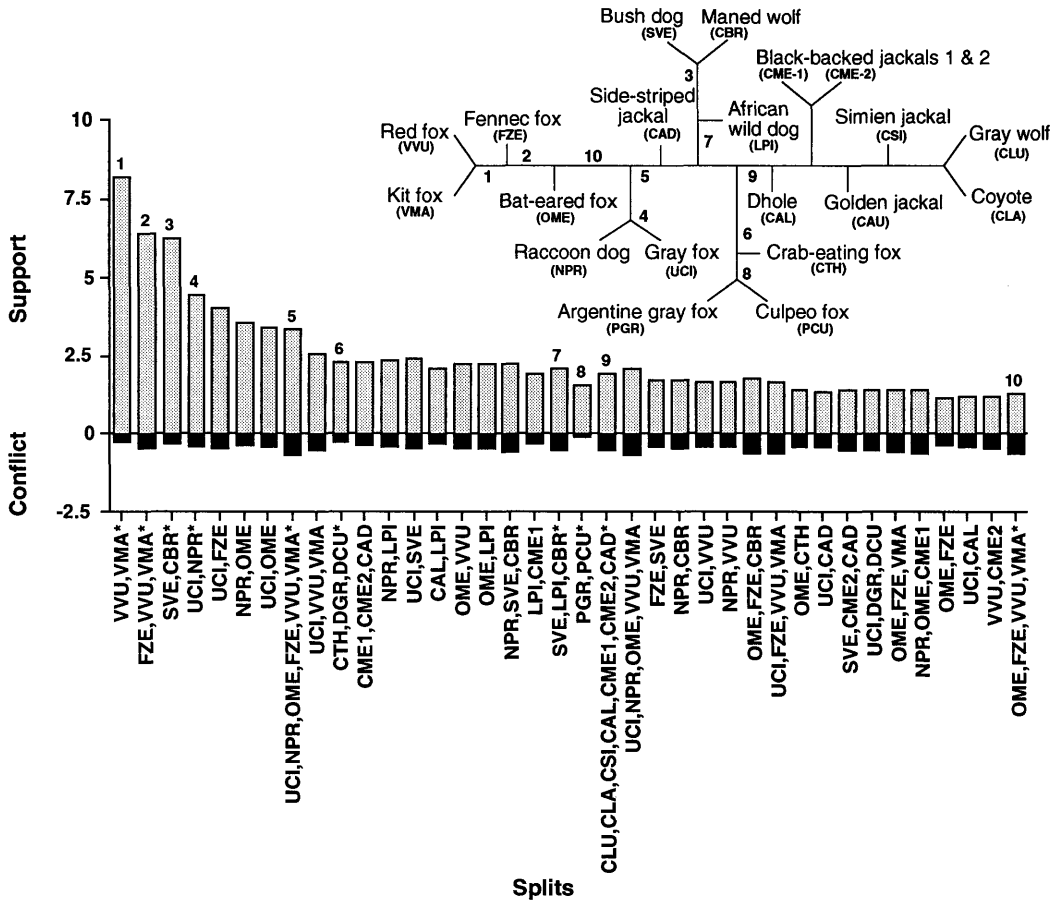


FIGURE 5. Support/conflict spectrum from the spectral analysis of 1,964 bp of canid mitochondrial sequence. Support and conflict represent the number of nucleotide positions in support and in conflict with the indicated split (see Lento et al., 1995). The splits with asterisks (e.g., VVU, VMA) are nodes found in the closest tree (shown). Numbers above graph histogram columns correspond to numbered nodes on the tree.

sion ratios of 6 and 10 and the Kimura two-parameter and paralignear/LogDet neighbor-joining trees), falls basal to all other wolflike canids and the maned wolf/bush dog grouping (maximum parsimony, and maximum likelihood with a transition/transversion ratio of 2 and parsimony with fourfold degenerate sites), or falls basal to other wolflike canids and the South American canids (parsimony with transversions only and fourfold degenerate site transversions only, and spectral analysis). The spectral analysis shows the South American foxes lying between the more derived wolflike canids and a bush dog/

maned wolf/African wild dog clade (Fig. 5).

The exact position of the African wild dog is uncertain. In the parsimony tree (Fig. 2a), closest tree (Fig. 5), and fourfold degenerate and maximum likelihood (transition/transversion ratio = 2) trees, it is associated with the bush dog and maned wolf. In all other trees, the African wild dog is sister group to the wolflike canids. The only other conflicts among the presented trees are in the position of the bat-eared fox and the small-eared dog. The unstable associations of these two taxa likely are due to long-branch attraction

TABLE 3. Sequence divergences based on a Kimura two-parameter correction for multiple hits (above diagonal) and total number of observed sequence differences (below diagonal) between canid taxa. Boxes enclose distances within major monophyletic groups (Fig. 2). PVI = the outgroup *Ploca vitulina* (harbor seal). See Table 1 for all other species abbreviations.

PVI	UCI	NPR	OME	FZE	VVU	VMA	SVE	CLU	CLA	CSI	CAL	CMEI	CME2	CAD	CAU	LPI	CTH	PGR	PCU	PGY	PSE	LVE	AMI	CBR
—	0.233	0.241	0.236	0.233	0.240	0.247	0.254	0.238	0.244	0.251	0.238	0.241	0.243	0.232	0.254	0.245	0.235	0.248	0.251	0.251	0.244	0.255	0.236	0.255
UCI	—	0.167	0.170	0.173	0.165	0.168	0.186	0.175	0.173	0.175	0.164	0.173	0.167	0.162	0.170	0.187	0.176	0.179	0.186	0.181	0.188	0.185	0.169	0.181
NPR	378	286	—	0.163	0.174	0.170	0.161	0.196	0.173	0.163	0.164	0.177	0.168	0.169	0.174	0.172	0.169	0.161	0.169	0.169	0.175	0.171	0.152	0.177
OME	371	289	279	—	0.145	0.170	0.173	0.190	0.176	0.170	0.178	0.174	0.184	0.174	0.180	0.180	0.160	0.163	0.164	0.163	0.165	0.173	0.156	0.169
FZE	369	295	296	253	—	0.134	0.128	0.161	0.160	0.151	0.149	0.151	0.163	0.162	0.149	0.152	0.153	0.152	0.154	0.158	0.157	0.154	0.164	0.149
VVU	381	282	291	290	237	—	0.087	0.182	0.168	0.176	0.167	0.182	0.173	0.164	0.170	0.184	0.160	0.155	0.160	0.161	0.161	0.162	0.154	0.188
VMA	388	285	275	295	229	161	—	0.187	0.181	0.166	0.171	0.174	0.174	0.177	0.162	0.161	0.176	0.166	0.158	0.159	0.157	0.163	0.162	0.178
SVE	400	315	329	321	279	330	317	—	0.144	0.134	0.134	0.139	0.138	0.130	0.134	0.140	0.134	0.132	0.136	0.131	0.140	0.138	0.121	0.116
CLU	378	299	295	299	277	309	307	256	—	0.046	0.055	0.088	0.087	0.092	0.102	0.051	0.117	0.115	0.113	0.115	0.116	0.111	0.122	0.106
CLA	386	295	280	290	263	289	284	240	89	—	0.051	0.087	0.085	0.083	0.098	0.056	0.113	0.124	0.115	0.119	0.117	0.114	0.125	0.106
CSI	395	299	281	303	261	302	293	240	104	97	—	0.089	0.092	0.093	0.096	0.061	0.114	0.117	0.119	0.124	0.128	0.122	0.131	0.105
CAL	379	283	301	296	263	288	297	248	163	161	165	—	0.089	0.094	0.103	0.088	0.113	0.119	0.124	0.128	0.127	0.122	0.131	0.105
CMEI	383	296	287	297	283	311	298	247	162	159	171	165	—	0.057	0.094	0.085	0.120	0.119	0.114	0.121	0.120	0.119	0.127	0.106
CME2	387	287	289	312	281	298	302	235	170	155	173	175	109	—	0.103	0.088	0.117	0.121	0.112	0.121	0.120	0.116	0.123	0.101
CAD	369	278	285	298	261	283	280	240	187	179	176	189	174	188	—	0.086	0.113	0.112	0.112	0.118	0.116	0.118	0.123	0.105
CAU	397	289	294	306	265	291	278	249	97	106	115	163	158	160	162	—	0.113	0.119	0.110	0.118	0.115	0.116	0.123	0.104
LPI	384	313	291	306	266	311	300	239	211	204	207	205	217	212	205	205	—	0.126	0.120	0.129	0.131	0.135	0.117	0.141
CTH	375	300	289	277	266	279	287	258	209	224	213	216	217	220	204	215	227	—	0.071	0.073	0.072	0.076	0.076	0.051
PGR	389	302	276	281	269	269	273	236	204	208	187	223	206	203	204	199	216	134	—	0.020	0.022	0.034	0.032	0.048
PCU	393	313	287	282	275	276	275	243	208	214	203	229	218	218	214	213	232	138	39	—	0.018	0.020	0.022	0.057
PGY	393	305	287	281	273	278	271	234	209	211	200	228	217	217	211	208	231	135	42	35	—	0.038	0.026	
PSE	383	316	297	282	266	277	278	249	201	207	195	219	215	210	212	208	232	142	66	57	54	—	0.035	
LVE	397	311	289	295	283	279	279	246	218	224	212	233	228	221	222	221	240	142	61	42	51	67	—	
AMI	361	278	254	261	252	258	250	210	186	186	177	184	187	178	186	183	205	95	88	104	99	104	106	—
CBR	400	308	302	290	265	319	304	211	241	230	237	224	242	232	230	243	250	220	236	244	241	242	257	184

given that they represent relatively long and unbroken terminal branches and that they lie topologically close to the long unbroken outgroup branch (e.g., see Felsenstein, 1978; Hendy and Penny, 1989). Additionally, topological relationships within the South American foxes and the association of the Simien jackal to other wolflike canids differs among trees.

The paralineal/LogDet tree is a hybrid between the maximum likelihood and neighbor-joining trees (Figs. 2b, 2c): the relationships among the wolflike canids (excluding the maned wolf and bush dog) are the same as in the maximum likelihood tree, as are the relationships among the gray fox, raccoon dog, and bat-eared fox. Otherwise its topology is identical to that of the neighbor-joining tree.

Among the trees not shown, only one deviates substantially from those presented, i.e., the two shortest trees from the parsimony analysis of the first and second sites. Those trees have the following deviations from the maximum parsimony tree (Fig. 2a): (1) the two most basal South American foxes, the crab-eating fox and Argentine gray fox, fall at the base of the wolflike canid clade, and (2) in one of the two most-parsimonious trees, the crab-eating fox is the sister group to the side-striped jackal, which lies basal to the Argentine gray fox, which in turn lies basal to the remaining wolflike canids. In the other shortest tree, the Argentine gray fox is basal to all wolflike canids, the crab-eating fox is the sister group of the maned wolf and bush dog, and the African wild dog and side-striped jackal form a clade that is the sister group to all other wolflike canids. However, in a bootstrap analysis, these anomalous groupings are only weakly supported, and the 50% majority consensus bootstrap tree is consistent with the parsimony tree based on all sites (Fig. 2a). Apparently, first and second site changes are too few to provide a well-resolved phylogeny of the Canidae.

#### *Specific Phylogenetic Hypotheses*

1. *Relationships of the raccoon dog, gray fox, and bat-eared fox.*—The gray fox is always

found at the base of the canid trees, and the raccoon dog and bat-eared fox also always fall very deep in the topologies, although their exact positions differ from one analysis to the next (Fig. 2). The specific branching order of the three taxa also differs among analyses. The difficulties in identifying their exact phylogenetic position is reflected in the results of the spectral analysis by ambiguity in the assignment of these taxa to specific partitions. As seen in Figure 5, there is substantial support for partitions of the gray fox (UCI) and raccoon dog (NPR) with a variety of other taxa (e.g., UCI with FZE [fennec fox], UCI with OME [bat-eared fox], UCI with VVU [red fox], UCI with SVE [bush dog], NPR with LPI [African wild dog], and NPR with OME). The fact that the strongest support for groups not found in the closest tree involve these two taxa and other long-branch taxa supports the hypothesis that they are early canid divergences. Moreover, as suggested by the appreciable sequence divergence between these taxa, there may be sufficient numbers of unique sequence identities among several of these taxa that are due to parallel evolution, which has masked the unique sequence identities that are the result of shared ancestry. Consequently, their uncertain position may be due to long-branch attraction. However, despite the uncertainties in the exact position of the raccoon dog, all of the phylogenetic analyses clearly indicate that the raccoon dog is not associated with the South American fox clade (which includes the crab-eating fox) nor is it particularly closely related to any other living canid.

2. *Monophyly of the South American foxes.*—A monophyletic grouping of South American foxes was strongly supported by the phylogenetic analyses of the mtDNA sequence data. This grouping was supported in all bootstrap replicates and in all trees except the parsimony analysis of the first and second sites (see above). The CP in the neighbor-joining analysis is also 99%. Within this group, the small foxes in the genera *Pseudalopex* and *Lycalopex* (see Table 1) form a monophyletic group supported in all trees and all bootstrap repli-

cations. The sequence divergences among these taxa are small, ranging from 2.0% to 3.5% (Table 3). The spectral analysis provides strong support for an Argentine gray fox (PGR)/culpeo fox (PCU) clade and for the sister group relationship between these two taxa and the only other South American fox included in the closest tree analysis, the crab-eating fox (splits 8 and 6 in the closest tree, ranked 10 and 20 among all possible splits, respectively; Fig. 5). The precise relationships among the South American foxes were not fully resolved, although in all cases (except the parsimony analysis of the first and second sites) the crab-eating fox and small-eared dog are basal (either as a clade or as a paraphyletic group).

3. *Relationships of the maned wolf and bush dog.*—Although the sequence divergence between these two taxa is large (11.6%), they form a monophyletic group in all trees, supported by 99% of the bootstrap analyses and at a 99% CP level. In the spectral analysis, the split defined by the bush dog and maned wolf versus all other canids is the third best supported partition in the data set and has relatively low levels of conflicting sites. Other possible splits involving either taxon (e.g., UCI and SVE, NPR and CBR [maned wolf]) have less than half the level of support and involve taxa characterized by long branch lengths that show affinities with a wide range of other taxa, all with an approximately equal level of support (Fig. 5).

4. *Evolution of the trenchant heel.*—The association of the three trenchant-heeled dogs differs among the four trees (Figs. 2, 5). The parsimony tree is consistent with the hypothesis that the trenchant heel (Fig. 2a) evolved only once in the Canidae and that it was lost in parallel in the maned wolf and the ancestor of the other wolflike canids. However, it is equally parsimonious to hypothesize three independent acquisitions of the character. In contrast, in both the neighbor-joining and maximum likelihood trees, the side-striped jackal, a canid without a trenchant heel, lies between the trenchant-heeled canids, the African wild dog, and dhole, rendering the

independent acquisition of the trenchant heel in three lineages more parsimonious than a single origin of the character followed by three losses. However, the internode distances in these regions of the trees are very short and the topologies are not well supported by the bootstrap analyses. Therefore, at this stage of analysis, we cannot discount a single origin of the trenchant heel, although support for the hypothesis is not strong.

The spectral analysis indicates that the uncertainty in the branching order near the base of the wolflike canids is primarily due to the uncertainty in the position of the trenchant-heeled African wild dog. There is strong support for several mutually contradictory positions of the African wild dog, including support for splits that include the African wild dog and (1) the bush dog and maned wolf (included in shortest tree) (2) the raccoon dog, (3) the dhole (CAL), (4) the bat-eared fox, and (5) the black-backed jackal (CME-1) (Fig. 5).

5. *Monophyly of the wolflike canids.*—A monophyletic group of wolflike canids including the Simien, golden, and black-backed jackals, the dhole, the gray wolf, and the coyote is well supported in all trees (Fig. 2). The spectral analysis, where this group is ranked 21st of all possible partitions, also indicates a relatively high level of support for this grouping (node 9, Fig. 5). However, the relationships of the other two wolflike canids, the African wild dog and the side-striped jackal, are problematic. Both species probably represent early divergences in the evolution of wolflike canids. The spectral analysis suggests that the side-striped jackal lineage may be basal to the entire clade of wolflike and South American canids, although there is relatively little support for this grouping (the grouping of splits 6, 7, and 9 on the closest tree that leaves the side-striped jackal basal is one of the most weakly supported nodes on this tree). The two transversion parsimony analyses also support this grouping. Although this basal position of the side-striped jackal is not supported in the neighbor-joining, parsimony, or maximum likelihood trees, its exact posi-

tion in the parsimony tree does not have strong bootstrap support. Moreover, in a maximum likelihood analysis, a tree with the side-striped jackals basal to all South American and wolflike canids is not significantly more likely than the maximum likelihood analysis presented in Figure 2. Similarly, in the parsimony analysis, a tree with the side-striped jackal basal to the South American and wolflike canids is 12 steps longer than the shortest tree (2,670 vs. 2,682 steps). Therefore, the hypothesis that the side-striped jackal lineage is basal to all the South American and wolflike canids is not clearly refuted by the mtDNA data.

In sum, with the exceptions of the African wild dog and the side-striped jackal, all analyses of the mtDNA data (Figs. 2, 5) suggest that the wolflike canids form a well-supported monophyletic group. The dhole lineage represents an early divergence within the group. Within the group of wolflike canids, two apparent associations are suggested. First, the golden jackal is clearly associated with the larger wolflike canids, the gray wolf, coyote, and Simien jackal. Second, the Simien jackal is clearly a close relative of gray wolves and coyotes. The Simien jackal is more than twice the size of the other jackal species and has a very distinct dentition (Clutton-Brock et al., 1976; Sillero-Zubiri and Gottelli, 1994). The surprising observation that domestic dogs, a recent descendant of the gray wolf, hybridize and produce fertile offspring with Simien jackals can be understood given their close genetic relationship (Gottelli et al., 1994). Golden jackals, coyotes, and gray wolves can also hybridize with domestic dogs (Gray, 1972; Lehman et al., 1991).

6. *Status of the jackals.*—In all analyses, the jackals are rendered paraphyletic by the inclusion of the gray wolf and coyote deep within a clade consisting of the Simien jackal and golden jackal and by the inclusion of the dhole or of a clade consisting of the black-backed jackals and dhole. In all cases, the side-striped jackal lies outside the clade consisting of the other jackals, gray wolf, coyote, and dhole.

7. *Relationships of the fennec fox.*—All four figured trees support the grouping of the fennec, red, and kit foxes (Fig. 2). Bootstrap and confidence percentages are near or at 100%. Moreover, in the spectral analysis, the split that includes these three taxa is the second most strongly supported partition (Fig. 5). The bat-eared fox is the sister group to these three taxa in the parsimony (bootstrap = 76%), parsimony with first and second positions only, and closest trees, but this is not the case in the maximum likelihood and neighbor-joining trees.

#### *Combined Analysis of mtDNA and Morphological Characters*

In all 10,000 replicates of the homogeneity partition test, the original partition into mtDNA and morphological components was shorter than the randomized data, implying significant incongruence between the two data sets ( $P < 0.001$ ).

#### *Absolute Rates of mtDNA Divergences in Canids*

Although molecular evolutionary rates clearly vary among taxonomic groups and genes (e.g., Avise, 1994; Marshall et al., 1994), in this study the same gene regions were analyzed in a closely related group of canids and estimated divergence times ranged over a narrow interval (0.3 to about 12 MYA; Wayne et al., 1991). Therefore, rate discrepancies may be less pronounced. Moreover, the relative uniformity of terminal branch lengths in all trees (Fig. 2) suggests molecular rates are fairly regular within the group.

However, using the fossil record to date the divergence times between lineages is far from straightforward (Marshall, 1990), and although the fossil record of some canids is quite rich (e.g., the dire wolf, *Canis dirus*), the paucity of fossils and uncertainty in taxonomic position of most fossil taxa make it difficult to estimate divergence times for the majority of nodes on the mtDNA trees. Only three internodes can be dated with sufficient precision to warrant the calculation of rates of mtDNA divergence. To estimate an average rate of

TABLE 4. Estimated rates of mtDNA divergence within the canids.

Internode (Fig. 2c)	Estimated divergence time (MYA)	Pairwise distances ( $\bar{x} \pm SD$ )		Estimated rate (%/million years)
		Above internode (%)	Below internode (%)	
1 (fennec fox)	9.5	13.1 $\pm$ 0.4	16.4 $\pm$ 1.1	1.4–1.7
2 (African wild dog)	6.7	11.5 $\pm$ 0.3	11.7 $\pm$ 0.8	1.7–1.7
3 (coyote)	3.5	4.6 <sup>a</sup>	5.3 $\pm$ 0.3	1.3–1.5

<sup>a</sup> Only one pairwise distance.

mtDNA divergence within the group, averages of the pairwise sequence divergences across the dated internodes were used after being corrected for multiple substitutions using the Kimura two-parameter correction. Because these distances also were used to generate the neighbor-joining tree, the topology of the neighbor-joining tree was used when deciding which pairwise distances to use to compute the average mtDNA distances. Others have used the number of transversions versus time to estimate divergence rates (e.g., Irwin et al., 1991; Allard et al., 1992); however, many of the nodes we wish to date have very recent divergence times and include taxa with few or no transversion differences. Consequently, we use corrected Kimura distances based on the total number of changes to increase the information content of our estimate and to decrease the among-comparison variance.

The oldest fossil assigned to the genus *Vulpes* is *Vulpes* sp. from 9–10-million-year-old sedimentary rocks from the Black Butte Local Fauna, Juntura Formation, Oregon (Shotwell et al., 1963). We have assumed that this fossil is the sister group to the fennec fox/*Vulpes* clade, and hence the divergence of the fennec fox/*Vulpes* clade from the wolflike and South American clades predates 9–10 MYA. Consequently, the average of the pairwise distances between the three taxa of the fennec fox/*Vulpes* clade and wolflike and South American clades divided by 9.5 million years gives a maximum estimate of the average rate of mtDNA evolution in the group (Table 4). A minimum estimate of the average rate of mtDNA evolution in the group is obtained by assuming that the fennec fox/*Vulpes* divergence postdates 9–10 MYA and divid-

ing the average of the pairwise distances between the fennec fox and the two *Vulpes* species analyzed by 9.5 million years (Table 4).

The first appearances of *Canis* in the fossil record is "*Canis*" *cipio* from Los Manusetos, Spain, dated at 6–7.4 MYA (Rook, 1992; Werderlin, in press). To estimate a rate for mtDNA evolution we used the middle of the range of dates for this fossil (6.7 MYA) and assumed that it postdates the divergence of the South American foxes from the wolflike canids to give a maximum estimate and that it predates the divergence of the African wild dog from the other wolflike canids to give a minimum estimate (Table 4).

The first appearance of a taxon that is the sister group to both the gray wolf and coyote is the 3–4-million-year-old *Canis lepophagus*, from Hagerman Local Fauna, Glenns Ferry Formation, Idaho (Munthe, in press). We assumed that the divergence of the Simien jackal lineage and the gray wolf/coyote clades predates 3.5 MYA to give a minimum estimate and that the divergence of the gray wolf and coyote postdates 3.5 MYA to give a maximum estimate of the rate of mtDNA evolution in this group (Table 4).

All estimates of sequence divergence rates based on these three internodes (see Fig. 2) are in close agreement, with a range of 1.3–1.7%/million years (Table 4). The estimates for the canid mtDNA rates have not been corrected for intraspecific variation, but where this variation has been estimated (e.g., Gorman et al., 1993; Mercure et al., 1993; Gottelli et al., 1994) it most often ranges between 0.1% and 0.3%.



## DISCUSSION

The phylogenetic analyses of the 2,001 bp of mtDNA sequences clearly suggest the presence of four monophyletic groups within the Canidae: (1) the South American foxes; (2) the bush dog and maned wolf; (3) the black-backed, Simien, and golden jackals, gray wolf, coyote, and dhole; and (4) the red, kit, and fennec foxes. The raccoon dog, gray fox, and possibly the bat-eared fox are not closely associated with any of these monophyletic groups, and all appear to be basal to these groups, except perhaps for the bat-eared fox.

*Canid Invasion of South America*

The topologies of the mtDNA trees, in conjunction with their branch lengths and with the estimated rates of canid mtDNA evolution, can be used to generate hypotheses concerning the invasion and radiation of canids in South America. Minimally, the topology of the mtDNA trees indicates that at least two canid invasions of South America are required to account for the phylogenetic distribution of the extant species: the bush dog/maned wolf clade and the South American fox clade. However, the large sequence divergence between the bush dog and maned wolf coupled with the estimated rates of mtDNA sequence divergence (Table 4) suggest these taxa diverged from each other 6–7 MYA, considerably before the time of formation of the Panamanian land bridge about 2–3 MYA. Therefore, at least three invasions are required to explain both the topology and DNA sequence divergence among extant South American canids.

Both the bush dog and maned wolf appear relatively recently in the South American fossil record, in the mid-Pleistocene (about 1 MYA; Berta, 1984), although fossils of maned wolves may have been found in the North American Blancan from Mexico and Arizona about 3–4 MYA (pers. comm. from R. H. Tedford to A. Berta, 1987). The putative maned wolf fossils suggest that both the maned wolf and bush dog have a relatively long unrecorded evolutionary history in Central and

North America and support the conclusions derived from the degree of mtDNA divergence between the maned wolf and bush dog that these two lineages had already diverged before the canid invasion of South America.

The sequence divergence data indicate the possibility that the foxlike South American canids may also have had multiple origins outside of South America. The sequence divergence between the crab-eating fox and the other South American foxes is between 5.1% and 7.6%. The fastest estimate of the rate of mtDNA divergence (Table 4) and the smallest DNA divergence (Table 3) give an estimated divergence time of 3 MYA, the outer limit for the time of formation of the Panamanian land bridge (dated at 2–3 MYA [Marshall, 1985]). Lower estimates of the mtDNA divergence rates (Table 4) and the largest estimates of DNA divergence of the crab-eating fox from the other South American foxes give an estimate of 4.5–5 MYA for the divergence, a time that likely predates the formation of the Panamanian land bridge. Hence, two fox lineages may have invaded South America: the crab-eating fox and a lineage that gave rise to the other South American foxes. However, given the variance in DNA divergence rates, the uncertainties in the fossil divergence times used to estimate the rates of mtDNA evolution in the canids, and the difficulty in determining when the corridors for terrestrial invasion into South America first formed, it is also possible the entire radiation of South American foxes occurred in South America.

However, the fossil record lends support to the hypothesis that the crab-eating fox had its origin outside of South America; the genus has been described from the late Miocene deposits of North America (3–6 MYA; Berta, 1984, 1987). If the fossil crab-eating fox is a firm sister group of the extant crab-eating fox (as opposed to being the sister group to all the extant South American foxes), then the case for two successful invasions of foxes into South America is greatly strengthened.

The mtDNA divergence between the

small-eared dog and the other foxes (excluding the crab-eating fox) is between 4.8% and 5.8%, distances that would place its divergence from these other taxa to the time of, or just before, the formation of the Panamanian land bridge. However, the small-eared dog may not be the sister group to the *Pseudalopex* and *Lycalopex* foxes but rather to the crab-eating fox (a divergence of 5.1%) (Fig. 2c). Hence, within the uncertainties associated with the use of molecular clocks to estimate divergence times, a divergence from the crab-eating fox before or after the formation of the Panamanian isthmus cannot be determined.

The divergences among the four species of *Pseudalopex* and *Lycalopex* range from 1.8% to 3.5%. These distances are sufficiently small to suggest that this group radiated somewhere during the interval of 1.0–2.5 MYA and, given the absence of non-South American fossils of these taxa, constitute an endemic South American radiation. Also, the generic distinction given to *Pseudalopex* and *Lycalopex* does not reflect much genetic differentiation, and in the absence of appreciable morphological differences we suggest these species should be assigned to a single genus.

In summary, the fossil record, the branch lengths of the mtDNA phylogeny, and the estimated rates of DNA sequence divergence suggest minimally that there were three canid invasions of South America. Although less secure, a similar analysis using sequence divergences and estimated rates of mtDNA sequence divergence of the South American fox clade suggests the possibility that two fox lineages invaded South America. Thus, four lineages of canids may have invaded South America after the formation of the Panamanian land bridge. We conclude that the endemism of the extant canids in South America apparently is not due solely to speciation in South America but also is due to extinction of the founder lineages in North and Central America.

#### *Evolution of the Trenchant Heel*

The three trenchant-heeled species, the bush dog, dhole, and African wild dog, are

considered the most carnivorous canids and share reduced postcarnassial molars (Ewer, 1973; Van Valkenburgh, 1990). The maximum parsimony tree suggests the possibility that the trenchant heel may have evolved early in the history of the wolflike canids and subsequently been retained only in the bush dog, dhole, and African wild dog (Fig. 2a). Simpson's (1945) intuition that the trenchant heel was a homologous character in the three species may have been right, although its independent loss in some of the descendants of the first trenchant-heeled dogs was not considered when using this character to group them in a separate subfamily. However, even in the most-parsimonious tree, three independent acquisitions of the character is as parsimonious as a single origin, and multiple origins for the character are implied by maximum likelihood or neighbor-joining trees. A clearer picture of the evolution of the trenchant heel may emerge with additional sequence data but will remain uncertain if the topology of the most-parsimonious tree is correct.

#### *Karyological and Biochemical Studies*

Allozyme genetic distance phenograms of 18 canid species support some of the groupings found in our mitochondrial DNA study (allozymes, Fig. 1b). In the distance phenogram, the gray fox, raccoon dog, and bat-eared fox are the most divergent of the canids, and the monophyly of the red, kit, and fennec foxes is also supported. However, although the bush dog is grouped with the wolflike canids, the maned wolf, although highly divergent, is clustered with the South American foxes. In general, there is a significant association of allozyme genetic distance and sequence divergence (Fig. 6,  $r = 0.64$ , Mantel's test:  $P < 0.01$ ), suggesting that these indices of genetic divergence are related. In contrast, the association between morphological dissimilarity (Clutton-Brock et al., 1976) and sequence divergence is not significant (Fig. 6,  $r = 0.07$ , Mantel's test:  $P = 0.36$ ), suggesting that the overall pattern of topological relationships based on the two distance measures is incompatible.

G-banded chromosomal data, using the cat (*Felis catus*) as an outgroup (Wayne et al., 1987b), showed that the raccoon dog and the gray fox have primitive canid genotypes and indicated an association of the maned wolf with the bush dog (Fig. 1a). The karyotype of the bat-eared fox was associated with the karyotype of the fennec fox, an association weakly supported by the mitochondrial parsimony and closest tree cladograms. However, the fennec fox is associated with red-fox-like canids in the mtDNA trees. The split of the fennec, red, and kit foxes versus all other canids had the second highest ranking of all possible splits in the spectral analysis (Fig. 5). In the karyological reconstruction, the fennec and bat-eared foxes were not grouped with the red-fox-like canids. Consequently, although the karyological data support the early divergence of the raccoon dog and gray fox, the association of the fennec fox to red-fox-like canids is not supported.

#### *Morphological Studies*

Many aspects of the phylogenetic pattern supported by the mtDNA analyses conflict with previous morphological studies. For example, in an initial study of morphological similarity, Clutton-Brock et al. (1976) suggested that the gray fox clusters with red, kit, and fennec foxes and the raccoon dog showed weak affinity to some of the South American foxes (Fig. 1c). Except for the grouping of the fennec fox with the red and kit foxes, none of the monophyletic groups observed in the present study were supported in Clutton-Brock et al.'s study. Since then, two studies using discrete characters and cladistic methods (Berta, 1987; Tedford et al., 1995) included a subset of taxa used in this study (Figs. 1d, 1e). In contrast to our results, these workers found that (1) the raccoon dog was a sister taxon to the crab-eating fox; (2) this clade was the sister group to the bush dog and small-eared dog clade; and (3) the gray fox and bat-eared fox were grouped with the red and kit foxes in a single clade. Hence, in their analyses, the South American foxes are paraphyletic (Figs. 1d, 1e). In our analysis, the crab-eat-

ing fox and the small-eared dog are part of a monophyletic South American fox clade. In both morphological trees, the bush dog is associated with the small-eared dog, which in turn lies within a clade dominated by the South American foxes, but in our mtDNA sequence study the bush dog was clustered with the maned wolf in a highly supported grouping that lies at or near the base of the clade dominated by *Canis* species. In Tedford et al.'s study, the maned wolf was included in the clade dominated by the South American foxes; in our tree and Berta's (1987) tree, the maned wolf has a closer affinity to *Canis*.

In contrast to the hypothesis of a single origin of the trenchant heel, Tedford et al.'s parsimony analysis of morphological characters suggests there were two independent acquisitions of the trenchant heel, once in the bush dog and once in the dhole/African wild dog clade. Thus, although our phylogenetic analyses of the mtDNA sequence data suggest that a single evolution of the trenchant heel is conceivable (followed by parallel losses in the wolflike canids and the maned wolf), the morphological data do not support this hypothesis.

The genus level analysis of Tedford et al. placed *Canis* as the sister group to a dhole/African wild dog clade. In our analyses, there is never a dhole/African wild dog clade. The dhole always lies either outside or deep within the *Canis* complex (excluding the side-striped jackal). In addition, the African wild dog usually lies outside the entire dhole/*Canis* complex, although in some cases the side-striped jackal lies even more basal. Our results strongly suggest the possibility that the genus *Canis* is not monophyletic and therefore that future systematic treatments of *Canis* and related taxa should be done at the species level.

#### *Combined mtDNA and Morphological Analysis*

The homogeneity partition test showed significant discordance between Tedford et al.'s (1995) morphological data and our

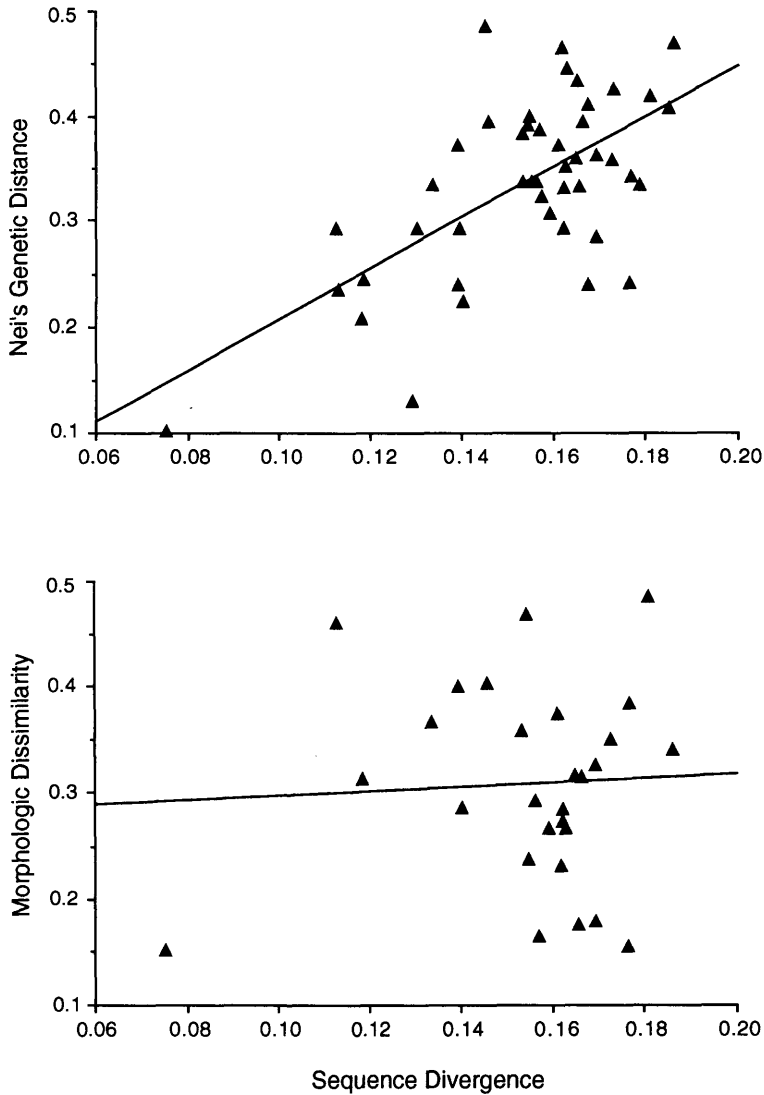


FIGURE 6. The relationship of Nei's genetic distance and morphological dissimilarity with sequence divergence based on 2,001 bp of canid mtDNA sequence. Nei's genetic distance values were taken from Wayne and O'Brien (1987: table 2) and the morphological dissimilarity values were taken from Clutton-Brock et al. (1976: table 1, bottom) by subtracting the "mean similarity" values from 1. In the morphological study, only generic means were provided so that only the values of monospecific genera could be compared directly with the pairwise sequence divergence values of this study.

mtDNA sequence data. The most significant discrepancy between the morphological (Fig. 1e) and mtDNA (Figs. 2, 5) trees is in the position of the raccoon dog, followed by the positions of the bush dog and maned wolf. To determine if the incongruence between the two data sets is just due to these taxa the homogeneity partition

test was repeated after elimination of the raccoon dog, after elimination of the raccoon dog and the bush dog, and after elimination of the raccoon dog, bush dog, and maned wolf. For each reduced data set 100 replicates were performed. In all three cases there was still significant incongruence ( $P < 0.01$ ) between the data sets. It

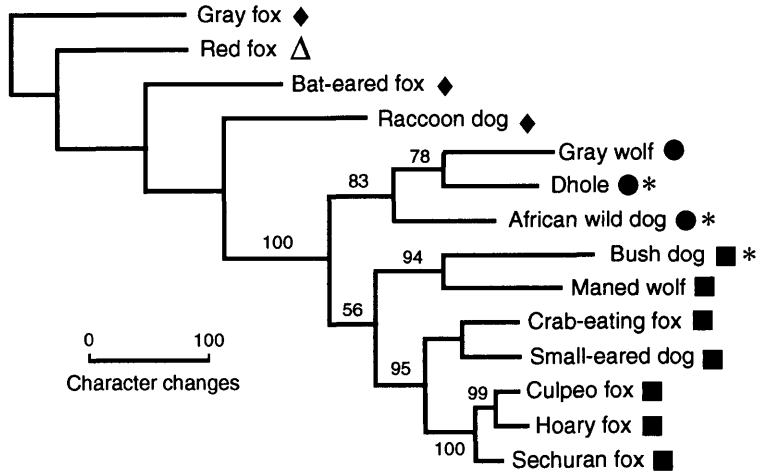


FIGURE 7. Most-parsimonious tree in combined analysis of the 2,001 bp of canid mtDNA and the 57 canid morphological characters from Tedford et al. (1995). \* = trenchant heel; ■ = South American; △ = red-fox-like; ● = wolflike. The numbers on the cladogram are the percentage of 1,000 bootstrap runs performed using branch and bound search in PAUP 3.1.1. Only values >50% are reported. Tree length = 1,787; consistency index = 0.486; retention index = 0.425; rescaled consistency index = 0.207; homoplasy index = 0.514.

appears that the incongruence is not just associated with one or two rogue taxa.

There has been considerable debate over whether to combine or not combine disparate types of data (e.g., see the recent reviews by de Queiroz et al., 1995; Huelsenbeck et al., 1996). The significant incongruity between the morphological and molecular data for the Canidae strongly suggests that these data should not be combined. However, in the spirit of data exploration and hypothesis generation, we performed a combined analysis. Figure 7 shows the resulting most-parsimonious tree. The tree is broadly similar to the mtDNA trees (Figs. 2, 5), particularly in the basal position of the raccoon dog, the sister group relationship of the maned wolf and bush dog, and the closer affinity of the dhole to *Canis* than to the African wild dog. These similarities with the mtDNA trees may be in part due to the fact that the mtDNA data set contributes many more characters than does the existing morphological data set.

Perhaps the most striking feature of the combined analysis is the presence, although with weak bootstrap support, of a monophyletic South American canid clade, a feature not seen in any of the separate

analyses. This grouping warrants further investigation, although the fossil evidence and large mtDNA divergences between many of these taxa still implies that the existing South American canid fauna is the result of at least three and perhaps four separate North and Central American invasions. Also, the combined analysis does not support a single origin of the trenchant heel.

The combined tree (Fig. 7) was rooted with the gray fox. However, rooting the tree between the bat-eared fox and raccoon dog renders a red fox/gray fox/bat-eared fox clade, similar to the morphological tree (Fig. 1e). Given the uncertainties associated with rooting the mtDNA trees with the single distant harbor seal outgroup, Tedford et al.'s morphological tree, which was rooted with three fossil taxa, probably gives a more reliable picture of the relationships of these basal canids.

The detailed reasons for data set incongruence are rarely understood (although see Marshall, 1992a, 1992b, for two counter cases). The morphological studies of canids may have failed to identify the clades that appear well supported in the mtDNA trees because of unidentified character complexes that evolved independently as a

consequence of the independent evolution of similar feeding strategies. For example, within each of the three groups, the wolf-like canids, red-fox-like canids, and South American canids, species exist with adaptations for carnivory, insectivory, and frugivory. The habits and habitats are equally diverse in each group, ranging from arid desert to temperate and tropical forest (Bueler, 1973; Nowak, 1991; Sheldon, 1992). Therefore, many of the quantitative skull and limb size proportions used by Clutton-Brock et al. (1976) and discrete morphological features used by Berta (1987) and Tedford et al. (1995), such as relative size of the canine teeth, muscle processes of the mandible, and even characters of the frontal sinus, may reflect different behavioral, ecological, or physiological adaptations that evolved independently in these different lineages. The taxonomic covariance in the distribution of many of Tedford et al.'s morphological characters may be the result of independent adaptations to similar environments rather than the result of shared ancestry. In support of this conjecture, Faith (1989) noted that genera of Anseriformes (ducks, geese, and other waterfowl) that share similar homoplastic characters also tend to use their habitats in the same way for feeding; much of the morphological homoplasy in these birds apparently is driven by similar but independently derived adaptations to similar ecological factors. The mtDNA phylogenies open up the possibility of assessing functionally driven parallelism (*sensu* Patterson, 1982) that may have been much more difficult to detect, or test, without an independent source of phylogenetic information.

#### *Conclusions and Perspectives*

Our molecular analysis appears to have resolved six of the seven specific taxonomic questions (Table 2). First, the lineages leading to the raccoon dog, bat-eared fox, and gray fox diverged early in the history of the extant Canidae. These taxa are not closely associated with any living canid. The hypothesis that the raccoon dog is a sister taxon to the South American crab-

eating fox is not supported nor is the association of the gray fox with any other foxlike taxa. Second, the South American foxes are monophyletic. Third, the bush dog and maned wolf define a well-supported monophyletic group. The bush dog is not associated with the small-eared dog, the crab-eating fox, or the raccoon dog. Similarly, the maned wolf is not a sister taxon to *Canis* nor does it lie within the South American fox lineage. Fourth, the gray wolf, coyote, and Simien jackal are monophyletic, with the golden jackal as the most likely sister group to this clade, followed by the black-backed jackals and dhole in an undetermined order. The African wild dog, the bush dog/maned wolf clade, and the side-striped jackal are basal to the other wolflike canids, but their relationships are not well resolved. Fifth, the jackals are paraphyletic. Sixth, the fennec fox is the sister group of the red fox and kit fox, consistent with the morphological similarity between the fennec fox and these canids. The phylogenetic relationships of the trenchant-heeled dogs is still not well resolved; some of the molecular trees are consistent with a single evolution of the character whereas others suggest it evolved at least twice independently. In addition, the mtDNA trees suggest that there have been three major radiations of extant canids: one within the red-fox-like clade, another associated with the wolflike canids, and another associated with the South American foxes.

In conjunction with the fossil record, at least three, and possibly four, invasions of South America can be inferred from our data. The bush dog and maned wolf represent two of these lineages, and both have highly derived morphologies. The South American fox clade represents a third invasion, although the most basal member of this lineage, the crab-eating fox, may have already diverged prior to the invasion. These results imply that the endemism in South America is as much due to selective extinction in North and Central America as to speciation within South America. They also suggest that an important evolution-

ary gap exists in the fossil record of the New World Canidae.

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## APPENDIX 1

Multiple alignment of mitochondrial DNA sequences (light strand, 5' to 3') from 23 canid taxa and 1 phocid outgroup taxon. N = unknown base. Cytochrome *b*: base pairs 1–729; cytochrome *c* oxidase I: base pairs 730–1,317; cytochrome *c* oxidase II: base pairs 1,318–2,001. See Table 1 for species abbreviations.

	10	20	30	40	50	60	70	80	90	100
CLU	NNGACCAACATTCGAAAAACCACCCACTAGCCAAAATTGTTAATAACTCATTTCAGCTCCCGAGCCGGCTCAACATCTCTGCTTGATGGAACCTCG									
CLA	AT	T	T				A	C		T
CSI	AT	NN					A			T
CAU	NNNNNNNNNNNA		T				A			T
CMES	AT	A	T	T	C		A	A	C	A
CMET	AT	A	T	T	T	C	G	T	A	C
CAL	AT	G	T	T			A	A	C	C
LPI	NNNNNNNNNNNN		T		A	C	C	T	A	A
CBR	NN	T	T	TT	A			A	A	CA
SVE	AT	T	GT	T	A	C	C	T	A	A
CAD	NNNNNNNNNNNA		T				A			T
CTH	AT		T		C		T		A	A
AMI	NNNNNNNNNNNN		T		C		T		A	A
PSE	AT	T	T	T	C		T	T	A	A
PGY	NNNNNNNNNNNN		T		C		T	T	A	A
LVE	NNNNNNNNNNNN		T		C		T	T	A	A
PCU	NNNNNNNNNNNN		T		C		T	T	A	A
PGR	NNNNNNNNNNNN		T		C		T	T	A	A
OME	NNNNNNNNNNNN				CG	T	T	T	C	A
FZE	NNNNNNNNNNNN		T		CG	T	T	A	A	C
VVU	AT	CC	G	T	T	C	C	T	C	A
VMA	NNNNNNNNNNNN		G	T	T	C	C	T	C	A
NPR	AT	C	G	T	T	C	C	T	C	A
UCI	AT	T	G	T	C	C	C	G	C	A
PVI	AT	C	T	ATA	A	C	C	A	CA	A

	110	120	130	140	150	160	170	180	190	200
CLU	GATCCTTACTAGGAGTATGCTTGATCTACAGATTCTAACAGTTTATTCTTAGCTATGCACTATACATCGGACACAGCCACAGCTTTTTCATCAGTCAC									
CLA		C			T	A				C
CSI					T	A				C
CAU	G	G	GT	C	C		A	C		A
CMES	T	T	G	C	T	T	A	A	T	T
CMET	T	T	C	C	G	T	C	A	T	C
CAL						TC	A			C
LPI	C		TC	A		TC	A	A	C	A
CBR	G	G	G	A		T	A	C	T	T
SVE	G	C	G	C		CC	T	A	C	A
CAD	G	G	GT		GAC	TC	A	C	A	C
CTH		G			GC					C
AMI	G	T	GT		A		A		T	T
PSE	C			A		A		T		C
PGY	C	G		A	C			T		G
LVE	C	G		A	CC			T		G
PCU	C	G		A	CC			T		G
PGR	C	G		A	CC		A	T		G
OME	C	GT	C		GC	C	C	A	C	A
FZE	G	C	G	C	CT	G	C	TC	G	A
VVU	TC	C	G	T	C	T	G	C	A	A
VMA	C	C	T	C	C	C	A	C	C	A
NPR	AC	G	T	C	C	T	C	A	C	C
UCI	G	C	GT	A	C	C	T	A	C	T
PVI	TC	T	C	A	C	C	A	C	C	A

	210	220	230	240	250	260	270	280	290	300
CLU	CCACATCTGCCGAGACGTTAACTACGGCTGAATTATCCGCTATATGCACGAAATGGCGCTCCATATCTCTTATCTGCCATTTCATACATGTAGGACGA									
CLA	T	T			C	A	T		T	T
CSI	T	T	T			A	T		G	T
CAU	A				A	T	C		T	C
CMES	T	G	T	T	A	C				C
CMET	T	T	T		A	T	C	C		T
CAL	T	T			A	T		T		T
LPI	T	T	T	T	T	C	A	T	C	T
CBR	T		T		T	G	C	A	T	G
SVE	T	T	T		A	T	C	A		T
CAD	T	T	T		A	T	C	A		T
CTH	T	T	T		C	A	T	T	C	T
AMI	T	T	T		T	C	A	T		TT
PSE	T	T	T		C	A	T		C	T
PGY	T	T	C	T	C	A	T		C	T
LVE	T	T	T		T	C	A	T		C
PCU	T	T	T		T	C	A	T		C
PGR	T	T	T		T	C	A	T		C
OME		A		C	T	C	A	T		C
FZE	A		T	G	C	C	A	T		C
VVU	T		T	T	C	A	T		A	C
VMA	A	T		T	C	A	T		A	C
NPR	A		T	C	A	T		C	A	A
UCI	T	T	T	T	C	A	T		C	C
PVI		A		C	T	C	T		A	G

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	310	320	330	340	350	360	370	380	390	400
CLU	GGCCTATATACGGATCCTATGTAATTCATAGAAACATGAAACATTGGAATTGTACTATTATTCGCAACCATAGCCACAGCAATTCATGGCGTATGTANNNN									
CLA	... T . C . . . . . C . . . . . C . . . . . A . . . . . NNNN									
CSI	... C . . . . . C . . . . . C . . . . . A . T . . . . . ACTC									
CAU	... C . . . . . C . . . . . T . . . . . C . . . . . A . . . . . CACCC									
CMES	... C . T . . . . . T . . . . . T . . . . . T . . . . . A . T . C . CACTC									
CMET	... C . C . . . . . T . . . . . A . C . CC . G . T . . . . . T . . . . . A . T . C . CACTC									
CAL	... T . G . . . . . T . . . . . T . . . . . T . . . . . T . . . . . T . . . . . A . . . . . C . NNNN									
LPI	... C . T . . . . . T . . . . . C . . . . . C . . . . . TAT . . . . . T . . . . . A . T . C . ACCC									
CBR	... C . T . . . . . T . A . . . . . C . . . . . G . C . . . . . G . C . . . . . T . . . . . T . . . . . A . . . . . NNNC									
SVE	... T . C . . . . . G . . . . . A . . . . . T . . . . . C . T . . . . . C . TT . . . . . T . . . . . A . T . . . . . ACTC									
CAD	... C . . . . . C . . . . . G . . . . . C . . . . . C . . . . . A . . . . . ACCC									
CTH	... T . G . C . T . . . . . A . . . . . T . . . . . G . C . . . . . T . . . . . T . . . . . A . . . . . ACCC									
AMI	... T . G . A . A . . . . . C . . . . . C . . . . . C . . . . . T . . . . . T . . . . . A . . . . . ACCC									
PSE	... C . T . . . . . A . . . . . T . . . . . C . . . . . C . G . . . . . A . NNNNNNNNNNN									
PGY	... C . T . . . . . A . . . . . T . . . . . C . T . C . . . . . A . . . . . ACTC									
LVE	... C . T . . . . . A . . . . . T . . . . . C . T . C . . . . . A . . . . . ACTC									
PCU	... C . T . . . . . A . . . . . T . . . . . C . T . C . . . . . A . . . . . ACTC									
PGR	... C . T . . . . . A . . . . . T . . . . . C . . . . . C . . . . . A . . . . . ACTC									
OME	... T . G . C . T . . . . . T . C . . . . . T . C . . . . . A . TT . CC . . . . . A . . . . . T . . . . . C . C . NNNC									
FZE	... T . C . T . C . . . . . C . . . . . T . . . . . C . . . . . A . CT . CC . . . . . G . . . . . T . . . . . A . T . . . . . NNNC									
VVU	... T . C . T . C . . . . . C . . . . . T . . . . . A . C . CC . G . T . . . . . T . . . . . A . . . . . C . CACTC									
VMA	... T . C . T . . . . . C . . . . . C . . . . . C . . . . . CT . C . . . . . T . . . . . T . A . T . C . . . . . ACCC									
NPR	... C . T . . . . . AC . . . . . G . . . . . C . . . . . G . AA . C . . . . . A . G . . . . . T . . . . . G . . . . . A . T . C . C . NNNN									
UCI	... A . C . T . . . . . AC . . . . . TG . . . . . A . C . . . . . C . T . G . T . . . . . A . . . . . C . NNNN									
PVI	... A . G . . . . . C . . . . . CAC . . . . . C . G . . . . . C . C . . . . . A . C . C . . . . . A . CGT . . . . . T . . . . . C . CACCC									

	410	420	430	440	450	460	470	480	490	500
CLU	NNCCACATATTAACCTGAGTGATATTTCCATATCGCCTATGCTATCCTACGATCCATTCCTAATAAATTAGGAGCGTACTCGCCCTAGTATTTCCTCAT									
CLA	... NNN . . . . . C . A . . . . . C . . . . . T . . . . . C . . . . . C . . . . . C . . . . . T . . . . . T .									
CSI	... CT . . . . . C . . . . . C . . . . . T . . . . . C . . . . . C . . . . . GC . . . . . T . . . . . T .									
CAU	... CT . . . . . C . . . . . A . . . . . C . . . . . C . . . . . T . . . . . C . . . . . T . . . . . T .									
CMES	... CT . . . . . C . C . . . . . A . . . . . C . . . . . C . . . . . T . . . . . T . . . . . T . . . . . T .									
CMET	... CT . . . . . C . . . . . A . . . . . T . . . . . C . . . . . T . . . . . C . . . . . T . . . . . T .									
CAL	... NN . . . . . C . . . . . A . . . . . C . . . . . T . . . . . C . . . . . C . . . . . T . . . . . T . . . . . T .									
LPI	... CT . . . . . T . . . . . A . . . . . C . . . . . T . . . . . C . . . . . T . . . . . C . . . . . T . . . . . C . T .									
CBR	... CT . . . . . C . A . G . . . . . C . . . . . C . . . . . C . . . . . C . . . . . T . . . . . T . . . . . T .									
SVE	... CT . . . . . C . C . . . . . A . . . . . C . . . . . C . . . . . G . . . . . C . . . . . C . . . . . T . . . . . T .									
CAD	... CT . C . . . . . C . . . . . A . . . . . C . . . . . C . C . . . . . T . . . . . C . . . . . T . . . . . T .									
CTH	... CT . . . . . C . A . . . . . C . . . . . T . . . . . C . . . . . G . T . C . . . . . C . . . . . C . . . . . T . . . . . A .									
AMI	... CT . . . . . C . . . . . C . A . G . . . . . T . . . . . C . . . . . T . C . . . . . C . G . . . . . T . A . . . . . A .									
PSE	... NNNNNNN . . . . . C . A . G . C . T . . . . . T . . . . . TT . . . . . C . . . . . C . . . . . C . . . . . T . . . . . A . . . . . T .									
PGY	... CT . . . . . C . A . . . . . C . . . . . T . . . . . C . . . . . T . . . . . G . T . C . . . . . C . C . . . . . T . . . . . A . . . . . T .									
LVE	... CT . . . . . C . A . . . . . C . . . . . T . . . . . C . . . . . T . . . . . G . T . C . . . . . C . C . . . . . T . . . . . A . . . . . T .									
PCU	... CT . . . . . C . A . . . . . C . . . . . T . . . . . C . . . . . T . . . . . G . T . C . . . . . C . C . . . . . T . . . . . A . . . . . T .									
PGR	... CT . . . . . C . . . . . A . . . . . T . . . . . C . . . . . T . . . . . G . T . C . . . . . C . C . . . . . T . . . . . A . . . . . T .									
OME	... CT . . . . . C . C . . . . . C . A . G . . . . . T . . . . . C . . . . . T . . . . . A . . . . . C . C . . . . . A . C . T . . . . . G . T .									
FZE	... CT . . . . . C . C . . . . . C . A . G . . . . . T . . . . . T . . . . . C . . . . . T . . . . . C . . . . . C . . . . . A . T . . . . . G . . . . . T .									
VVU	... CC . . . . . C . . . . . C . A . G . C . . . . . T . . . . . T . . . . . T . C . G . . . . . C . . . . . C . . . . . A . C . A . T . . . . . G . A .									
VMA	... CT . . . . . C . . . . . C . . . . . C . . . . . C . . . . . T . C . G . T . C . . . . . C . C . . . . . G . A . C . . . . . T . . . . . A .									
NPR	... NNNNNNNNNNN . . . . . C . A . . . . . C . . . . . C . C . . . . . G . A . C . . . . . C . C . . . . . A . C . . . . . T . . . . . A .									
UCI	... NNNNNNNNNNN . . . . . C . A . . . . . C . . . . . C . C . . . . . T . . . . . A . C . C . C . . . . . A . G . . . . . G . . . . . A .									
PVI	... CA . . . . . C . . . . . C . A . G . C . . . . . T . . . . . C . A . T . . . . . C . C . C . C . . . . . A . . . . . A . . . . . C . . . . .									

	510	520	530	540	550	560	570	580	590	600
CLU	CCTAATCTTGGCAATTCATTCCACTCCTCCACACATCTAAGCAACGAGCATAATATTCGGGCCCTTAAGCCAAATGCCTATTCGTGACTTCTAGTCCGGCAT									
CLA	... C . . . . . T . . . . . A . . . . . T . . . . . A . . . . . T . . . . . T . . . . . T . . . . . T . . . . . T .									
CSI	... C . A . . . . . T . . . . . C . A . . . . . T . . . . . T . A . T . C . T . . . . . T . . . . . T . . . . . T .									
CAU	... T . A . . . . . T . . . . . T . . . . . T . . . . . T . . . . . A . . . . . C . . . . . T . . . . . T . . . . . T .									
CMES	... C . . . . . C . TT . . . . . T . . . . . A . . . . . T . . . . . T . A . . . . . C . . . . . T . . . . . T . . . . . T .									
CMET	... T . . . . . C . A . . . . . C . TT . . . . . T . . . . . A . . . . . T . . . . . T . A . T . C . . . . . T . . . . . T . . . . . T .									
CAL	... T . . . . . C . . . . . T . . . . . CT . . . . . C . . . . . A . . . . . T . . . . . T . C . . . . . T . C . . . . . T . . . . . C .									
LPI	... T . . . . . C . A . . . . . CT . . . . . C . . . . . C . . . . . T . A . . . . . T . . . . . T . . . . . G . T . C . . . . . C .									
CBR	... T . . . . . C . AAT . . . . . G . . . . . C . T . . . . . A . . . . . A . . . . . T . A . G . C . . . . . T . . . . . T . . . . . ACT .									
SVE	... C . . . . . C . AT . C . . . . . C . . . . . T . . . . . C . A . G . . . . . T . . . . . T . . . . . C . . . . . T . . . . . CT . C . T . C .									
CAD	... C . A . . . . . CC . CT . . . . . C . . . . . A . . . . . T . . . . . T . A . T . . . . . T . . . . . T . T . AC . T . C .									
CTH	... T . G . C . A . . . . . C . TT . . . . . C . . . . . T . . . . . C . . . . . T . . . . . A . . . . . T . . . . . T . AC . T . A .									
AMI	... C . A . C . T . . . . . T . T . . . . . C . A . T . . . . . T . . . . . T . . . . . C . . . . . T . . . . . T . AT . C . A . .									
PSE	... C . A . C . . . . . T . T . T . . . . . C . . . . . T . . . . . A . . . . . T . . . . . T . . . . . T . . . . . CT . C . A . . C .									
PGY	... C . A . C . . . . . T . T . T . . . . . C . . . . . T . . . . . T . . . . . T . . . . . T . . . . . T . . . . . CT . C . . . . C .									
LVE	... C . A . C . . . . . NT . T . T . . . . . C . . . . . T . . . . . T . . . . . T . . . . . T . . . . . T . . . . . CT . C . . . . C .									
PCU	... C . A . C . . . . . T . T . T . . . . . C . . . . . T . . . . . T . . . . . T . . . . . T . . . . . T . . . . . CT . C . . . . C .									
PGR	... C . A . C . . . . . T . T . . . . . C . . . . . T . . . . . T . . . . . T . . . . . T . . . . . AT . C . T . . . . . T .									
OME	... C . A . C . T . . . . . TA . A . . . . . C . . . . . G . A . . . . . G . A . . . . . A . A . . . . . T . . . . . T . . . . . C . . . . . ACT . C .									
FZE	... TC . A . . . . . C . CT . A . . . . . C . A . A . . . . . G . A . . . . . T . A . . . . . T . . . . . T . . . . . AC . . . . . C .									
VVU	... A . . . . . C . T . C . C . A . A . . . . . C . A . A . . . . . TG . A . . . . . A . . . . . T . T . . . . . AC . . . . . A . C .									
VMA	... A . . . . . A . C . . . . . C . TA . . . . . T . C . A . A . . . . . G . A . . . . . A . . . . . C . T . . . . . C . AC . A . C .									
NPR	... C . A . T . . . . . A . C . . . . . C . A . . . . . TG . A . . . . . A . A . T . . . . . A . G . . . . . A . TACA . T . . . . .									
UCI	... T . . . . . C . . . . . A . T . . . . . A . A . . . . . G . A . . . . . G . . . . . A . T . C . T . . . . . TT . G . . . . . T . ACT . . . . .									
PVI	... T . TG . C . C . TA . . . . . A . C . A . . . . . A . A . . . . . AG . A . . . . . A . A . C . . . . . T . . . . . T . C . . . . . A . A . C .									

	610	620	630	640	650	660	670	680	690	700
CLU	CTTCTCACTTTA	ACATGAAT	TGGAGGACA	ACCAGTTGA	ACCCTTTTCAT	CATTATCGGACA	AGTCGCTTCAG	TTTTATATTT	CCACCATCTT	TATGATCC
CLA	C					C		C		T
CSI	C	C		A	C	T	C		C	T
CAU		T	G		G	A		C	A	C
CMES	T		G	A			A	C		C
CMET	T		T	G	A		C	A	T	C
CAL		C		G	A		A	C		C
LPI	C	T			A	C	T	C	T	G
CBR	C	T	C		C	T	G	A		T
SVE	T	CC			A	G		T	T	C
CAD	C		G	A		C	T	T	T	A
CTH	T		G	A		C	T	T	T	A
AMI	G		G	G	A		T		A	C
PSE				A	T	T		A	C	CA
PGY	T	C		A				A	C	CA
LVE	T	C	NN		A	T	T		A	C
PCU	T	C		A	T	T		A	C	CA
PGR	T	C		A	T	T		A	C	CA
OME	A	AC		G	C		G		C	T
FZE	C	A		G	C		G		C	T
VVU	C	C	T		G	G	G	A		C
VMA	C	C	C		G	A		C	T	C
NPR	C	C		C	C	G	C		C	A
UCI	C	T	AC		C	T	G		G	
PVI	A	AC		C		AT		CCG	T	T

	710	720	730	740	750	760	770	780	790	800
CLU	TAATACCAACAG	TAGCGTTAT	CGAAAAC	TTCGATTCT	TCGGGCAT	CTCGAAGTT	TAACATTC	TTATCTCG	CCCGGATT	CGGAATAAT
CLA	T	A		T		A				
CSI		TA								
CAU	G	CA		T		A			AG	G
CMES	GT	A		C		T		A		G
CMET	G	A		C		T		A		G
CAL	T	A	C		T		A			
LPI	C		A	A	C		T		A	
CBR	A	C	A		T	T	T		A	
SVE	GA	A		T		T		A		C
CAD	A	C	TA	C		T	T		A	
CTH	GA	C	A		T		A		T	
AMI	GA	C	A		T		N	A	N	
PSE	TG	A		T		A		T	C	A
PGY	TG	A		T		A		T	C	A
LVE	TG	A		T		A		T	C	A
PCU	TG	A		T		A		T	C	A
PGR	GA	A		T		A		T	C	A
OME	G	TCA	C	A	A		T		T	A
FZE	G	TA	C	A		T		T	A	
VVU	G	CA	A		T		T		A	
VMA	G	CA	A		T		T		A	
NPR	CA	CG	TA	C		T		T	A	
UCI	G	TA	C	A		T		T	A	
PVI	C	TT	CC	A	C		T		A	

	810	820	830	840	850	860	870	880	890	900
CLU	CACCTACTACTC	AGGAAAAAG	AGCCCTT	TCGGTTAT	TATAGGAAT	AGTATGAG	CAATAATAT	CTATTGGG	TTTTAGGC	TTATCGTAT
CLA	T			C	G	A		G		
CSI				C				G	G	
CAU				C				G		
CMES	T	C		T						C
CMET	T	C		T						C
CAL	C	T		C				G		C
LPI	C			C				G		C
CBR	T	T		A	C	T		G		G
SVE	T	T		T	G	G		C		G
CAD	T			T	G	G		C		G
CTH	T			A	T			C		C
AMI	N	N		T	N			NN		N
PSE	T	T		T	C	C		T		C
PGY	T			T	C	C		T		C
LVE	T	T		T	C	C		G		T
PCU	T	T		T	C	C		G		T
PGR	C	T		T	C	C		G		T
OME	G	C		A		T		G	C	
FZE	C			C	T	C		G	T	G
VVU	C			T	A	G		C	T	C
VMA	C			T	A	G		C	T	C
NPR	T	A		C		A		T		T
UCI	A			T	G	A		T		T
PVI	T	C		T	A			A		T

	910	920	930	940	950	960	970	980	990	1000
CLU	ATGTTTACCCTAGGAATAGATG	TAGACACACAGCATACTTT	ACGTCGCCACATAA	TATTCGCTATTCCAAC	GGGAGTAAAGTATTTAGTTGACTGG					
CLA	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CSI	. . . . . G	. . . . . C	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . A	. . . . . G	. . . . . T
CAU	. . . . . T	. . . . . C	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . C	. . . . . A	. . . . .	. . . . . T
CMES	. . . . .	. . . . . C	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . C	. . . . . A	. . . . .	. . . . . T
CMET	. . . . .	. . . . . C	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . C	. . . . . A	. . . . .	. . . . . T
CAL	. . . . .	. . . . . C	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . C	. . . . . A	. . . . .	. . . . . T
LPI	. . . . .	. . . . . C	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . C	. . . . . A	. . . . .	. . . . . T
CBR	. . . . . A	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . C	. . . . . A	. . . . . T
SVE	. . . . . A	. . . . . T	. . . . . C	. . . . . T	. . . . . G	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . G
CAD	. . . . . A	. . . . .	. . . . . C	. . . . . T	. . . . . G	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . . G
CTH	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . .	. . . . . G	. . . . .	. . . . . A	. . . . . T	. . . . . G
AMI	. . . . . A	. . . . . N	. . . . .	. . . . . N	. . . . . N	. . . . . N	. . . . .	. . . . . N	. . . . . N	. . . . . N
PSE	. . . . . A	. . . . . T	. . . . .	. . . . .	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T	. . . . . A
PGY	. . . . . A	. . . . . T	. . . . .	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
LVE	. . . . . A	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
PCU	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
PGR	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
OME	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
FZE	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
VVU	. . . . . A	. . . . .	. . . . . C	. . . . .	. . . . . T	. . . . . C	. . . . .	. . . . . G	. . . . . T	. . . . . C
VMA	. . . . . A	. . . . .	. . . . . C	. . . . .	. . . . . T	. . . . . C	. . . . .	. . . . . G	. . . . . T	. . . . . C
NPR	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
UCI	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
PVI	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
	1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
CLU	CAACACTTCATGGAGGCAATATTA	ATAATGATCTCCAGCTATG	TATGAGCTTTAGGGTTTATTTT	TCTATTTACAGTAGGGCGG	CTAACAGGTATTTGCTCT					
CLA	. . . . . C	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . G	. . . . .	. . . . . C	. . . . . T	. . . . . A
CSI	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . . A	. . . . . G	. . . . .	. . . . . C	. . . . . T	. . . . . A
CAU	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . . A	. . . . . G	. . . . .	. . . . . C	. . . . . T	. . . . . A
CMES	. . . . . A	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . G	. . . . .	. . . . . C	. . . . . T	. . . . . A
CMET	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . G	. . . . .	. . . . . C	. . . . . T	. . . . . A
CAL	. . . . .	. . . . . C	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . T	. . . . . G
LPI	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . C	. . . . . A
CBR	. . . . .	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . T	. . . . . G
SVE	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . T	. . . . . C	. . . . .	. . . . . A	. . . . . T	. . . . . G
CAD	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . T	. . . . . C	. . . . .	. . . . . A	. . . . . T	. . . . . G
CTH	. . . . .	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . T	. . . . . G
AMI	. . . . . N	. . . . . N	. . . . .	. . . . . N	. . . . . N	. . . . .	. . . . . N	. . . . . N	. . . . . N	. . . . . N
PSE	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
PGY	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
LVE	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
PCU	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
PGR	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
OME	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
FZE	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . T	. . . . .	. . . . . G	. . . . . T
VVU	. . . . . C	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . . G
VMA	. . . . . C	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . . G
NPR	. . . . . C	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . . G
UCI	. . . . . C	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . . G
PVI	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . . A	. . . . . T
	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
CLU	AGCTAAITTCGTCCTTAGACATCG	TTCTTTCATGATACATATTA	TATGTTGTAGCTCATTTT	CATACTGCTTTTCACTATG	CTTTTCACATGCTTTTCA	ATAGGAGCAGTTTTTGG	CATTATATGGCAATATGGGA			
CLA	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CSI	. . . . .	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CAU	. . . . .	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CMES	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CMET	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CAL	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
LPI	. . . . .	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CBR	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
SVE	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
CAD	. . . . .	. . . . . C	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .
CTH	. . . . .	. . . . . T	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
AMI	. . . . .	. . . . . N	. . . . .	. . . . . N	. . . . .	. . . . . N	. . . . .	. . . . .	. . . . . N	. . . . . N
PSE	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
PGY	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
LVE	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . . A	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
PCU	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
PGR	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OME	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
FZE	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
VVU	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
VMA	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
NPR	. . . . . G	. . . . .	. . . . . C	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
UCI	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
PVI	. . . . . C	. . . . .	. . . . . A	. . . . .	. . . . . T	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .

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	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CLU	GGATTGGCCACTGATTCCTTTATTCCTCAGGTTATACCTCTTAAACGATCTTGAGCAAAGATTCACCTTTACAATTTATGTTTGTGGGAGTAAATATAACTT									
CLA										
CSI						A				
CAU		C						A		
CMES		C		T					G	
CMET				C	T		T			
CAL	T	C	C	C	T	C	A	A		
LPI	C	C	C	C	T	C	A	C	C	C
CBR	T	T	T	C	C	C	T	C	C	A
SVE	G	T	CC	G	G	C	C	T	A	G
CAD		C		T		A		C		A
CTH	G	T	C	C	C	C	T	A	A	A
AMI	N	G	N	NC	N	C	N	N	A	N
PSE	G	CC	C	C	C	C	A	T	C	A
PGY	G	G	CC	G	C	C	A	T	C	A
LVE	G	C	G	C	C	A	T	C	A	A
PCU	G	G	C	G	C	C	A	T	C	A
PGR	G	C	G	C	C	A	T	C	A	A
OME	T	C	T	T	CC	G	T	G	A	C
FZE	G	T	CC	T	C	C	A	T	C	C
VVU	G	T	CC	T	C	C	A	T	C	C
VMA		CC		A	T	C	C	G	A	C
NPR	T	CC	N	C	C	A	T	C	C	A
UCI	C	T	CC	G	C	T	C	A	C	G
PVI	C	T	T	CC	G	C	T	C	A	C

	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400
CLU	TCTTCCCTCGGCATTTTCATGGGGTACCCATTTCAACTCGGATTACAGGACGCAACCTCCCTCATTATAGAGGAGCTACTTTCATTTTCATGACCACACACT									
CLA		A								T
CSI	AA	CC	C							T
CAU	A			C						TG
CMES	C	AA	T	T						
CMET	C	AA	T							T
CAL	C	AA	T							A
LPI	AA	C	C	T	C	T	T	T	C	C
CBR	T	AA	C	C	T	C			A	T
SVE	T	C	AA	C	C				A	C
CAD	T	C	AA	C	T				C	C
CTH	AA	C	T	C					C	C
AMI	N	N	AAN	C	N				C	A
PSE	AA	C	T	C					C	C
PGY	AA	C	T	C					C	C
LVE	T	C	AA	C	T	C			C	C
PCU	AA	C	T	C					C	T
PGR	AA	C	T	C					C	T
OME	T	AA	C	T	C	G			C	C
FZE	T	AA	C	T	C	G			C	T
VVU	AA	C	T	C	G				T	T
VMA	AA	C	T	C	G				T	T
NPR	AA	C	T	C	A				C	T
UCI	T	C	A	C					T	GT
PVI	T	C	AA	C	A	CC	A	A	CC	A

	1410	1420	1430	1440	1450	1460	1470	1480	1490	1500
CLU	AATAATTTGATTCCTTAATCAGTTCTTTAGTTCTCTATATCATTTTACTAATATTGACTACAAAATTAACCCATACAAGCACAATAGACGCACAAGAAGTG									
CLA				C	G	A	G			A
CSI			A	C				T		A
CAU				C	C			T		A
CMES	G	T	C	C	T	GT	A	C	T	C
CMET		T		T	GT	G	A	C		A
CAL		C	T	C	T	C	A	T		A
LPI		C	T	C	T	T	C	A	T	A
CBR	G	G	TC	CC	G	C	T	CTC	A	C
SVE	G	G	TC	CC	G	C	T	CTC	A	C
CAD		C	C	T	C	T	GT	C	A	G
CTH	C	T	C	C	T	T	C	A	T	C
AMI		C		T	T	A		T	C	A
PSE		C		T	C	T	C	A	T	C
PGY		C		T	T	C	A	G	T	C
LVE		T	C		T	T	C	A	T	C
PCU		C		T	T	C	A	G	T	C
PGR		C		T	T	C	A	G	T	C
OME		C	C	T	C	T	C	A	T	C
FZE		C	AC	T	C	T	A	CT	C	A
VVU		C	A	T	C	T	A	CT	C	A
VMA		C	A	T	C	T	A	CT	C	A
NPR		TC	T	C	A	C	T	A	C	T
UCI		C	T	C	A	C	T	A	C	T
PVI		G	C	T	C	A	C	G	C	C

	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600
CLU	GAAACAGTATGAACCAATCTTACCGCCAATATCCTAATCCTAATCGCTCTACCTTCCCTCCGAATCCTTTATATATAATGGACGAAATTAATAACCCCTCTT									
CLA	T	C	C		G		C	A		C
CSI	G	T	C					A		A
CAU	T	C						A	C	C
CMES	T		T		T	A	G	T	T	C
CMET	T		C	T	T	A		T	T	C
CAL	T			T	A		T		G	A
LPI	G	C		TCG	A	C	T	T	C	A
CBR	T	C	A	C	G	GT		G	A	C
SVE	T	CT	A	C	G		AT		G	A
CAD	G	T		T		A		T	C	A
CTH	T		A		G	G	T	A		T
AMI	G	T	C	G	A	TT	A		G	
PSE	G		C	A		T		A		T
PGY	G	T	C	A		T		A		T
LVE	G		C	A		T		AT		T
PCU	G	G	T	C	A		T		A	
PGR	G	G	T	C	A		T		A	
OME	G		C		A	T		T	T	G
FZE	T				G	G	T	C	C	T
VVU	C		C	A		G	T	T	T	A
VMA	C	A	A			G	C	T	A	G
NPR	G	C	T	C	A		AT	T	A	T
UCI	C		T	C	A		T		G	A
PVI	G	G	C	C	TT	T	C	T	C	A

	1610	1620	1630	1640	1650	1660	1670	1680	1690	1700
CLU	TAACCGTGAACAATAGGCCACCAATGATACGAAGCTATGAATATAC TGACTATGAAGACTTAAACTTTGACTCCTACATAATCCCAACACAAGAATT									
CLA							T			
CSI	T	A	G				T	C		
CAU	T				C	T				
CMES	T		G					T		C
CMET	T	G					G			C
CAL	A	G			T			T		C
LPI	A		T	G		T		T	T	GC
CBR	G	A	T	T	T		C	G		C
SVE	A		C	G	T	T		G		C
CAD	T		T	T	G			T		C
CTH	A				T	G	C	C	T	
AMI	A				T		C		T	C
PSE	T	A	G			C		T	T	G
PGY	T	A				C	C		T	G
LVE	T	A		G			C	C	T	G
PCU	T	A					C		T	G
PGR	T	A					C	C	T	G
OME	A			G			C	G	C	T
FZE	A			G	T		C			
VVU	A			T			C	G	C	TC
VMA	T	A			T		C	C	C	C
NPR	A	A			G		C	C	C	T
UCI	A		G		G	G		C	A	C
PVI	G	A		T	A	T	G		C	C

	1710	1720	1730	1740	1750	1760	1770	1780	1790	1800
CLU	AAAGCCAGGAGAACTGCGACTATTAGAAGTAGACAACCGAGTTGTCTCCCAATAGAAAATAACCATCCGAATACTTATCTCTTCAGAAGACGTTTTGCAT									
CLA			C		G			G		
CSI			C					T		C
CAU			C							
CMES			C			T		TG	T	CG
CMET	A		G	T				TG	T	
CAL			A			T		T		G
LPI	A		C					G	T	T
CBR	A		A	C				G	T	T
SVE	A		T		G			T	T	G
CAD	A		C					T	G	G
CTH	A		C			T		AG	T	C
AMI	A		G	C		T		AG	T	C
PSE	A		G	T		G		AG	T	C
PGY	A		G	T		G		AG	T	C
LVE	A		G	T		G		AG	T	C
PCU	A		T		G			AG	T	C
PGR	A		G	T		G		AG	T	C
OME	A			G	C			C	T	C
FZE			C					G	T	T
VVU	A		G	C				TG		T
VMA	A		G	G	T			G		T
NPR	A		G	T		GC		C	T	GA
UCI	A		A			C		T		T
PVI	C		A		GC			T	A	

Table with 19 columns (1810-1900) and 28 rows (CLU-PVI) showing nucleotide characters at specific positions. Each cell contains a single nucleotide (A, C, G, T) or a dash (-) indicating a missing or unsequenced position.

Table with 11 columns (1910-2000) and 28 rows (CLU-PVI) showing nucleotide characters at specific positions. Each cell contains a single nucleotide (A, C, G, T) or a dash (-) indicating a missing or unsequenced position.

APPENDIX 2
Data matrix of 57 morphological characters from Tedford et al. (1995), used in the combined analysis. See Tedford et al. (1995) for description of characters.

Table with 6 columns (character ID and positions 10, 20, 30, 40, 50) and 16 rows (HESPEROCYONINAE, BOROPHAGINAE, LEPTOCYON, VULPES, UROCYON, OTOCYON, PSEUDOLOPEX, LYCALOPEX, CHRYSOCYON, CERDOCYON, NYCTEREUDES, ATELOCYNUS, SPEOTHOUS, CANIS, CUON, LYCAON). It contains binary data (0s and 1s) representing the presence of specific morphological traits.

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