DNA Sequence Analysis of Familial Relationships Among Dasyuromorphian Marsupials

Article *in* Journal of Mammalian Evolution · January 2000

DOI: 10.1023/A:1009454515671

CITATIONS
13

READS
20

3 authors:

Carey Krajewski
Southern Illinois University Carbondale
187 PUBLICATIONS **2,264** CITATIONS
SEE PROFILE

Mark J Blacket
State Government Victoria
197 PUBLICATIONS **1,387** CITATIONS
SEE PROFILE

Michael Westerman
La Trobe University
369 PUBLICATIONS **4,146** CITATIONS
SEE PROFILE

Journal of Mammalian Evolution, Vol. 7, No. 2, 2000

DNA Sequence Analysis of Familial Relationships Among Dasyuromorphian Marsupials

Carey Krajewski,[1–3] Mark J. Blacket,[2] and Michael Westerman[2]

Although modern morphological and molecular analyses support the monophyly of the Australasian marsupial order Dasyuromorphia, there is much less certainty about relationships among its constituent families (Dasyuridae, Myrmecobiidae, and Thylacinidae). While most authors regard Dasyuridae as monophyletic, a few have suggested that thylacines, numbats, or both have their closest relatives among dasyurids. Recent morphocladistic studies have identified several basicranial characters as putative synapomorphies of dasyurids, but no features that clearly implicate thylacinids, myrmecobiids, or both, as the sister group of Dasyuridae. Only two previous DNA studies have included both thylacine and numbat sequences along with dasyurids, and neither provided strong resolution of interfamilial relationships. In this study, we report a more thorough analytical treatment of complete cytochrome *b*, 12S rRNA, and protamine P1 gene sequences from dasyuromorphians than has heretofore been attempted. Our results concur with previous morphological studies in showing that Dasyuridae is monophyletic and with immunological findings that thylacinids and dasyurids are sister groups, apart from myrmecobiids. However, the level of support for nodes is highly dependent on the method of phylogenetic analysis employed. Our results also suggest that partitioning of sequence data sets to account for substitutional heterogeneity within and among genes does not necessarily lead to a major reduction in the precision of estimated phylogenies.

KEY WORDS: phylogeny; cytochrome *b*; 12S rRNA; protamine P1; partitioning.

INTRODUCTION

The marsupial order Dasyuromorphia, as delimited by Aplin and Archer (1987) and Marshall *et al.* (1990), consists of three families: the recently extinct Thylacinidae ("marsupial wolves"), the monotypic Myrmecobiidae (numbat), and the speciose Dasyuridae. Archer (1982) also considered three Miocene fossils (*Ankotarinja*, *Dasylurinja*, and *Keeuna*) as dasyurids, but these were regarded by Wroe (1997) as dasyuromorphians of uncertain family affiliation. The dasyuromorphian fossil record extends from the latest Oligocene with the thylacinid *Badjcinus* (Muirhead and Wroe, 1998), and dasyurids date from the early or middle Miocene (*Barinya*; Wroe, 1999). In contrast, there are no pre-Pleistocene fossils of myrmecobiids (Archer, 1984).

[1] Department of Zoology and Center for Systematic Biology, Southern Illinois University, Carbondale, Illinois 62901-6501.
[2] Department of Genetics, La Trobe University, Bundoora, Victoria 3083, Australia.
[3] To whom correspondence should be addressed. E-mail: careyk@siu.edu

1064-7554/00/0600-0095$18.00/0

Debate over the monophyly of Dasyuromorphia has centered on the ordinal affinities of thylacinids. Several early authors (e.g., Sinclair, 1906) noted the similarity of thylacinids to extinct South American borhyaenids, a proposition that Archer (1976b) considered plausible on the basis of dental characters. In contrast, Archer's (1976a) survey of basicranial anatomy suggested that thylacinids are primitive with respect to both borhyaenids and dasyurids. Lowenstein *et al.* (1981) found that serum albumin of *Thylacinus* showed more immunological cross-reactivity with dasyurid antisera than with antisera from American marsupials. Moreover, an "immunological clock" suggested that thylacinids and dasyurids diverged only 7 million years ago (Mya), as compared to an apparent 24 Mya divergence of myrmecobiids. Although the former divergence date is obviously wrong in light of the thylacinid fossil record, Szalay's (1982) analysis of tarsal morphology provided strong support for placement of thylacinids among australidelphians. Archer (1982, 1984) accepted the balance of evidence favoring dasyurid affinities of thylacines, and Aplin and Archer (1987) listed three putative synapomorphies for Dasyuromorphia: incisor number reduced to four uppers and three lowers, enlargement of the epitympanic sinus, and loss of the intestinal cecum. There has been little subsequent debate about dasyuromorphian monophyly, although two DNA studies (Thomas *et al*., 1989; Krajewski *et al*., 1997b) have provided further support for it.

Kirsch's (1977) comparative serology results demonstrated that numbats and dasyurids form a clade apart from other marsupials (except perhaps thylacines, which were not included in the serological comparisons). Following Kirsch's work, concern over myrmecobiid systematics has focused on the numbat's distinctness and taxonomic rank with respect to dasyurids. Long considered a member of Dasyuridae (Bensley, 1903; Ride, 1968), numbats were first recognized as a monotypic family by Archer and Kirsch (1977). One consequence of this was the necessity to find synapomorphies documenting the monophyly of Dasyuridae *sensu stricto.* In addition to the serological results, Archer (1984) adduced three such features (a tendency to reduce the lower third premolars, enlargement of stylar cusp D, and loss of posterolateral palatal foramina); two others (presence of a hypoconulid notch and a large alisphenoid tympanic wing) were given by Aplin and Archer (1987). Wroe (1997) rejected all five characters as unique synapomorphies, but noted two new features (presence of a periotic hypotympanic sinus and a tubal foramen) that unite dasyurids. In his description of the Miocene *Barinya*, Wroe (1999) found two additional dasyurid synapomorphies (presence of a paraoccipital hypotympanic sinus and a deeply invasive sulcus with the posteroventral lip formed by a mesially directed process in the pars petrosa), as well as four features that unite modern dasyurids apart from barinyaines.

Although opinions on the composition and taxonomy of Dasyuromorphia have converged on the classification of Aplin and Archer (1987), phylogenetic relationships among the three families have never been clear. Evidence favoring a monophyletic Dasyuridae contradicts the notion that the sister groups of thylacinids and/or numbats are specific subgroups of dasyurids (Tate, 1947; Case, 1989; Szalay, 1994). Dasyurid monophyly was further supported by a study of cytochrome *b* (*cytb*) gene sequences (Krajewski *et al*., 1992) showing that 13 dasyurids formed a clade apart from *Thylacinus* with moderate bootstrap resolution (≤75%), although, unfortunately, *Myrmecobius* was not included in this data set. Interestingly, the first DNA study to include all three dasyuromorphian families (Krajewski *et al*., 1997b) could not resolve a monophyletic Dasyuridae apart from

Myrmecobius and *Thylacinus*, based on sequences of *cytb*, 12S rRNA (*12S*), and protamine P1 (*P1*) loci.

Archer (1984) suggested that the presence of a "foramen pseudovale" (an opening for the mandibular branch of the fifth cranial nerve between the alisphenoid, basioccipital, and periotic bones) is a synapomorphy uniting dasyurids and myrmecobiids, apart from thylacinids. Case (1989), Muirhead (1994), and Wroe (1997), however, argued that the "foramen pseudovale" is a primitive feature in marsupials, relative to the "foramen ovale" (alisphenoid enclosure of the foramen). Wroe (1997) noted conflicting potential synapomorphies among dasyuromorphian families: numbats and dasyurids share an expanded alisphenoid tympanic wing, whereas some thylacinids and dasyurids show an enlarged stylar cusp D and loss of the hypoconulid notch. Wroe (1997) considered all three characters to be of questionable homology.

In a recent study of combined, complete DNA sequences of *cytb*, *12S*, and *P1* from nearly all modern dasyuromorphians, Krajewski *et al.* (2000) found support for both dasyurid monophyly and a sister pairing of thylacinids and dasyurids. These nodes, however, were only weakly resolved in bootstrapped parsimony analyses (69 and 62%, respectively). It is important to note that the size of the data matrix in the Krajewski *et al.* (2000) study was sufficiently large (>70 taxa, 2.7 kb of sequence) that few computationally intensive analyses could be performed. Moreover, the complex gap structure of *P1* alignments precluded the use of model-based distance and likelihood analyses that could have been applied to *cytb* and *12S* sequences. Previous studies (Krajewski *et al.* 1997c; Armstrong *et al*., 1998; Blacket *et al*., 1999) focusing on relationships within dasyurid tribes have used the *cytb–12S–P1* data set with fewer taxa and more sophisticated analyses, but to date no such analyses have been performed to address family-level phylogeny. In this paper, we provide such analyses, with emphasis on the phylogenetic implications of the mitochondrial *cytb* and *12S* sequences. In addition, we discuss some of the implications of our results for methodological issues relating to data partitioning and phylogenetic precision.

MATERIALS AND METHODS

Taxa and Sequences

Published *cytb* and *12S* sequences are available for 62 of 68 dasyuromorphian species, but such a data set poses too large a computational problem for multiple analyses involving maximum likelihood. We therefore chose 15 exemplar species from the four major clades of dasyurids (tribes in Table I). There exemplars include the 13 dasyurids used by Krajewski *et al.* (1997b), as well as *Ningaui yvonnae* and *Planigale ingrami.* The latter were included to ensure that each tribe is represented by at least two species. Thylacinidae is represented by sequences from the recently extinct *Thylacinus cynocephalus*; all other thylacinids are known only from fossils. Myrmecobiidae is represented by the numbat, *Myrmecobius fasciatus.* We chose outgroups from the remaining Australasian marsupial orders, including the marsupial mole (*Notorcytes typhlops*, Notoryctemorphia), two bandicoots (*Perameles* and *Isoodon*, Peramelina), a kangaroo (*Macropus*, Diprotodontia), a possum (*Trichosurus*, Diprotodontia), and koala (*Phascolarctos*, Diprotodontia). The *P1* sequence from *Perameles gunnii* was linked with *cytb*

Table I. GenBank Accession Numbers for Taxa and Sequences Employed in This Study

Species[a]	Cytochrome *b*	*12S* rRNA	Protamine *P1*
Dasyuromorphia			
Dasyuridae			
Dasyurinae			
Dasyurini			
Dasykaluta rosamondae	M99456	L28085	L35325
Dasyurus hallucatus	M99460	U87400	L35341
Dasyurus viverrinus	U07582	U97401	AF010273
Parantechinus apicalis	M99457	U87403	L35326
Phascolosorex dorsalis	M99462	AF009895	L35339
Pseudantechinus macdonnellensis	M99458	AF009885	L35337
Sarcophilus harrisii	M99465	U87402	L35324
Phascogalini			
Antechinus stuartii	M99454	AF038305	L35335
Antechinus swainsonii	M99453	AF038304	L35338
Murexia longicaudata	M99455	U21177	L35336
Phascogale tapoatafa	M99459	U33497	L35327
Sminthopsinae			
Sminthopsini			
Ningaui yvonnae	U07587	AF001938	AF001589
Sminthopsis crassicaudata	M99463	L28087	L32743
Planigalini			
Planigale ingrami	U10319	AF001942	L32745
Planigale sp. (Pilbara planigale)	M99464	U87788	AF001595
Thylacinidae			
Thylacinus cynocephalus	M99452	U87405[b]	U87140
Myrmecobiidae			
Myrmecobius fasciatus	U82329	U87404[b]	U87139
Notoryctemorphia			
Notoryctes typhlops	U87135	AF240636[b]	L35446
Diprotodontia			
Macropus giganteus	U87137	X86941	L35333
Phascolarctos cinereus	AF166348	U61076	U87789
Trichosurus vulpecula	U87138	U21190	L32744
Peramelina			
Isoodon macrourus	AF139057	U61074	L35518
Perameles nasuta	M99466[b]	AF131245	—
Perameles gunnii	—	—	L35305

[a] Suprageneric classification follows Kirsch *et al.* (1997). Alignments are available upon request from the senior author.
[b] These files have been updated since their original publication/submission.

and *12S* sequences from *Perameles nasuta* to form a composite sequence for combined analyses. A list of taxa and GenBank numbers is given in Table I. Sequence lengths (including gaps) are 1146 bp for *cytb*, 995 bp for *12S*, and 750 bp for *P1*.

Combinability and Analytical Constraints

Krajewski *et al.* (1997a, 2000) and Blacket *et al.* (1999) noted two potential gene-tree conflicts between mitochondrial and protamine (*P1*) sequences from 67 dasyurids, one involving the sminthopsin *Antechinomys* and the other the Pilbara planigale. Neither affects the branching order considered here, so we considered the two linkage groups combinable and constructed "total-evidence" trees under the maximum parsimony (MP)

criterion applied to all three genes. *P1* analyses are limited to parsimony because of the numerous indel events in *P1*'s evolution; no model-based methods are available that account for such events, and most of our previous attempts to use conventional sequence-evolution models with *P1* failed to produce meaningful trees. The phylogenetic information in indels, however, is critical and can be captured for MP analysis with the homologous gap-coding strategy of Retief *et al.* (1995) (see also Krajewski *et al*., 1997a,b,c; Blacket *et al*., 1999).

Process Partitions, Parameter Estimates, and Weights

Each of the three codon positions in *cytb* constituted a process partition for our study, as did stem and loop sites in *12S* (we used the secondary structure model of Springer and Douzery, 1996). These are not the only possible partitions for these genes, but they do represent subsets of sites with distinct evolutionary dynamics (Krajewski *et al*., 1999). Each partition was characterized for three parameters relevant to Felsenstein's (1993) "F84" model: base composition, an expected ratio of transitions to transversions (ts/tv), and relative substitution rate. Base compositions were taken as the mean frequency of each nucleotide over all species. The ts/tv value was estimated as the mean observed ratio of transition to transversion mismatches over all sequence pairs that were ≥90% identical. Relative substitution rates were calculated from regression coefficients of pairwise partition distances on overall distances computed under the F84 model and standardized to the lowest value (i.e., *cytb* second positions) (Krajewski *et al*., 1997a,b,c). Krajewski *et al.* (1999) showed that these methods of estimating ts/tv and relative rates for *cytb* partitions yield values comparable to tree-based maximum-likelihood estimates. We employed the F84 model for two reasons: first, to maintain comparability between this study and our previous papers on dasyuromorphian phylogeny; second, because the F84 parameters (base composition, ts/tv, and rate categories) capture what appear to be the major constraints on *cytb* and *12S* sequence evolution.

Previous analyses of marsupial *cytb* and *12S* rRNA genes (Springer *et al*., 1994; Krajewski *et al*., 1997a,b,c; Armstrong *et al*., 1998; Blacket *et al*., 1999) suggested that transversion weighting has obvious and beneficial effects on phylogenetic estimation at deeper nodes. For *cytb*, the "3TV" weighting scheme, in which transitions at third positions are omitted from the analysis, has been most often employed. Because past *cytb* studies focused on intrafamilial relationships, however, we also performed a "TV" (transversion parsimony) scheme in which transitions at all positions are eliminated. The 3TV method is motivated by the rapid rate of change at third codon positions, whereas the TV approach exploits the lower rate of transversions at all three positions. The use of partition-based weights for *12S* is complicated by three confounding aspects of its evolution: stems have a higher ts/tv value than loops, loops have a higher relative rate than stems, and stems show an appreciable frequency of compensatory substitutions (61% in mammals, according to Springer *et al*., 1995). Thus, only TV weighting (of stems *and* loops) was applied to *12S* sequences.

Phylogenetic Analyses

Alignments of informative sites were constructed for each gene (*cytb*, *12S*, *P1*) and two combinations of genes (*cytb* + *12S* and *cytb* + *12S* + *P1*) under each weighting scheme. There were four weightings for *cytb* + *12S* alignments: all transitions and trans-

versions included (ALL); third-position transitions omitted from *cytb* (3TV); third-position transitions omitted from *cytb* and all transitions omitted from *12S* (3TV + TV); all transitions omitted from *cytb* and *12S* (TV). To investigate how site-based weights perform in comparison to transversion weighting, we constructed MP trees from first- and second-position sites alone ("12" weighting). We also obtained an MP tree from amino acid sequences of the cytb protein. Phylogenetic trees were estimated with the DNAPARS and PROTPARS programs of PHLYLIP 3.572c (Felsenstein, 1993), using three random input orders of taxa. Resolution on the MP trees was assayed by bootstrap resampling (Felsenstein, 1985) with 300 replicates.

Pairwise distances (d) assuming an unpartitioned F84 model of sequence evolution were calculated from alignments of all sites for each of the weighting schemes described above (except those including *P1*), using the "maximum likelihood" option of the DNADIST program in PHYLIP 3.572c. Values of ts/tv and base-composition parameters were calculated as weighted averages across partitions. For transversion-weighted partitions, sites were recorded as Y (C or T) and R (A or G) and ts/tv was set to the lowest value (0.6–0.7) consistent with the implied base compositions.

Pairwise distances for a partitioned F84 model were calculated as the weighted-average ($\bar{d}$) of partition-specific distances (d_i):

$$\bar{d} = \sum_i f_i d_i \tag{1}$$

where f_i is the fraction of total sites in partition i, and d_i is estimated under the F84 model with parameter values appropriate for i (Slade *et al*., 1994; Krajewski *et al*., 1997a,b,c). Note that different relative rates among partitions are accounted for in this calculation because d_i values scale in proportion to partition-specific rates. Weighted-average distances were calculated with the WAVEBOOT program of Krajewski *et al.* (1999). Phylogenetic trees were estimated from distances with the weighted least-squares method of Fitch and Margoliash (1967) as implemented in the FITCH program of PHYLIP 3.572c. Tree searches employed three random input orders of taxa and global branch swapping. Resolution was assayed by bootstrapping with 300 replicates; bootstrap searches omitted global rearrangements and used a single random input order of taxa.

ML tree-searches under the F84 model were performed on each of the alignments described above using the DNAML program of PHYLIP 3.572c with global branch swapping and three random input orders of taxa. The "partition" treatment available for DNAML involves treating each partition as a rate category with a specified relative rate and frequency of occurrence (we refer to this treatment as "MLC"). Sites are not fixed in categories but rather are assigned to them in a way that maximizes the likelihood of each candidate topology, i.e., the hidden Markov method of Felsenstein and Churchill (1996). We constrained this process by constructing input alignments in which all sites within a partition were contiguous, then specifying the level of autocorrelation among them (i.e., the average "patch size"). Values of ts/tv and base composition for the overall alignment were taken as weighted averages across partitions. Resolution was assayed by bootstrap resampling with 100 replicates; bootstrap tree searches omitted global rearrangements and used a single random input order of taxa to limit computation times.

Tree Comparison Tests

We assessed the power of analytical treatments to reject alternative topologies by comparing each optimal topology to a suboptimal one on which the node of interest was absent. To construct an alternative to dasyurid monophyly, we constrained *Thylacinus* to be sister to whichever basal division of Dasyuridae contained the dasyurin species (usually a dasyurin-phascogalin clade). This alternative was motivated by the hypotheses of Case (1989) and Szalay (1994) noted above, and by the tendency of some *cytb* analyses to place *Thylacinus* as sister to dasyurins. The alternative to a thylacine-dasyurid clade was a topology on which dasyurids and *Myrmecobius* were sisters, as suggested by Archer (1984) and by *P1* analyses. Statistical tree comparisons were performed using the versions of Kishino and Hasegawa's (1989) test for MP and ML criteria implemented in PHYLIP 3.572c (we relaxed the assumption of autocorrelation among sites to implement the ML test). No such test exists for distance trees, so we computed the difference in "average percent standard deviations" (APSDs; Nei, 1987, p. 300) between optimal and suboptimal FITCH topologies, and noted instances where this difference exceeded 1%. Experience suggests that this heuristic approach is conservative in that optimal and random FITCH trees tend to differ by roughly 10% APSD for our data set, whereas rearrangement of branches on short internodes changes APSDs by <1%.

RESULTS

Estimated Phylogeny from Combined Sequences

The uniformly weighted *cytb* + *12S* + *P1* (ALL) alignment contains 976 informative sites and DNAPARS recovered two minimum-length trees, the strict consensus of which (not shown) resolves a monophyletic Dasyuromorphia with 100% bootstrap support, and unites Myrmecobiidae with a monophyletic Dasyuridae, apart from Thylacinidae, with a 59% bootstrap value. The *cytb* + *12S* + *P1* (3TV) alignment contains 844 informative sites and parsimony analysis yielded three optimal trees, the strict consensus of which is shown in Fig. 1. This arrangement of families was recovered by 62 of 68 analytical treatments over all genes and gene combinations. Dasyuromorphian monophyly was strongly supported in all analyses with *P1* and almost all analyses with *12S*, but only with transversion-distance analysis for *cytb.* Consistent with earlier findings, 3TV weighting reversed the positions of *Myrmecobius* and *Thylacinus*, linking the latter to Dasyuridae. Analyses of 3TV + TV (710 informative sites, one tree) and TV (635 sites, four trees) alignments also recovered dasyurid monophyly and the dasyurid-thylacinid clade, although with only weak to moderate bootstrap values (Table II). The Kishino and Hasegawa (1989) test rejected the alternative to dasyurid monophyly for the ALL, 3TV, 12, and 12 + TV weightings of *cytb* + *12S* + *P1* data. None of the treatments rejected the sister pairing of numbats and dasyurids in favor of thylacinids + dasyurids. Given these results and the findings of Krajewski *et al.* (2000), we take the thylacinid + monophyletic dasyurid arrangement as the generally optimal point estimate of phylogeny from all available sequence data. Because these nodes are not highly resolved by the MP analyses appropriate for *P1*, however, we consider their levels of support from the wider range of analyses available for *cytb* and *12S.*

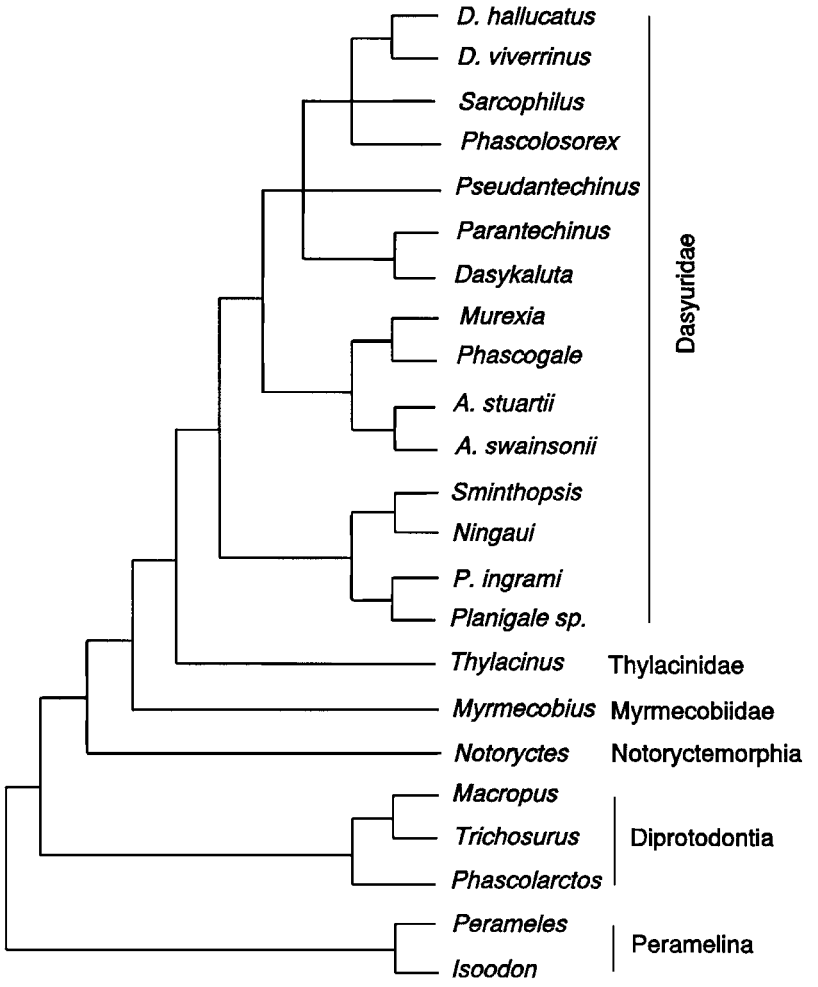

Fig. 1. Strict consensus of three minimum-length trees for combined cytochrome *b*, *12S* rRNA, and protamine sequences (844 informative sites, 3052 steps per tree). Transitions at third codon positions in cytochrome *b* were given 0 weight (3TV treatment). The tree was obtained with the DNAPARS program of PHYLIP 3.572c using three random input orders of taxa. Families of Dasyuromorphia and outgroup orders are identified along the right side. Genus abbreviations: A. = *Antechinus*; D. = *Dasyurus*; P. = *Planigale*. See Table I for species names of taxa labeled with genus name only. The root is placed arbitrarily on the branch to Peramelina.

Precision of Mitochondrial Trees

Dasyurid and dasyurid + thylacinid nodes were frequently absent from parsimony trees obtained from *cytb* alone (not shown), and were never well-resolved (bootstraps ≤50%). This includes the protein parsimony tree, on which only one ingroup node (pairing of the two *Antechinus* species) was recovered with >50% bootstrap support. Trees from *12S* (not shown) had much better resolution on interfamilial nodes, with TV weights giv-

Table II. Bootstrap and Tree-Comparison Results for Parsimony Analyses Involving Protamine

Alignment	Weighting[a]	Node		
		Dasyuridae	Dasyuridae + Thylacinidae	Dasyuromorphia
1. *P1*	ALL	(31)[b]	(16)	100[d]
2. *P1* + *cytb* + *12S*	ALL	67[c]	(40)	100[d]
3. *P1* + *cytb* + *12S*	3TV	66[c]	80	100[d]
4. *P1* + *cytb* + *12S*	3TV + TV	60	73	100[d]
5. *P1* + *cytb* + *12S*	TV	69	58	100[d]
6. *P1* + *cytb* + *12S*	12	52[c]	61	100[d]
7. *P1* + *cytb* + *12S*	12 + TV	54[c]	53	100[d]

[a] ALL = all sites and substitution types equally weighted; 3TV = transitions at third positions of *cytb* omitted; 3TV + TV = transitions at third positions of *cytb* and transitions at all positions of *12S* omitted; TV = transitions at all positions of *cytb* and of *12S* omitted; 12 = third positions of *cytb* omitted; 12 + TV = third positions of *cytb* and transitions at all positions of *12S* omitted.
[b] Parentheses indicate that the node did not occur on the optimal topology.
[c] $p < .05$ for Kishino and Hasegawa (1989) test of alternative topology.
[d] $p < .01$ for Kishino and Hasegawa (1989) test of alternative topology.

ing moderate bootstraps on Dasyuridae (73%) and dasyurids + thylacinids (69%). When *cytb* and *12S* were combined for MP analyses, most weighting schemes produced modest improvements in bootstrap resolution (Table III).

Estimates of parameter values for the F84 model for each partition of *cytb* and *12S* are given in Table IV. As with parsimony, FITCH trees from *cytb* have low resolution on interfamilial nodes (not shown), although dasyurid and dasyurid + thylacinid nodes do occur on all but one optimal tree. Unpartitioned treatments give the highest bootstraps on both nodes. Distance trees from *12S* (not shown) also gave highest resolution with unpartitioned analyses. Partitioning drastically lowers the bootstrap on the dasyurid node with the ALL treatment, but loss of resolution is only moderate with the partitioned TV treatment. Unpartitioned and weighted-average distances from combined *cytb* and *12S* sequences gave generally similar bootstraps in the range of 61 to 99% on both nodes (Table V).

Table III. Bootstrap and Tree-Comparison Results for Parsimony Analyses of Combined *cytb* and *12S* Sequences

Node	Weighting scheme[a]					
	ALL	3TV	3TV + TV	TV	12	12 + TV
Dasyuridae	57	68	64[b]	64	66	74
Dasyuridae + Thylacinidae	65	84	82	68	83	76

[a] ALL = all sites and substitution types equally weighted; 3TV = transitions at third positions of *cytb* omitted; 3TV + TV = transitions at third positions of *cytb* and transitions at all positions of *12S* omitted; TV = transitions at all positions of *cytb* and of *12S* omitted; 12 = third positions of *cytb* omitted; 12 + TV = third positions of *cytb* and transitions at all positions of *12S* omitted.
[b] $p < .05$ for Kishino and Hasegawa (1989) test of alternative topology.

Table IV. Estimated Parameter Values for the F84 Model

Partition	Base composition (A : T : C : G %)	ts/tv	Relative rate			Fraction of total sites
			ALL	3TV	TV	
cytb 1st positions	28 : 23 : 27 : 22	4	3	3	5	.179
cytb 2nd positions	21 : 41 : 25 : 13	4	1	1	1	.179
cytb 3rd positions	40 : 21 : 36 : 3	4	31	26	37	.179
12S stems	24 : 26 : 24 : 26	12	1	1	1	.231
12S loops	46 : 22 : 20 : 12	2	4	4	8	.232

ML trees from *cytb* (not shown) are weakly resolved at best, with bootstraps on the dasyurid and dasyurid + thylacinid nodes ≤65% (resolution is best with unpartitioned 3TV treatments). Unweighted *12S* analyses (not shown) produce stronger resolution than TV methods on most nodes, with no clear distinction between unpartitioned and partitioned treatments. ML trees from combined *cytb* and *12S* sequences had bootstraps in the range of 66–90% (Table VI), with comparable levels of resolution from partitioned and unpartitioned treatments.

Tree Comparison Tests

No test found the dasyurid + thylacine clade significantly better than its alternative, nor could the alternative to dasyurid monophyly be rejected for any *cytb* treatment. Based on *12S* alone, dasyurid monophyly had a significantly higher likelihood than nonmonophyly only for the ALL treatment. For combined *cytb* and *12S*, dasyurid monophyly was significantly better than its alternative under one MP and four ML treatments (Tables III and VI).

Table V. Bootstrap and Tree-Comparison Results for Distance Analyses of Combined *cytb* and *12S* Sequences

Node	Weighting scheme[a]					
	ALL	3TV	3TV + TV	TV	12	12 + TV
Dasyuridae						
Unpartitioned (d)	99	98	85	91	96	88
Partitioned ($\overline{d}$)	68	85	92	92	93	90
Dasyuridae + Thylacinidae						
Unpartitioned (d)	61	80	95	82	74	83
Partitioned ($\overline{d}$)	80	87	85	85	63	67

[a] ALL = all sites and substitution types equally weighted; 3TV = transitions at third positions of *cytb* omitted; 3TV + TV = transitions at third positions of *cytb* and transitions at all positions of *12S* omitted; TV = transitions at all positions of *cytb* and of *12S* omitted; 12 = third positions of *cytb* omitted; 12 + TV = third positions of *cytb* and transitions at all positions of *12S* omitted.

Table VI. Bootstrap and Tree-Comparison Results for Maximum-Likelihood Analyses of Combined *cytb* and *12S* Sequences

Node	Weighting scheme[a]					
	ALL	3TV	3TV + TV	TV	12	12 + TV
Dasyuridae						
Unpartitioned (ML)	86	85[b]	72[b]	71[b]	84	83
Partitioned (MLC)	89[b]	90	80	72	82	77
Dasyuridae + Thylacinidae						
Unpartitioned (ML)	85	78	75	74	76	78
Paritioned (MLC)	76	66	76	79	79	79

[a] ALL = all sites and substitution types equally weighted; 3TV = transitions at third positions of *cytb* omitted; 3TV + TV = transitions at third positions of *cytb* and transitions at all positions of *12S* omitted; TV = transitions at all positions of *cytb* and of *12S* omitted; 12 = third positions of *cytb* omitted; 12 + TV = third positions of *cytb* and transitions at all positions of *12S* omitted.
[b] $p < .05$ for Kishino and Hasegawa (1989) test of alternative topology.

DISCUSSION

Dasyuromorphian Phylogeny

Our results provide strong support for the monophyly of modern Dasyuromorphia, consistent with morphological evidence summarized by Aplin and Archer (1987) and with our previous molecular work (Krajewski *et al*., 1997b, 2000). The evidence from *cytb*, *12S*, and *P1* supporting the monophyly of modern dasyurids apart from thylacines and numbats is less overwhelming, with *cytb* + *12S* + *P1* parsimony trees showing weak bootstrap support for this node and rejecting its alternative in only four of seven treatments. Mitochondrial data alone provide stronger support for Dasyuridae, particularly in distance and likelihood analyses. Thus, the currently available DNA sequence data concur with Wroe's (1997, 1999) morphocladistic results in supporting dasyurid monophyly and tells against the hypotheses of Case (1989) and Szalay (1994) that numbats and/or thylacines arose from dasyurid ancestors.

Our data are less conclusive on relationships among dasyuromorphian families. Combined *cytb* and *12S* analyses uniformly support Thylacinidae as the sister group of Dasyuridae, as do all but one of the *cytb* + *12S* + *P1* analyses, but this arrangement is not significantly stronger than the numbat + dasyurid grouping for any treatment. Bootstrap support for Dasyuridae + Thylacinidae from combined sequences varies from weak (53% for 12 + TV parsimony analysis of *cytb* + *12S* + *P1*) to strong (95% for unpartitioned 3TV + TV distance analysis of *cytb* + *12S*), with most values in the range of 65 to 85%. These results thus concur with previous immunological work (Lowenstein *et al*., 1981) and provide some support for Wroe's (1997) suggestion that development of a hypoconulid notch and enlargement of stylar cusp D may be synapomorphies for dasyurids and thylacinids. Clearly, however, relationships among dasyuromorphian families require further study.

The Price of Partitioning

Partitioned distance ($\overline{d}$) and likelihood (MLC) analyses performed remarkably well in our comparisons. Tables III, V, and VI reveal no trend suggesting that bootstraps from

partitioned treatments are systematically lower than their unpartitioned counterparts, nor is there evidence of reduced statistical power in tree comparison tests based on partitioned analyses. This is in contrast to the finding of Yang *et al.* (1994) that a more complex model of sequence evolution entailed loss of power to discriminate alternative topologies. Yang *et al.* specifically considered a model in which substitution rates among sites varied according to a gamma, rather than a Poisson, distribution. The latter model is obviously incorrect for *cytb* and *12S* (and many other) sequences and this has led some authors (e.g., Sullivan and Swofford, 1997) to advocate use of gamma models for phylogenetic inference from sequences with heterogenous rates. How such models compare with partitioned treatments of the sort employed here has not been well studied (but see Armine and Springer, 1999).

Accounting for among-site rate variation in *cytb* and *12S* sequences can be handled in a number of ways. Average substitution rates clearly vary among codon positions of *cytb*, and between stems and loops of *12S.* By treating these partitions as discrete rate categories in distance and likelihood analyses, much of the among-site rate variation is incorporated into phylogenetic estimates without the assumption of a gamma-rate distribution (Yang, 1996). Moreover, such partitioning captures biologically relevant information about informational and structural constraints on sequence evolution and does not rely on fitting a single distribution of rates to a complex dynamic process. Partitioned distance analyses currently have the additional advantage that parameters other than substitution rate (e.g., transition bias and base composition) can vary among partitions without introducing a large computational burden into tree searches. Data-filtering strategies such as transversion weighting may be applied to some partitions to enhance phylogenetic signal without concomitantly dampening it in others. Partitioning also opens the possibility that residual rate variation within partitions could be accommodated by employing a gamma model for some or all of them, with shape parameters estimated separately for each. The cost in precision and power of such a parameter-rich treatment may be substantial, but may be compensated by improved accuracy on estimates of phylogeny, branch lengths, and divergence times (Yang, 1996).

Our results complement those of Krajewski *et al.* (1999), who compared the accuracy and precision of partitioned and unpartitioned treatments of *cytb* and ND6 genes in a recently evolved group of birds (cranes; divergences <10 million years). In those analyses, partitioned treatments performed as well as or better than unpartitioned ones (including gamma-rates models), but it was unclear whether partitioning would remain well-behaved at deeper divergences where sequences have begun to saturate. Dasyuromorphian results suggest optimism on this front, particularly when appropriate weighting and partitioning strategies are combined.

ACKNOWLEDGMENTS

We thank Lori Armstrong, Larry Buckley, and Jodie Young for assistance in the laboratory, Steve Wroe for discussions of dasyuromorphian anatomy and paleontology, and Mark Springer for information on 12S secondary structure and evolution. Matt Fain, William D. Moore, and Greg Moyer provided helpful comments on the manuscript. This research was supported by funds from the National Science Foundation (grant DEB-9419907 to C.K.), La Trobe University, and Southern Illinois University.

LITERATURE CITED

Aplin, K. P., and Archer, M. (1987). Recent advances in marsupial systematics with a new syncretic classification. In: *Possums and Opposums: Studies in Evolution*, M. Archer, ed., pp. xv–lxi, Surrey Beatty, Sydney.

Archer, M. (1976a). The basicranial region of marsupicarnivores (Marsupialia), interrelationships of carnivorous marsupials, and affinities of the insectivorous marsupial peramelids. *J. Linn. Soc. Lond. (Zool.)* **59:** 217–322.

Archer, M. (1976b). The dasyurid dentition and its relationship to that of didelphids, thylacinids, borhyaenids (Marsupicarnivora) and peramelids (Peramelina: Marsupialia). *Aust. J. Zool. Suppl. Ser.* **39:** 1–34.

Archer, M. (1982). Review of the dasyurid (Marsupialia) fossil record, integration of data bearing on phylogenetic interpretation and suprageneric classification. In: *Carnivorous Marsupials*, M. Archer, ed., pp. 397–443, Royal Zoological Society of New South Wales, Mossman.

Archer, M. (1984). The Australian marsupial radiation. In: *Vertebrate Zoogeography and Evolution in Australia*, M. Archer and G. Clayton, eds., pp. 633–708, Hesperian Press, Carlisle.

Archer, M., and Kirsch, J. A. W. (1977). The case for the Thylacomyidae and Myrmecobiidae, Gill, 1872, or why are marsupial families so extended? *Proc. Linn. Soc. N.S.W.* **102:** 18–25.

Armine, H. M., and Springer, M. S. (1999). Maximum likelihood analysis of the tethythere hypothesis based on a multigene data set and a comparison of different models of sequence evolution. *J. Mammal. Evol.* **6:** 161–176.

Armstrong, L. A., Krajewski, C., and Westerman, M. (1998). Phylogeny of the dasyurid marsupial genus *Antechinus* based on cytochrome-*b*, *12S*-rRNA, and protamine-*P1* genes. *J. Mammal.* **79:** 1379–1389.

Bensley, B. A. (1903). On the evolution of the Australian Marsupialia; with remarks on the relationships of the marsupials in general. *Trans. Linn. Soc. Lond. (Zool.)* **9:** 83–217.

Blacket, M. J., Krajewski, C., Labrinidis, A., Cambron, B., Cooper, S., and Westerman, M. (1999). Systematic relationships within the dasyurid marsupial tribe Sminthopsini–a multigene approach. *Mol. Phylogenet. Evol.* **12:** 140–155.

Case, J. A. (1989). Cranial isometry in Australian carnivorous marsupials and phyletic relationships of the dog-like thylacines. *J. Vertebr. Paleontol. Abstr.* **9:** 16A.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Felsenstein, J. (1993). PHYLIP (phylogeny inference package). Distributed by the author, Department of Genetics, University of Washington, Seattle, WA.

Felsenstein, J., and Churchill, G. A. (1996). A hidden Markov model approach to variation among sites in rate of evolution. *Mol. Biol. Evol.* **13:** 93–104.

Fitch, W. M., and Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155:** 279–284.

Kirsch, J. A. W. (1977). The comparative serology of Marsupialia, and a classification of marsupials. *Aust. J. Zool. Suppl. Ser.* **52:** 1–152.

Kirsch, J. A. W., Lapointe, F.-J., and Springer, M. S. (1997). DNA-hybridisation studies of marsupials and their implications for metatherian classification. *Aust. J. Zool.* **45:** 211–280.

Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* **29:** 170–179.

Krajewski, C., Driskell, A. C., Baverstock, P. R., and Braun, M. J. (1992). Phylogenetic relationships of the thylacine (Marsupialia: Thylacinidae) among dasyuroid marsupials: evidence from cytochrome-b DNA sequences. *Proc. Roy. Soc. Lond. B* **250:** 19–27.

Krajewski, C., Blacket, M., Buckley, L., and Westerman, M. (1997a). A multigene assessment of phylogenetic relationships in the dasyurid marsupial subfamily Sminthopsinae. *Mol. Phylogenet. Evol.* **8:** 236–248.

Krajewski, C., Buckley, L., and Westerman, M. (1997b). DNA phylogeny of the marsupial wolf resolved. *Proc. Roy. Soc. Lond. B* **264:** 911–917.

Krajewski, C., Young, J., Buckley, L., Woolley, P. A., and Westerman, M. (1997c). Reconstructing the evolutionary radiation of dasyurine marsupials with cytochrome *b*, *12S* rRNA, and protamine gene trees. *J. Mammal. Evol.* **4:** 217–236.

Krajewski, C., Fain, M. G., Buckley, L., and King, D. G. (1999). Dynamically heterogenous partitions and phylogenetic inference: an evaluation of analytical strategies with cytochrome *b* and ND6 gene sequences in cranes. *Mol. Phylogenet. Evol.* **13:** 302–313.

Krajewski, C., Wroe, S., and Westerman, M. (2000). Molecular evidence for the pattern and timing of cladogenesis in dasyurid marsupials. *Zool. J. Linn. Soc.*, in press.

Lowenstein, J. M., Sarich, V. M., and Richardson, B. J. (1981). Albumin systematics of the extinct mammoth and Tasmanian wolf. *Nature* (*London*) **291:** 409–411.

Marshall, L. G., Case, J. A., and Woodburne, M. O. (1990). Phylogenetic relationships of the families of marsupials. In: *Current Mammalogy*, Vol. 2, H. H. Genoways, ed., pp. 433–505, Plenum Press, New York.

Muirhead, J. (1994). *Systematics, Evolution, and Palaeobiology of Recent and Fossil Bandicoots (Peramelemorphia, Marsupialia)*, Ph.D. dissertation, University of New South Wales, Sydney.

Muirhead, J., and Wroe, S. (1998). A new genus and species, *Badjcinus turnbulli* (Thylacinidae: Marsupialia), from the Late Oligocene of Riversleigh, Northern Australia, and an investigation of thylacinid phylogeny. *J. Vertebr. Paleontol.* **18:** 612–626.

Nei, M. (1987). *Molecular Evolutionary Genetics.* Columbia University Press, New York.

Retief, J. D., Krajewski, C., Westerman, M., Winkfein, R. J., and Dixon, G. H. (1995). Molecular phylogeny and evolution of marsupial protamine *Pl* genes. *Proc. Roy. Soc. Lond. B* **259:** 7–14.

Ride, W. D. L. (1968). On the past, present and future of Australian mammals. *Aust. J. Sci.* **31:** 1–11.

Sinclair, W. J. (1906). Mammalia of the Santa Cruz beds. *Rep. Princeton Univ. Expedition to Patagonia* **4:** 333–460.

Slade, R. W., Moritz, C., and Heideman, A. (1994). Multiple nuclear-gene phylogenies: application to pinnipeds and comparison with a mitochondrial DNA gene phylogeny. *Mol. Biol. Evol.* **11:** 341–356.

Springer, M. S., and Douzery, E. (1996). Secondary structure and patterns of evolution among mammalian mitochondrial *12S* rRNA molecules. *J. Mol. Evol.* **43:** 357–373.

Springer, M. S., Westerman, M., and Kirsch, J. A. W. (1994). Relationships among orders and families of marsupials based on *12S* ribosomal DNA sequences and the timing of the marsupial radiation. *J. Mammal. Evol.* **2:** 85–115.

Springer, M. S., Hollar, L. J., and Burke, A. (1995). Compensatory substitutions and the evolution of the *12S* rRNA gene in mammals. *Mol. Biol. Evol.* **12:** 1138–1150.

Sullivan, J., and Swofford, D. L. (1997). Are guinea pigs rodents? The importance of adequate models in molecular phylogenetics. *J. Mammal. Evol.* **4:** 77–86.

Szalay, F. S. (1982). A new appraisal of marsupial phylogeny and classification. In: *Carnivorous Marsupials*, M. Archer, ed., pp. 621–640, Royal Zoological Society of New South Wales, Mossman.

Szalay, F. S. (1994). *Evolutionary History of the Marsupials and An Analysis of Osteological Characters.* Cambridge University Press, Cambridge.

Tate, G. H. H. (1947). Results of the Archbold Expeditions No. 56. On the anatomy and classification of the Dasyuridae (Marsupialia). *Bull. Amer. Museum Nat. Hist.* **88:** 97–156.

Thomas, R. H., Schaffer, W., Wilson, A. C., and Paabo, S. (1989). DNA phylogeny of the marsupial wolf. *Nature* (*London*) **340:** 465–467.

Wroe, S. (1997). A reexamination of morphology-based synapomorphies for the families of Dasyuromorphia (Marsupialia). I. Dasyuridae. *J. Mammal. Evol.* **4:** 19–52.

Wroe, S. (1999). The geologically oldest dasyurid, from the Miocene of Riversleigh, northwest Queensland. *Paleontology* **42:** 501–527.

Yang, Z. (1996). Maximum-likelihood models for combined analyses of multiple sequence data. *J. Mol. Evol.* **42:** 587–596.

Yang, Z., Goldman, N., and Friday, A. (1994). Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. *Mol. Biol. Evol.* **11:** 316–324.