

Molecular Evidence for a Deep Clade of Dunnarts (Marsupialia: Dasyuridae: *Sminthopsis*)

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Abstract *Sminthopsis* is the most speciose genus of living dasyurid marsupials and, along with its close relatives *Antechinomys* and *Ningauai*, constitutes the clade Sminthopsini. Phylogenetic relationships among the 23 species in this clade have been the subject of much morphological and molecular investigation, including a recent integration of penis morphology (in *Sminthopsis*) with molecular systematics. Several phylogenetic issues remain open, however, including the monophyly of *Sminthopsis* and branching order among early sminthopsin lineages. In this study, we revisit sminthopsin systematics with an expanded molecular data set, including new DNA sequences from mitochondrial (valine transfer-RNA and 16S ribosomal RNA) and nuclear (interphotoreceptor retinoid binding protein and beta-fibrinogen) loci, along with previously published sequences of cytochrome *b*, 12S ribosomal RNA, control region, and protamine P1. Our results again fail to establish the monophyly of *Sminthopsis*, but do provide a clearer resolution of early sminthopsin branching. Specifically, our phylogeny suggests three major groups of *Sminthopsis*

species: *S. longicaudata* (perhaps the sister of *Antechinomys*); the Macroura species group of previous authors (*S. crassicaudata*, *S. macroura*, *S. virginiae*, *S. douglasi*, and *S. bindi*); and the remaining 13 species allied with the Murina species group. Our results depart from previous molecular findings by reuniting *S. ooldea* with the Murina group, while resolving *S. psammophila* as sister to the hairy-footed dunnarts (*S. hirtipes* and *S. youngsoni*). We suggest that this conflict traces to anomalous phylogenetic signal in previously published cytochrome *b* sequences. Penis morphology maps reasonably well onto our phylogeny, requiring parallel origination of only one of the ten morphotypes described for *Sminthopsis*.

Keywords Marsupial evolution · Dasyurid · Sminthopsinae · Sminthopsini · *Sminthopsis* · *Ningauai* · *Antechinomys*

Introduction

The dasyurid marsupial clade Sminthopsini includes 19 species of dunnarts (*Sminthopsis*), three ningauis (*Ningauai*), and the monotypic kultarr (*Antechinomys*) (Table 1). Dunnarts are the most speciose dasyurid genus, ningauis are among the tiniest marsupials, and the kultarr is notable for its greatly elongated hindlimbs and quadrupedal-bounding gait. These small-bodied carnivores inhabit arid and semi-arid regions of Australia, though some also occur in moister areas of the continental margin; two dunnart species have ranges extending into southern New Guinea.

Morphological and molecular data (summarized by Krajewski and Westerman 2003) have consistently identified *Planigale* (Planigalini) as the sister-group of sminthopsins, and the two groups together are recognized as the dasyurid clade Sminthopsinae (Groves 2005). Krajewski et al. (2000) used a linearized tree approach with sequence

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Table 1 Classification of Sminthopsinae (Krajewski and Westerman 2003) and source data for tissue specimens used in this study

Taxon	Source data ^a
Sminthopsini	
<i>Antechinomys</i>	
<i>A. laniger</i>	LTU colony female 46
<i>Ningau</i>	
<i>N. ridei</i>	Collected by G. Friend (16S, Val); SAM FCAT10 34 (IRBP, bfib7)
<i>N. yvonnae</i>	SAM A16 FGAWLER
<i>N. timealeyi</i>	AM M29385 (16S, Val); AM M29399 (IRBP, bfib7)
<i>Sminthopsis</i>	
<i>S. psammophila</i>	SAM M14288 NP0502
<i>S. longicaudata</i>	WAM M24531
<i>S. granulipes</i>	WAM M20647
<i>S. hirtipes</i>	WAM M20796 = QM JM5239
<i>S. aitkeni</i>	SAM M16158
<i>S. griseoventer</i>	QM JM18178 (16S, Val, bfib7); WAM W1017 (IRBP)
<i>S. youngsoni</i>	QM JM10029
<i>S. ooldea</i>	SAM M17477
<i>S. crassicaudata</i>	Collected by J. Reid (C003), Coongie Lakes, South Australia
Species Group Macroura	
<i>S. macroura</i>	LTU laboratory bred 40/89 (16S, Val) and 38/89 (IRBP); LTU 108 = SAM FCAT 11 18 (bfib7)
<i>S. virginiae</i>	ANWC M29835
<i>S. douglasi</i>	QM JM18113
<i>S. bindi</i>	NTM U0954 (16S, Val, IRBP, bfib7)
Species Group Murina	
<i>S. murina</i>	SAM FCAT 11 38 (16S, Val, IRBP, bfib7)
<i>S. gilberti</i>	QM JM18170
<i>S. leucopus</i>	MW Victoria (16S, Val, bfib7); MW Tasmania (IRBP)
<i>S. butleri</i>	NTM U4417 (16S, Val, IRBP, bfib7)
<i>S. dolichura</i>	QM JM18174
<i>S. archeri</i>	QM JM10959 (16S, Val, bfib7); QM JM10027 (IRBP)
Planigalini ^b	
<i>P. gilesi</i>	SAM FCAT10 3
<i>P. ingrami</i>	QM JM18677 (16S, Val); QM JM 10027 (bfib7, IRBP)
<i>P. maculata</i>	QM JM1486/1487 (16S, Val, IRBP, bfib7)

^aAcronyms: AM Australian Museum; ANWC Australian National Wildlife Collection; LTU La Trobe University; MW M. Westerman collection, La Trobe University; NTM Northern Territory Museum of Arts and Sciences; QM Queensland Museum; SAM South Australian Museum (F and FCAT = Evolutionary Biology Unit tissue collection); WAM Western Australian Museum

^bOnly *Planigale* species employed as outgroups in this study are listed

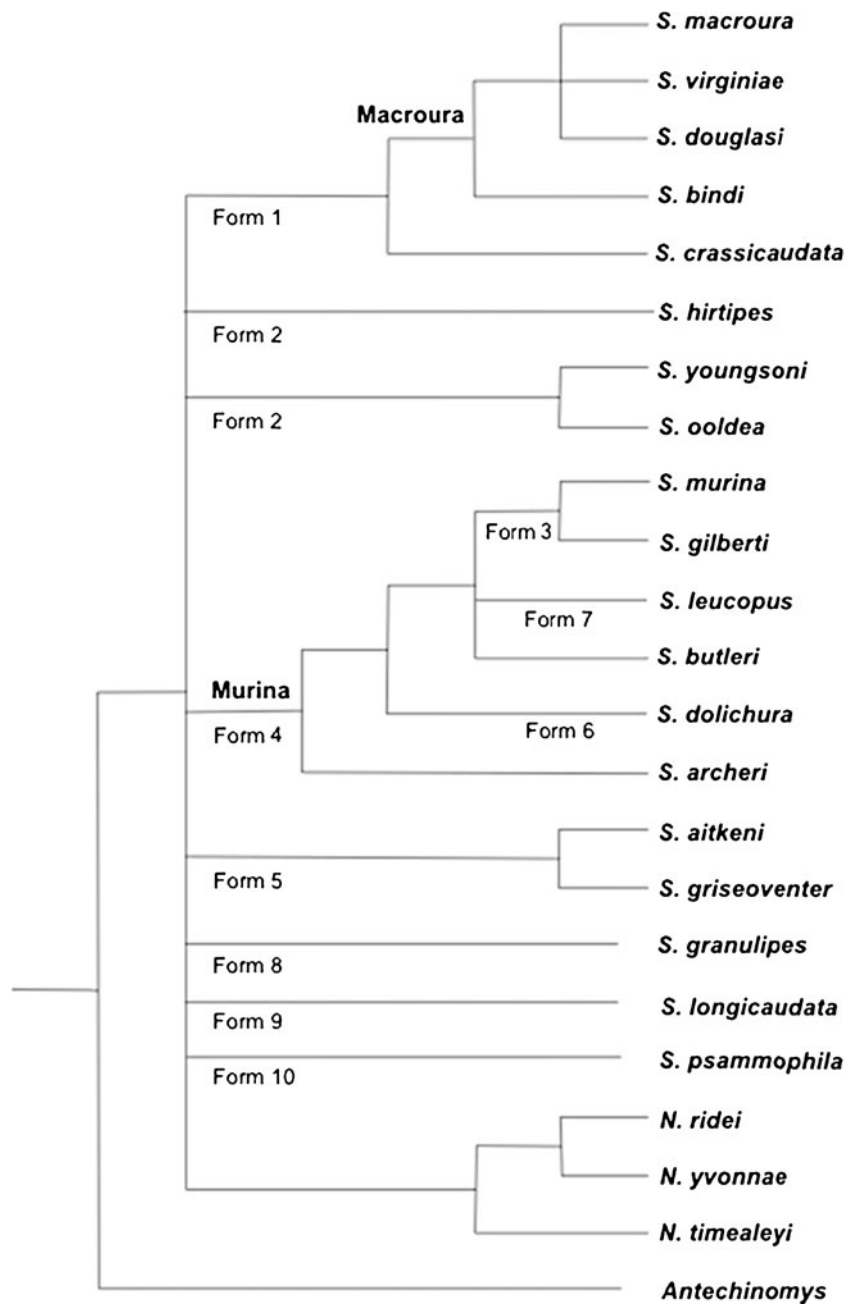
divergences of mitochondrial cytochrome *b* (*cytb*) and 12S rRNA (*12S*) genes from all dasyurids to suggest that sminthopsins and planigalins diverged some 20 million years ago (Mya) in the early Miocene. Sminthopsin lineages diversified rapidly around 15 Mya, such that cladogenic events leading to the modern species were complete before the end of the Miocene.

The history of systematic research on sminthopsins was summarized by Blacket et al. (1999), who also presented the first multigene phylogeny of the group. Blacket et al.'s parsimony analysis of four combined loci failed to resolve the monophyly of *Sminthopsis* apart from *Ningau*, though *Antechinomys* was supported as sister to the clade comprising the former two genera. Within the *Sminthopsis-Ningau* clade,

Blacket et al. (1999) identified two diverse species groups of dunnarts (Macroura and Murina; Table 1), as well as several other resolved nodes. Among the latter, *Ningau* appeared monophyletic, *S. crassicaudata* was sister to the Macroura group, *S. ooldea* was sister to *S. youngsoni*, and *S. griseoventer* was sister to *S. aitkeni* (Fig. 1). Clades similar, but not identical, to the Macroura and Murina groups were suggested by Archer (1981) and Van Dyck et al. (1994) on the basis of morphocladistic analyses.

Subsequent studies clarified relationships within the Macroura and Murina groups. Blacket et al. (2001) used mitochondrial DNA (mtDNA) sequences to show that *S. bindi* is sister to all other species within the Macroura group (Fig. 1).

Fig. 1 Phylogenetic tree for Sminthopsini based on the molecular results of Blacket et al. (1999, 2001, 2006). Polytomies represent nodes that have not received strong support in any of these studies. Genus abbreviations: *S.* = *Sminthopsis*, *A.* = *Antechinomys*, *N.* = *Ningauai*. Murina and Macrourea species groups are labeled. Form numbers on branches are penile morphotypes described by Woolley et al. (2007)



Blacket et al. (2006) reported mtDNA and omega-globin sequences from members of the Murina group, which resolved *S. murina* and *S. gilberti* as sisters within a clade that also includes *S. leucopus* and *S. butleri*. *Sminthopsis dolichura* and *S. archeri* formed successively earlier branches within the group (Fig. 1).

Woolley et al. (2007) described variation in penis morphology among, and its phylogenetic implications for, species of *Sminthopsis*. Based on combinations of six discrete characters, dunnarts display ten penile morphotypes. Although species groupings based on these morphotypes showed little congruence with clades suggested by Archer (1981) or Van

Dyck et al. (1994), they mapped with almost perfect consistency onto the composite molecular phylogeny of Blacket et al. (1999, 2001, 2006) (Fig. 1). In particular, penile Form 1 occurs only in members of the Macrourea group and its sister, *S. crassicaudata*. Penile Form 2 suggests a relationship between Blacket et al.'s (1999) *S. ooldea*-*S. youngsoni* clade and the unresolved *S. hirtipes*. Penile Form 5 occurs only in the sister-species *S. aitkeni* and *S. griseoventer*. Interestingly, the Murina group displays considerable diversity in penile morphology, with four distinct morphotypes occurring among its six member species. Although putative sisters *S. murina* and *S. gilberti* share penile Form 3, Form 4 appears as a

symplesiomorphy in *S. archeri* and *S. butleri*. All other dunnart species display unique morphotypes.

In this study we seek to improve the resolution of phylogenetic branching order within Sminthopsini by expanding Blacket et al.'s (1999) data set of DNA sequence characters. We complement available mtDNA gene sequences of *cytb*, *12S*, and Domain I of the control region (*CR*) with those of 16S rRNA (*16S*) and valine transfer RNA (*Val*). Nuclear protamine P1 (*PI*) gene sequences are complemented by those of interphotoreceptor retinoid binding protein exon 1 (*IRBP*) and beta-fibrinogen intron 7 (*bfib7*). Krajewski et al. (2004, 2007) used a similar combination of loci to resolve relationships among dasyurine marsupials.

Materials and Methods

Taxon Sampling

Sequences were obtained from a single exemplar of each recognized sminthopsin species. Although Blacket et al. (2001, 2006) identified potentially significant phylogeographic structure within some widespread *Sminthopsis* species, there was no indication that haplotypes from currently recognized species would fail to show reciprocal monophyly. Because resolution of nodes near the root is critical for clarifying sminthopsin relationships, we employed three *Planigale* species as outgroups. As noted above, *Planigale* is the sister of Sminthopsini, and multiple exemplars help subdivide its long outgroup branch. Source data for DNA samples used in this study are given in Table 1; similar data for sequences of *cytb*, *12S*, and *CRI* are given in Blacket et al. (1999).

Gene Sampling

Blacket et al. (1999, 2001, 2006) and Krajewski et al. (2000) showed that combined *cytb*, *12S*, and (in the case of sminthopsins) *CR* sequences provide limited resolution of close dasyurid relationships. However, these loci represent only a relatively small fraction (about 15 %) of the mtDNA molecule. Therefore, we attempted to increase resolution on our estimate of the mtDNA tree by adding sequences of *16S* and *Val* to our data set, bringing the number of mtDNA sites up to about 3,600 (roughly 23 % of the total molecule). To date, the only nuclear DNA sequences that have been brought to bear on overall sminthopsin relationships come from *PI*, and these provided very limited resolution (Blacket et al. 1999). Here we augment the sminthopsin nuclear alignment with sequences from *IRBP* and *bfib7*. *IRBP* evolves relatively slowly (as expected for a coding sequence), whereas *bfib7* shows higher rates of change (as expected for a noncoding sequence). The

total length of our nuclear DNA alignment (about 3,200 sites) is comparable to that for mtDNA.

DNA Amplification and Sequencing

DNA was extracted from tissue samples as described by Krajewski et al. (1997). PCR primers, as well as amplification and sequencing protocols, for *Val-16S*, *IRBP*, and *bfib7* are given in Krajewski et al. (2004). Cycle-sequencing employed Big Dye chemistry (Applied Biosystems, Inc.) followed by electrophoresis in an ABI 377 automated sequencer. Individual sequences were aligned by eye separately for Sminthopsini and *Planigale*, and consensus sequences of the two groups aligned to produce a final estimate of homologous sites. Alignments of *cytb*, *12S*, *CR*, and *PI* were taken from Blacket et al. (1999) without modification.

Data Analysis

New sequences were checked for authenticity in several ways: *IRBP* sequences were conceptually translated with the universal genetic code to check for premature stop codons, frameshift indels, or other indications of pseudogene amplification (none were found); *16S* sequences were folded into stem-loop secondary structures according to the model of Burk et al. (2002). Nucleotide compositions of each locus for each species, and the overall alignment, were obtained with MEGA 4 (Tamura et al. 2007). Variable sites of individual and combined loci were examined for compositional heterogeneity among taxa with the χ^2 test implemented by PAUP* (Swofford 2004).

Phylogenetic trees were obtained for data sets comprising individual genes and combinations via maximum likelihood analysis using RAxML 7.0.4 (Stamatakis 2006). For multi-locus data sets, several partitioning schemes were evaluated in preliminary analyses. In one scheme, a single substitution model was applied to the entire alignment (i.e., no partitioning). Data were also partitioned by locus and by locus and codon position. Partitioning by locus and codon position resulted in nine data partitions for the combined mitochondrial + nuclear data set, as follows: partitions for codon positions 1–3 pooled from all protein-coding loci (*cytb*, *PI* and *IRBP*); *12S*; *Val-16S*; *CR*; *PI* 5' and 3' flanking regions; *PI* intron; *bfib7*. Single-model RAxML analyses for all individual genes and combined data sets were performed under the general time-reversible model with among-site rate variation estimated by a discrete gamma approximation (GTR+G), and consisted of 1,000 nonparametric bootstrap replicates with 10 searches per replicate (RAxML options: -f i -b <bootstrap random number seed> -# 1000 -m GTRGAMMA -k -u 10 -p <parsimony random number seed>) for the bootstrap analysis and 500 inferences using 500 distinct randomized MP trees for the best-known

likelihood (BKL) tree search (RAxML options: `-f d -# 500 -m GTRGAMMA -p <parsimony random number seed>`). For partitioned analyses, GTR+G model parameters were estimated separately for each partition in RAxML 7.0.4. An appropriate partitioning scheme for each of the three combined data sets—mtDNA data only, nuclear data only, and all data—was chosen using a second-order correction of the Akaike Information Criterion (AICc), calculated using the total number of characters as the sample size (Posada and Buckley 2004).

Ancestral penis morphotypes (Woolley et al. 2007) were inferred on the combined data maximum-likelihood phylogeny using maximum parsimony (MP) and maximum likelihood (ML) in Mesquite (Maddison and Maddison 2010) under default settings. The Mk1 model (Lewis 2001) was used for ML reconstructions. ML reconstruction allows information about branch lengths to be taken into account during ancestral state reconstruction, and the Mk1 model is suitable for these data in that it allows reconstructions for multistate characters. However, it is a one-parameter model; it does not estimate (or allow) separate rates for gains and losses.

Results

Sequence Characteristics

The following alignments (and numbers of aligned positions) were assembled: *cytb* (1,146 sites); *12S* (971 sites); *Val-16S* (1,706 sites); *CR* (395 sites); *P1* (607 sites); *IRBP* (1,066 sites); *bfib7* (1,460 sites). Only partial sequences were recovered from a few species: *S. butleri cytb* (449 sites); *S. hirtipes bfib7* (844 sites); *P. maculata bfib7* (1,024 sites). The *bfib7* sequence of *S. longicaudata* contained a 595-site inversion relative to other sminthopsins; because the inverted region could be aligned easily with other sequences, we performed phylogenetic analyses with and without these sites. The *bifb7* sequence of *Antechinomys* included a unique 195-site deletion near the center of the intron. Base-composition tests showed no significant departures from homogeneity among taxa. GenBank accession numbers for *cytb*, *12S*, *CR*, and *P1* are given in Blacket et al. (1999); those for sequences generated in this study are JQ413947–JQ413972 (*Val-16S*), JQ687036–JQ687059 (*IRBP*), and JQ599227–JQ599251 (*bfib7*). *IRBP* and *bfib7* sequences and GenBank numbers from *S. crassicaudata* and *P. ingrami* were reported by Krajewski et al. (2007).

Trees from Mitochondrial Loci

Individual mtDNA loci showed very limited support for sminthopsin relationships (Fig. 2a): *12S*, *Val-16S*, and *CR*

recovered *Ningau* (with internal relationships as shown in Fig. 1), the Macroura group of *Sminthopsis*, and *S. griseoventer* + *S. aitkeni*; *Val-16S* and *CR* recovered the Murina group; *Val-16S* recovered the *S. macroura* + *S. virginiae* + *S. douglasi* clade; *CR* recovered *S. crassicaudata* as sister to the Macroura group (Fig. 2a). The only sminthopsin branch with strong bootstrap support from *cytb* united *S. ooldea* and *S. youngsoni* (98 %), in moderate conflict with *12S* and *Val-16S* clades uniting *S. youngsoni* with *S. hirtipes* and *S. psammophila* (79–83 %) (Fig. 2a).

The best-fitting partitioning scheme for all concatenated data sets (mtDNA data only, nuclear data only and all data) was the “gene/pooled codons” partitioning scheme (Table 2); here we only present results from analyses based on this partitioning scheme. Concatenated mtDNA sequences yielded a tree (Fig. 3) with much stronger support for many branches—in fact recovering nearly all resolved nodes shown in Fig. 1, and no others. The separation of *Antechinomys* from other sminthopsins, however, received only weak bootstrap support (58 %). Reanalysis of the mitochondrial alignment without *cytb* (not shown) placed *S. ooldea* as sister to the Murina group (78 %), and *S. youngsoni* with *S. hirtipes* and *S. psammophila* (94 %).

Trees from Nuclear Loci

Trees for *P1* (Fig. 2b) failed to provide strong support for any node within Sminthopsini. *IRBP* and *bfib7* (Fig. 2b) resolved the Macroura group with *S. crassicaudata* as its sister, along with *S. griseoventer* + *S. aitkeni*. In addition, *bifb7* resolved the monophyly of *Ningau*, with *N. ridei* and *N. yvonnae* as sisters, but united *Antechinomys* and *S. longicaudata* in a clade with 100 % bootstrap support. There were no conflicting nodes with >70 % bootstrap support among nuclear-locus trees. A tree based on concatenated nuclear sequences (Fig. 4) showed strong support for the following: (1) *Antechinomys* + *S. longicaudata*; (2) *Ningau*; (3) *S. crassicaudata* as sister to the Macroura group; (4) *S. griseoventer* + *S. aitkeni*; (5) *S. ooldea* as part of the Murina group; (6) *S. hirtipes* + *S. psammophila* + *S. youngsoni*; (7) a clade with all *Sminthopsis* species not part of groups (1)–(3) above. Association of *S. ooldea* with the Murina group conflicts significantly with the mtDNA tree (only when *cytb* is included), but the nuclear relationship is moderately supported by both *IRBP* and *bfib7* (82 % and 83 % bootstraps, respectively). Trees based on analyses of *bfib7* without the *S. youngsoni* inversion showed no significant differences from those that included the (inverted) inversion (trees not shown).

Trees from Combined Mitochondrial and Nuclear Loci

Concatenation of all mtDNA and nuclear sequences yielded a phylogeny (Fig. 5) with strong support for several key groups,

including all seven nodes resolved on the nuclear tree (Fig. 4). Three of these differ markedly from groups found by Blacket et al. (1999, 2001, 2006) and illustrated in Fig. 1. One is the linkage of *Antechinomys* and *S. longicaudata*, support for which comes primarily

from *bfib7*; trees estimated from a combination of all loci except *bfib7* lack this node. The others are *S. psammophila* + *S. hirtipes* + *S. youngsoni*, and *S. ooldea* + Murina, both relating to the conflict between *cytb* and other loci. Nuclear loci also resolve a node that was

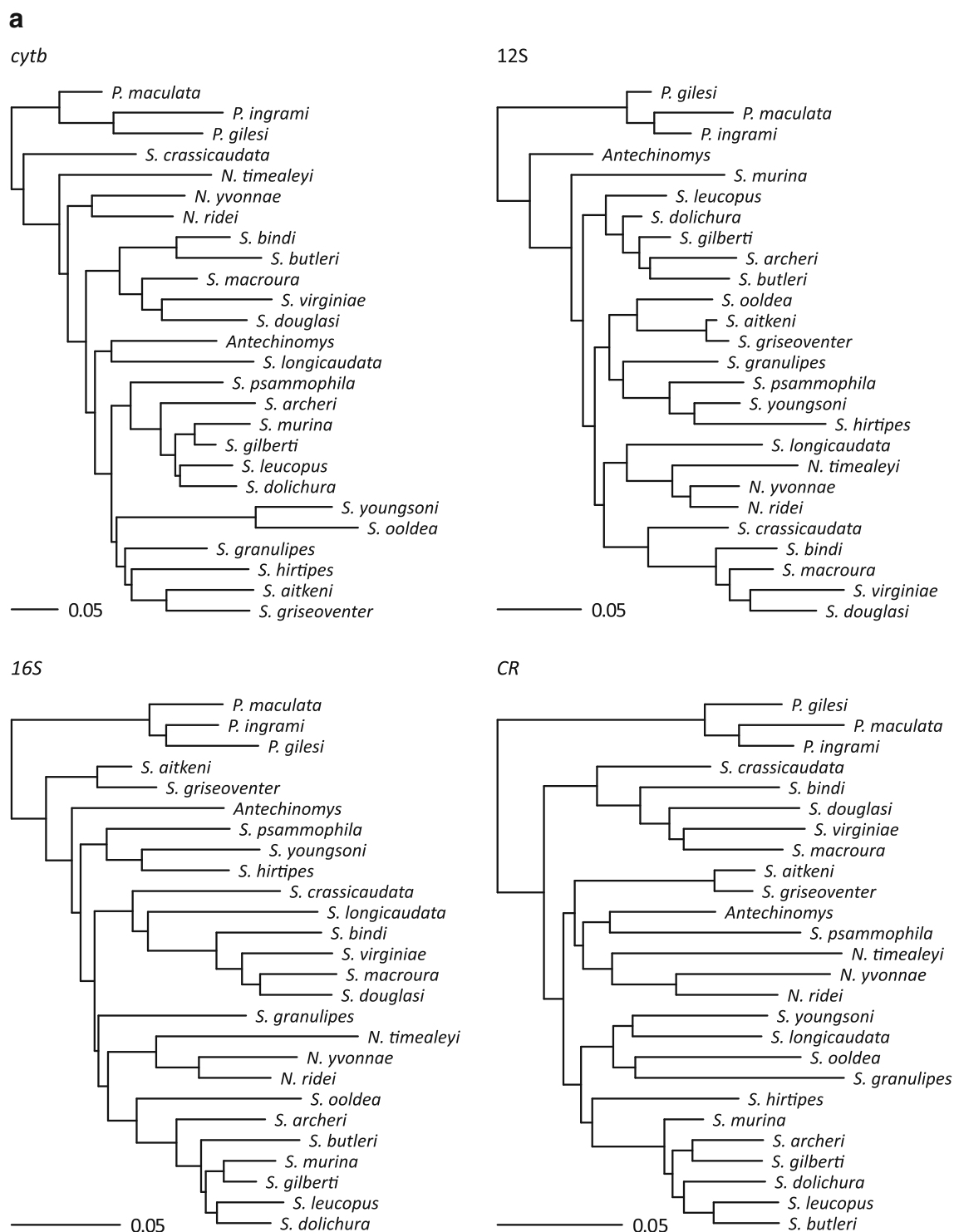


Fig. 2 Best-known likelihood trees for Sminthopsini based on analyses of individual loci, **a** mitochondrial loci, **b** nuclear loci; scale bars denote the estimated number of substitutions per site

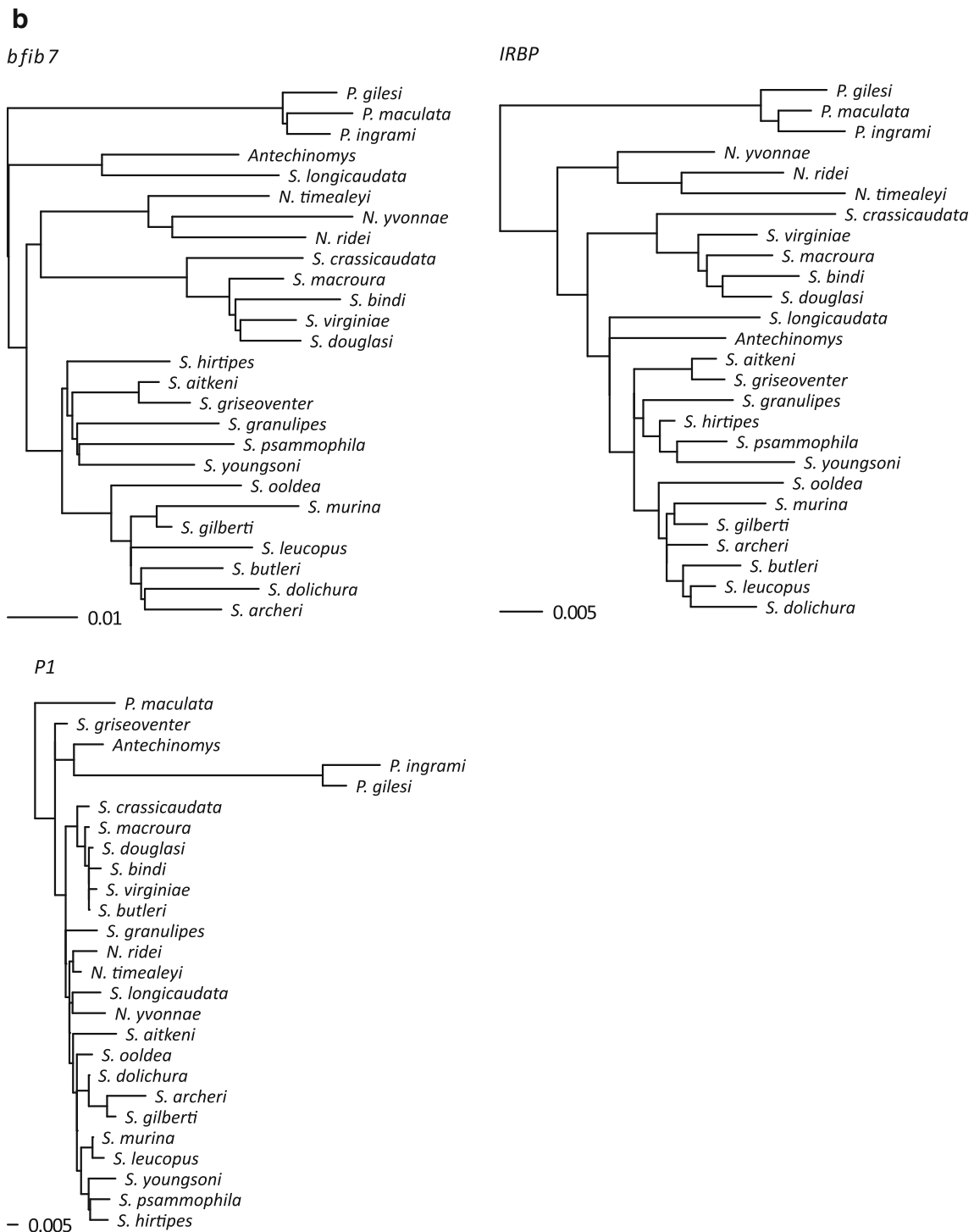


Fig. 2 (continued)

not apparent in previous analyses—namely, a clade of all *Sminthopsis* species other than *S. longicaudata*, *S. crassicaudata*, and the *Macroura* group (clade 7 above).

This branch is supported mostly by *IRBP* and *bfib7*, though neither gene alone provides greater than 75 % bootstrap value for it.

Table 2 AICc values for the RAxML BKL trees for each data set under three partitioning schemes. AICc values were calculated using all characters as the sample size. The number of parameters for each partitioning scheme is the sum of parameters for each substitution model (GTR+G in all cases) across all data partitions (one for the unpartitioned analysis, eight for the “genes” partitioning scheme and ten for the “genes/pooled codons” partitioning scheme). The partitioning schemes with the lowest AICc values for each data set are highlighted with asterisks (*)

Data set	Partitioning	Parameters	lnL	AICc
mtDNA	None	11	-29440.274216	58858.48566
	Genes	44	-29132.209942	58175.47093
	Genes/Pooled Codons	55	-28491.643062	56871.80607*
Nuclear	None	11	-12080.016909	24137.94923
	Genes	33	-11931.331948	23795.93979
	Genes/Pooled Codons	66	-11771.796613	23408.70869*
All data	None	11	-42415.545303	84809.05463
	Genes	77	-41226.189835	82296.72808
	Genes/Pooled Codons	99	-41138.37623	82076.02199*

Discussion

Interlocus Conflict

Results from *cytb* are clearly at odds with those of *12S*, *16S*, *IRBP*, and *bfib7* regarding placement of *S. ooldea* and *S. youngsoni* (Fig. 2). The *cytb*-*12S* discrepancy is apparent in the trees presented by Blacket et al. (1999), but *cytb* signal predominated when the loci were combined. The additional loci included here suggest, however, that *cytb* is anomalous. *Sminthopsis ooldea* + *S. youngsoni* formed the longest branch on the *cytb* distance tree of Blacket et al. (1999), and this is also true for the mtDNA tree reported here. The two sequences are not particularly similar, showing 12.9 % mismatch; their 148 variable sites are distributed along the length of the gene, except for a 175-base region (positions 518–692) where they are identical. The sequences were confirmed with data from a second individual of each species (M. Westerman, unpublished data), and neither show obvious signs of being a pseudogene. Further phylogenetic analyses revealed that their anomalous behavior is restricted

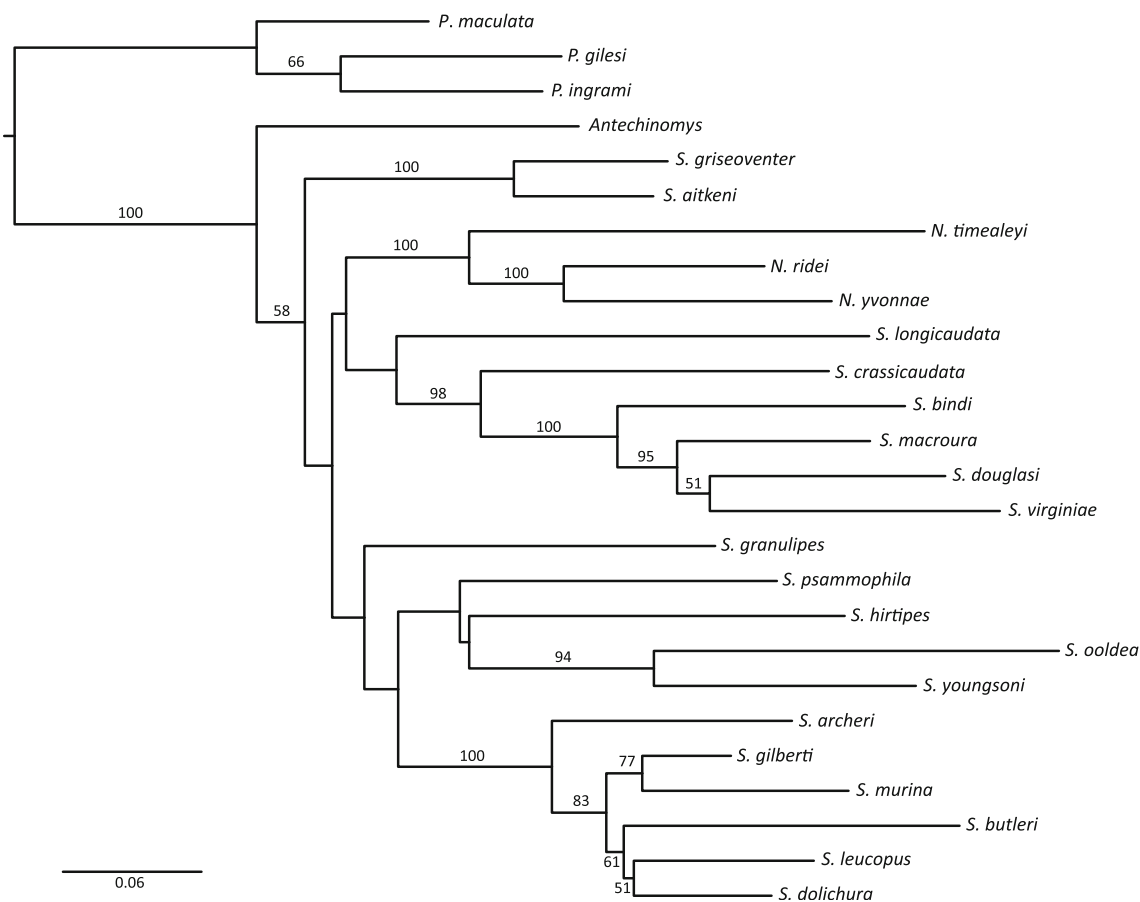


Fig. 3 Maximum-likelihood tree and bootstraps for combined mtDNA loci (*cytb*, *12S*, *16S*, and *CR* sequences). The tree was obtained with RAxML, partitioning the data by genes and pooled codon positions, with a separate GTR + Γ model of nucleotide substitution assigned to

each partition. Bootstrap values are based on 100 resamplings; only values above 50 % are shown. Branch length scale is substitutions per site. Genus abbreviations as in Fig. 2, and *P.* = *Planigale*

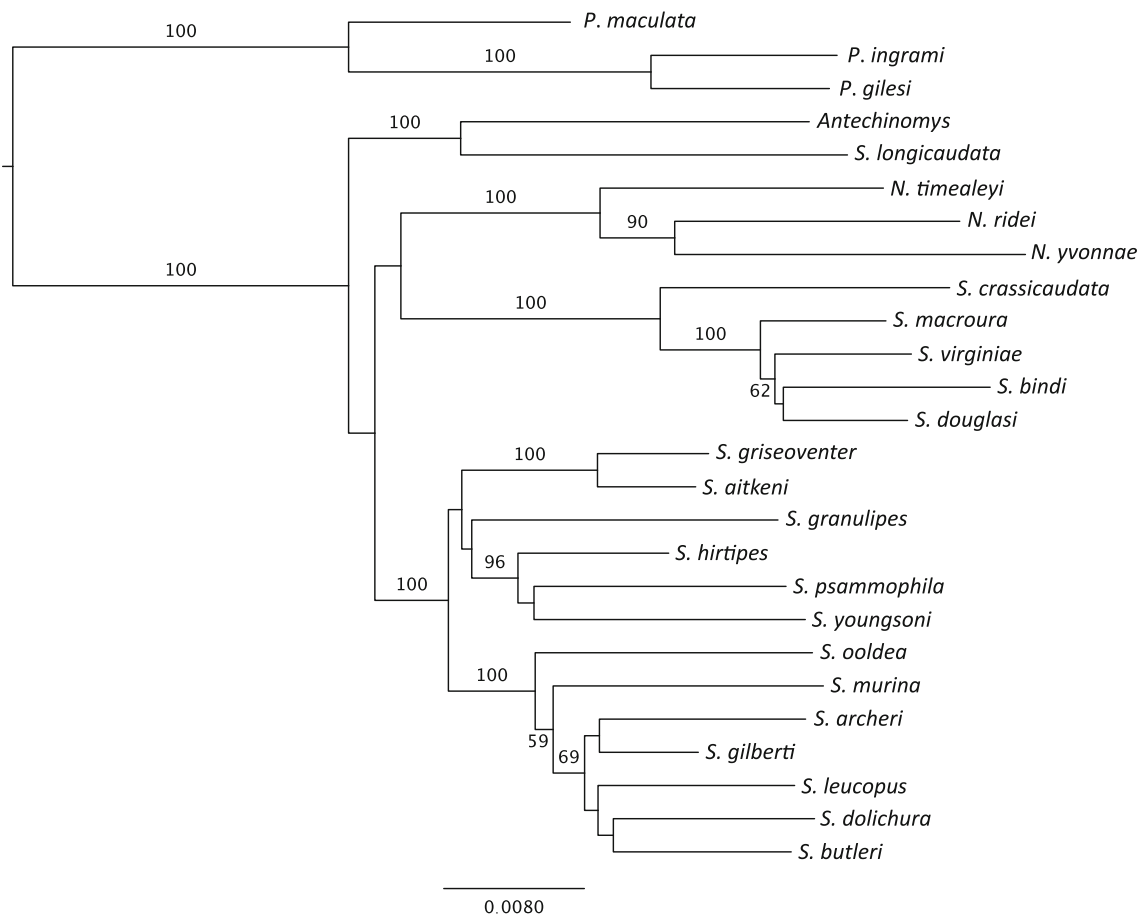


Fig. 4 Maximum-likelihood tree and bootstraps for combined nuclear loci (*P1*, *IRBP*, *bfib7*, and *eglob* sequences). Details and labeling conventions as for Fig. 3

to the identical region and all positions upstream from it; the downstream 454 bases yield trees consistent with Figs. 4 and 5. Although the cause of this phenomenon is unclear, we tentatively conclude that the *cytb* data for these two species are misleading.

Phylogeny of Sminthopsini

The cladogram in Fig. 5 represents a hypothesis of sminthopsin phylogeny based on our multilocus data set. These results fail to confirm the monophyly of *Sminthopsis* apart from *Antechinomys* and *Ningaui*. Based on parsimony analysis of combined *cytb*, *12S*, *CR*, and *P1* sequences, Blacket et al. (1999) resolved *Antechinomys* as sister to other sminthopsins (Fig. 2), a result strongly influenced by *12S* and *P1*. These loci also indicate separation of *Antechinomys* in our ML trees (45 % and 73 % bootstraps, respectively), but with much less support than *bfib7* provides for *Antechinomys* + *S. longicaudata* (100 %). The latter clade also appears on the *cytb* ML tree, but with a bootstrap of only 33 %;

indeed, its support drops from 90 % to 75 % when *cytb* is excluded from the combined analysis. Placement of *Antechinomys* within the *Sminthopsis* radiation is not without precedent—similar results were obtained from morphocladistic analyses by Archer (1981) and Van Dyck et al. (1994). The latter authors adduced three synapomorphies for nesting *Antechinomys* within *Sminthopsis*, and six cranial traits linking it with *S. longicaudata*. Although Blacket et al. (1999) echoed Archer's (1981) concern that these traits might be convergent, our results seem to vindicate Van Dyck et al.'s (1994) interpretation.

Archer (1981) first recognized *S. ooldea* as distinct from *S. murina*, but retained it in his Murina species group (along with *S. murina*, *S. leucopus*, and *S. longicaudata*). This group was expanded when Kitchener et al. (1984) described *S. aitkeni*, *S. dolichura*, *S. gilberti*, and *S. griseoventer* (all formerly parts of *S. murina*). Baverstock et al. (1984) found that *S. ooldea* and *S. griseoventer* were allozymically distinct from other Murina species (though *S. aitkeni* and *S. longicaudata* were not included in the study). Although Van Dyck et al.

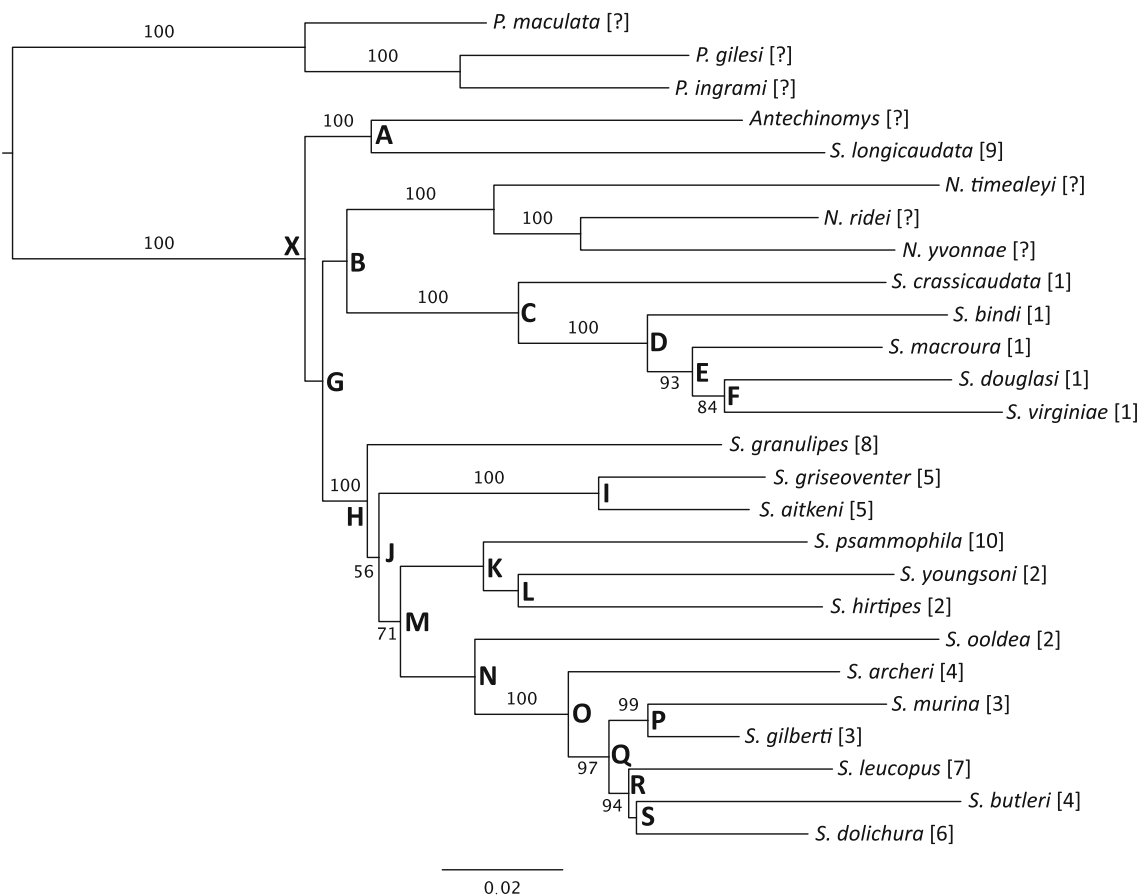


Fig. 5 Maximum-likelihood tree and bootstraps for combined mitochondrial and nuclear loci (*cytb*, *12S*, *16S*, *CR*, *PI*, *IRBP* and *bfb7* sequences). Details and labeling conventions as for Fig. 3. Letters are nodes corresponding to those in Table 3

(1994) found no morphocladistic support for a Murina group, Blacket et al. (1999) recovered a remnant of it that excluded *S. aitkeni*, *S. griseoventer*, and apparently *S. ooldea* (which they linked to *S. youngsoni*) (Fig. 2). Our results suggest that the latter grouping was an artifact of *cytb*, and that the true affinities of *S. ooldea* lie with the Murina group, within which it is sister to the remaining species. The many genetic and morphological similarities between *S. aitkeni* and *S. griseoventer* found by Kemper et al. (2011) are consistent with our results; indeed, these authors suggested that the two forms might be conspecific.

A close relationship between *S. hirtipes* and *S. youngsoni* was anticipated in the original description of the latter by McKenzie and Archer (1982), and by Van Dyck et al. (1994). Indeed, the common names of these species—hairy-footed and lesser hairy-footed dunarts, respectively (Van Dyck and Strahan 2008)—reflect their long-presumed relationship, here supported by molecular data for the first time. Placement of *S. psammophila* in a clade with *S. hirtipes* and *S. youngsoni* is a novel result, though Blacket et al. (1999) referred to a

poorly supported “Psammophila Group” that included these three along with *S. ooldea*. Both Archer (1981) and Van Dyck et al. (1994) considered *S. psammophila* as sister to *S. granulipes* on the basis of similarities in dentition and toe pads; until this study, no molecular data set has been able to identify close relatives of *S. granulipes* (see below).

A basal polytomy of lineages has been characteristic of molecular phylogenetic studies of sminthopsins (Fig. 1), including those based on allozymes (Baverstock et al. 1982), albumin immunology (Baverstock et al. 1990), and early DNA sequences (Blacket et al. 1999). Our multilocus analyses, however, resolve a deep node among dunnarts that has not previously been identified—namely, that comprising the Murina group (with *S. ooldea*), *S. hirtipes* + *S. youngsoni* + *S. psammophila*, *S. aitkeni* + *S. griseoventer*, and *S. granulipes*. The monophyly of this group apart from other sminthopsins is strongly supported by *IRBP* and *bfb7* sequences, and not contradicted by other loci. Resolution of this node results in four distinct lineages at the base of the sminthopsin radiation: *Ningau*, *Antechinomys* + *S. longicaudata*,

Table 3 Maximum parsimony (MP) and maximum likelihood (ML) reconstructions of ancestral penis morphotypes. Node IDs refer to Fig. 5. For the ML reconstructions, only morphotypes with a proportional likelihood greater than 0.05 are listed. If multiple morphotypes with proportional likelihoods greater than 0.05 are reconstructed for a given node, the morphotype with the highest proportional likelihood is shown in bold. Asterisks (*) indicate nodes where there is conflict between MP and ML reconstructions; question marks indicate that states could not be constructed for the node under ML because the penis morphotype for one of the two lineages descending from the node is unknown (*Antechinomys* for node A, *Ningauai* for node B). For details on penis morphotypes, see Woolley et al. (2007)

Node ID	Ancestral penis morphotype reconstruction	
	Parsimony	Proportional likelihood (Mk1 model)
A	1,2,5,8,9	?
B	1,2,5,8,9	?
C	1	1: 0.900
D	1	1: 0.995
E	1	1: 0.999
F	1	1: 0.999
G	1,2,5,8,9	1: 0.269, 2: 0.293 , 5: 0.109, 8: 0.077, 9: 0.085
H	1,2,5,8,9	1: 0.152, 2: 0.390 , 5: 0.138, 8: 0.094, 9: 0.050
I	5	5: 0.956
J	1,2,5,8,9	1: 0.126, 2: 0.424 , 3: 0.053, 4: 0.055, 5: 0.148, 8: 0.078
K	2	2: 0.691 , 5: 0.050, 10: 0.078
L	2	2: 0.799
M	2	1: 0.088, 2: 0.521 , 3: 0.061, 5: 0.104, 8: 0.056
N	2	1: 0.055, 2: 0.482 , 3: 0.123, 4: 0.127, 5: 0.064
O	4	2: 0.146, 3: 0.317, 4: 0.329 , 6: 0.062, 7: 0.055
P	3	3: 0.900
Q*	4	2: 0.065, 3: 0.448 , 4: 0.284, 6: 0.082, 7: 0.071
R*	4	2: 0.054, 3: 0.361 , 4: 0.296, 6: 0.126, 7: 0.110
S*	4	3: 0.338 , 4: 0.303, 6: 0.148, 7: 0.101
X	1,2,5,8,9	1: 0.256, 2: 0.279 , 5: 0.106, 8: 0.076, 9: 0.106

the Macroura group, and all other dunnarts (including the Murina group). It is the diversification of these groups that Krajewski et al. (2000) dated to the mid-Miocene.

Evolution of Penis Morphology in Sminthopsini

Woolley et al. (2007) documented a notable consistency between groups of dunnarts established by phylogenetic analysis of DNA sequences and those based on morphology of the penis (Fig. 1). Penile characters vary from evolutionarily conservative (e.g., a single morphotype in all members of the Macroura group) to labile (e.g., four morphotypes within the Murina group), but showed relatively little homoplasy (consistency index=0.79) on the tree in Fig. 1. This is less true for penile traits mapped onto the phylogeny in Fig. 5 (consistency index=0.62). However, parsimony reconstructions reveal only one ambiguous ancestor (that of *S. hirtipes* + *S. youngsoni* + *S. psammophila*) and suggest that penile morphotypes support at least two of the four lineages (Table 3). In the large *Sminthopsis* clade containing the Murina group, penile Forms 2–8 are most parsimoniously derived directly from Form 4 via 1–4 state-changes in terminal branches or their immediate ancestors. Only Form 2 arises in parallel (in *S. ooldea* and in

S. hirtipes + *S. youngsoni*), plausibly due to a single homoplastic step (ventral lobe absent → ventral lobe small) on each branch. All other forms in this group arise only once, but most (Forms 5–8 and 10) require two to four parallel character-changes each.

There is no published description of penis morphology for *Antechinomys*, though Woolley (1984) cited unpublished observations that it is distinct enough from that of *Sminthopsis* to warrant recognition of separate genera. At the level of gross anatomy, the penis of *Antechinomys* is quite distinct from that of *S. longicaudata*, other sminthopsins, and indeed all other dasyurids (Woolley, unpublished data). No descriptions of penis anatomy in *Ningauai* are available in the literature. However, Table 3 and Fig. 5 suggest that early sminthopsin lineages may have possessed distinct penis morphologies, with Form 1 ancestral for the Macroura clade, Form 4 ancestral for the Murina clade, and Form 9 ancestral for *S. longicaudata*. Data on the penis structures of *Antechinomys* and *Ningauai* may help elucidate the branching order among these lineages.

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