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A molecular phylogenetic analysis of the Scarabaeinae (dung beetles)

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Abstract

The dung beetles (Scarabaeinae) include *ca.* 5000 species and exhibit a diverse array of morphologies and behaviors. This variation presumably reflects the adaptation to a diversity of food types and the different strategies used to avoid competition for vertebrate dung, which is the primary breeding environment for most species. The current classification gives great weight to the major behavioral types, separating the ball rollers and the tunnelers, but existing phylogenetic studies have been based on limited taxonomic or biogeographic sampling and have been contradictory. Here, we present a molecular phylogenetic analysis of 214 species of Scarabaeinae, representing all 12 traditionally recognized tribes and six biogeographical regions, using partial gene sequences from one nuclear (28S) and two mitochondrial (*cox1*, *rrnL*) genes. Length variation in 28S (588–621 bp) and *rrnL* (514–523 bp) was subjected to a thorough evaluation of alternative alignments, gap-coding methods, and tree searches using model-based (Bayesian and likelihood), maximum parsimony, and direct optimization analyses. The small-bodied, non-dung-feeding *Sarophorus* + *Coptorhina* were basal in all reconstructions. These were closely related to rolling *Odontoloma* + *Dicranocara*, suggesting an early acquisition of rolling behavior. Smaller tribes and most genera were monophyletic, while Canthonini and Dichotomiini each consisted of multiple paraphyletic lineages at hierarchical levels equivalent to the smaller tribes. Plasticity of rolling and tunneling was evidenced by a lack of monophyly (S-H test, $p > 0.05$) and several reversals within clades. The majority of previously unrecognized clades were geographical, including the well-supported Neotropical Phanaeini + Eucraniini, and a large Australian clade of rollers as well as tunneling *Coptodactyla* and *Demarziella*. Only three lineages, Gymnopleurini, *Copris* + *Microcopris* and *Onthophagus*, were widespread and therefore appear to be dispersive at a global scale. A reconstruction of biogeographical characters recovered 38–48 transitions between regions and an African origin for most lineages. Dispersal–vicariance analysis supported an African origin with links to all other regions and little back-migration. Our results provide a new synthesis of global-scale dung beetle evolution, demonstrating the great plasticity of behavioral and morphological traits and the importance of biogeographic distributions as the basis for a new classification.

Keywords: Bayesian; Biogeography; Dispersal–vicariance; Evolution; Feeding behavior; Gap-coding; Length-variation; Likelihood; Nesting behavior; Scarabaeidae

1. Introduction

Dung beetles in the Scarabaeinae include *ca.* 5000 species and show a great diversity of morphology and nesting behaviors. This diversity is thought to arise primarily from their adaptation to feeding on a wide variety of vertebrate dung types and to breeding in a competitive environment. A number of theories have been advanced to explain the evolutionary history of complex behaviors and bizarre morphologies and these often are related to the evolution of different nesting strategies (Cambefort, 1991a), sexual selection (Emlen et al., 2005), historical biogeography (Cambefort, 1991b; Davis et al., 2002), and inter-species competition for an ephemeral resource (Emlen, 1997; Hanski and Cambefort, 1991). Evolutionary studies of Scarabaeinae have been greatly influenced by the classification of Balthasar (1963) with the recognition of two groups, Scarabaeinae and Coprinae (or Scarabaeini and Coprini

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of earlier authors, e.g. Janssens, 1949), separated based on their rolling and tunneling nesting behavior, respectively. Whereas tunnelers bury dung directly beneath the dung pat, many rollers display a spectacular behavior of forming dung balls and rolling them to distant sites for burial. Each of these two main groups were subdivided into six tribes to reflect their great morphological and biogeographical diversity, whereby two widespread tribes, the rolling Canthonini and tunneling Dichotomiini, were thought to be ancient lineages that pre-date the break up of Gondwanaland (Cambefort, 1991b; Davis et al., 2002). The biogeographically more localized tribes were considered to be derived from these widespread 'old' lineages to form the 'intermediate' and 'modern' tribes (Cambefort, 1991b).

Although plausible, this hypothesis of dung beetle evolution has not stood up to tests from phylogenetic analysis. Using genitalic characters, Zunino (1983) generally confirmed the Scarabaeinae–Coprinae dichotomy but found the 'intermediate' Onitini and Phanaeini (tunnelers) near the 'intermediate' Eucraniini (rollers), thus rendering the rolling tribes paraphyletic. Montreuil (1998) examined 27 genera of tunnelers in the 'modern' Coprini and 'old' Dichotomiini, and found both tribes to be paraphyletic, clearly refuting the existing evolutionary scenario. This was confirmed in a wider representation of tribes by Philips et al. (2004) who found neither of the 'old' tribes Dichotomiini and Canthonini monophyletic, while rolling and tunneling lineages were intermixed throughout the tree. Finally, phylogenetic analyses based on mitochondrial (Villalba et al., 2002) and nuclear (Ocampo and Hawks, 2006) gene sequences, examining local faunas in the Iberian Peninsula and southern South America, respectively, also broadly contradicted the Balthasar (1963) classification. The main findings in these studies for deep level phylogenetics were the demonstration of the affinity of Onthophagini, Oniticellini, and Onitini (Villalba et al., 2002) and the grouping of Dichotomiini, Phaenaeini, and Eucraniini (Ocampo and Hawks, 2006). Taken together, these recent studies have demonstrated clear insufficiencies in the older classification, with important implications for our understanding of the evolution of Scarabaeinae and biogeographical scenarios (Davis et al., 2002). Nonetheless, recent studies have been restricted taxonomically and geographically, with widely differing conclusions on phylogenetic relationships, and hence no new synthesis of evolutionary patterns in Scarabaeinae has emerged.

Here, we address basal relationships in Scarabaeinae using partial gene sequences from one nuclear and two mitochondrial genes. We focused on the geographic relationships between the major hypothesized areas of dung beetle evolution in the southern continents and islands, and we obtained broad taxonomic coverage with particular emphasis on the two 'old' groups of Canthonini and Dichotomiini. This analysis shows the importance of wide taxonomic sampling, in particular from the presumed ancient areas of their diversification in the southern hemisphere, in order to understand the biogeographical diversification and the evolution of nesting strategies of Scarabaeinae. The results are broadly consistent with an 'out-of-Africa' scenario of early lineages, followed by diversification and repeated switching between rolling and tunneling behavior in other southern continental areas.

2. Materials and methods

2.1. Taxon sampling, DNA extraction, and DNA sequencing

Taxa were collected from Neotropical, Palearctic, Afrotropical, Malagasy, Oriental, and Australasian regions (Appendix A). Fourteen species of Aphodiinae including three species of *Aphodius*, the presumed sister taxon of Scarabaeinae (Browne and Scholtz, 1999), were used as outgroups in the analysis. Most beetles were caught in dung or carrion traps or were taken from museum collections. Details of collecting methods are given in Inward (2003). DNA was extracted using phenol/chloroform methods (Vogler et al., 1993), Qiagen DNeasy columns, or Promega WizardSV extraction plates. Tissue typically was extracted from flight muscle or leg muscle tissue. For some small individuals (body length <5 mm), the whole specimen was used for extraction.

One nuclear and two mitochondrial gene regions were chosen in an attempt to resolve relationships at tribal and generic levels. Approximately 600 bp of nuclear 28S rRNA was amplified using newly designed 28SFF (5′ TTACACA CTCCTTAGCGGAT) and 28SDD (5′ GGGACCCGTC TTGAAACAC), or 28SKa (5′ ACACGGACCAAGGA GTCTAGCATG) and 28SKb (5′ CGTCCTGCTGTCT TAAGTTACC). The 3′ region of cytochrome oxidase I (*cox1*) was amplified using primers Pat and Jerry (Simon et al., 1994) and *ca.* 520 bp of the 3′ end of 16S ribosomal RNA (*rrnL*) was amplified using primers 16Sar (Simon et al., 1994) paired with either 16SB2 (CTCCGGTTTG AACTCAGATCA) or 16Sb2 (TTTAATCCAACATCGA GG). Forward and reverse strands were sequenced using the same primers and a BigDye v.3.1 (ABI) sequencing reaction. Chromatograms were assembled and edited using Sequencher (Genecodes Corp., Ann Arbor, MI, USA). All sequences used in the present study have been submitted to GenBank (Appendix A).

2.2. Multiple alignment and direct optimization

A major factor of uncertainty about the tree topology was due to length variation in *rrnL* (514–523 bp) and 28S rRNA (588–621 bp). This problem was addressed using two approaches. We used dynamic homology searches implemented in POY (Direct Optimization; Wheeler, 1996) and a two-step protocol of building fixed alignments based on a range of gap penalties in ClustalW (Higgins et al., 1996) followed by tree searches. This two-step protocol was used to determine preferred matrices for both model-based and parsimony-based analyses.

ClustalW multiple alignments were performed under a range of gap opening penalties to assess the congruence of markers and the effect of alignment on topology. A starting alignment for each gene was made using complete *rrnL* and 28S sequences for the full taxon set ($n = 225$) under the ClustalW (align.genome.jp) IUB weight matrix default parameters of gap opening and extension penalties of 15 and 6.66, respectively. The resulting alignments (matrix size *rrnL* = 544 bp, 28S = 650 bp) were used to establish a 'length-invariable' matrix, by removing length-variable regions (*rrnL* = 154 bp, 28S = 174 bp). The length-invariable *cox1* alignment was added to produce a combined matrix of 1668 bp. Parsimony searches using TNT (Goloboff et al., 2004) with 10 ratchet iterations, 10 cycles of tree drifting, and 3 rounds of tree fusing for each of 200 random addition sequences returned three shortest trees of 13,266 steps. This tree was then used to evaluate different alignments of the length-variable regions of *rrnL* and 28S by assessing the Incongruence Length Difference (ILD). We calculated the percent incongruence as the proportional increase in tree length when aligned characters of *rrnL* and 28S were added to the length-invariable characters and gaps were treated as a 5th base. We calculated this incongruence across a range of parameter space, using six different gap opening penalties of 1, 5, 10, 15, 20, and 25 for each gene region.

The process was repeated using 'simple coding' (Simmons and Ochoterena, 2000) implemented in the GapCoder method (Young and Healy, 2003). This approach recodes multiple-residue gaps as the unit of character change, rather than coding each nucleotide position as an individual character, to avoid the greater weight that may be afforded to indels with growing length. Under this procedure the original gap positions are coded as 'missing' and gaps with different beginning and/or endpoints in the aligned matrix are coded as separate characters whose presence/absence is scored and added as binary characters to the nucleotide matrix (Simmons and Ochoterena, 2000). Each of the six matrices was re-coded in this manner and congruence with the length-invariable matrix was assessed as above.

Direct optimization was performed using POY v. 3.0.11 (Wheeler et al., 2002) on the combined matrix of all three genes. The length-invariable *cox1* data were included in the tree searches as prealigned. Fragments of *rrnL* and 28S gene regions were divided into conserved and variable regions based on the starting alignment to reduce the computational effort. Only a single *Aphodius* was used as an outgroup to avoid the use of partially incomplete sequences in the remaining outgroup species in the POY searches (not shown). All tree searches were performed in parallel on a 14-node dual-processor (2.8 GHz P4, 2 GB RAM) cluster at Imperial College London. The search strategy was based on that of Giannini and Simmons (2003) and consisted of 25 iterations of random addition sequences, each followed by tree bisection reconnection (TBR) branch swapping, with nucleotide transformations minimized with a cost ratio of indels, transversions, and transitions of 1:1:1. All distinct trees from this initial step were then submitted to the more rigorous tree fusing (Goloboff, 1999), with up to 10,000 fusings allowed and up to 1000 tree fusing trees kept and exchange of subtrees of minimal size during fusing.

2.3. Phylogenetic analysis

Model-based phylogenetic analyses were performed on the alignments with lowest incongruence as determined with parsimony (described above). We conducted a maximum likelihood analysis on the matrix chosen with standard coding (gaps as a fifth state), using PhyML (Guindon and Gascuel, 2003) under a $\mathrm{GTR} + \mathrm{I} + \Gamma$ model with all parameters estimated from the data. Models of character variation could be improved if applied separately to functionally different data partitions that are presumably affected by different dynamics of sequence evolution. This was implemented in a Bayesian analysis on the preferred alignments from each of the two different codings (standard and 'simple'). We used parallel MrBayes v. 3.1 (Altekar et al., 2004; Ronquist and Huelsenbeck, 2003) running on 5 Macintosh nodes under POOCH (Dauger Research, California, USA). Partitioned Bayesian analyses (Brandley et al., 2005) were performed by separating the combined matrix into seven partitions: one for each codon position of *cox1*, and a length-invariable and a length-variable partition for each of *rrnL* and 28S. An added partition of simple-coded (binary) gap characters from GapCoder was added to each of *rrnL* and 28S for a 9-partition analysis. Searches were conducted using a $\mathrm{GTR} + \mathrm{I} + \Gamma$ model on all partitions, except for the two binary partitions which were examined using a F81 model with variable coding bias (see Ronquist and Huelsenbeck, 2003). Several initial analyses on partitioned and un-partitioned (i.e. all data in a single partition) data sets were used to explore the effects of partitioning, running relatively short (*ca.* 2,000,000 generations) MCMC searches. Parameter values and the success of cold chain swapping were evaluated from these initial runs to determine the appropriate settings.

Once the preferred scheme for partitioning had been established, in-depth analyses were conducted for 6,000,000 generations, using random starting trees and 2 runs of 4 heated and 1 cold Markov chains (heating of 0.05). Chains were sampled every 100 generations and a burn-in of 5,000,000 generations was selected based on the average standard deviation of split frequencies as well as by plotting $-\ln L$ against generation time. Because of the relatively high average standard deviation of split frequencies (*ca.* 0.14 for the 7-partition model), model parameters and trees were selected using the higher likelihood of the two runs. We calculated a Bayes factor as the ratio of the harmonic means of $-\ln L$ (calculated with the *sump* command in MrBayes, e.g.) to compare whether different

models gave significantly better fit to the data following Brandley et al. (2005).

Branch lengths were estimated on the Bayesian topology under maximum likelihood using a GTR + I + Γ model in PAUP* v 4.0b10 (Swofford, 2002). To generate clock-constrained branch lengths, relative ages of nodes were estimated using penalized likelihood and non-parametric rate smoothing as implemented in r8s v 1.7 (Sanderson, 2003). The outgroup was pruned from this tree and the ingroup node set to 100, an arbitrary number used in the absence of absolute node ages.

Maximum parsimony searches were conducted on the two preferred alignments (standard gaps and simple coding) using TNT with 10 ratchet iterations, 10 cycles of tree drifting, and 3 rounds of tree fusing for each of 200 random addition sequences. Bremer support was calculated for a subset of nodes on the resulting trees by constraining single nodes for non-monophyly and repeating TNT searches as above.

2.4. Biogeographical analysis

Biogeographical patterns were investigated by the study of character transformations on phylogenetic trees in MacClade v. 4.06 (Maddison and Maddison, 1992). Six biogeographical regions were defined as character states: Africa, Madagascar, the Neotropics, Australasia (including New Zealand and New Caledonia), Eurasia (Spain, Turkey), and the Oriental region (Nepal, Indonesia, Malaysia, Hong Kong). The evolutionary history of Scarabaeinae has traditionally been viewed as the result of a mixture of dispersal and vicariance patterns (Davis et al., 2002), thus we also investigated the character distribution with dispersal–vicariance analysis using DIVA (Ronquist, 1997). This method optimizes scenarios of biogeographical history on the phylogenetic trees, from inferring the build-up of physical barriers (vicariance) and establishment of (temporary) connections between areas of endemism (dispersal). The implementation of the method is constrained by the number of taxa in a single analysis. We therefore reduced the number of taxa in our analyses by using only a single representative for those monophyletic groups confined to the same geographic area based on our reconstruction. The DIVA procedure is conservative in assigning multiple areas (dispersal links) to nodes, where sister taxa emanate from these nodes with tip level taxa distributed in several areas. The output therefore is a list of several areas possibly linked at higher node levels, creating ambiguity in the assignment of areas at nodes. We reduced this ambiguity by limiting the areas assigned to each node where a simple sequence of nodal assignments could be established upward toward the tips of the tree with a subset of two states assigned to the nodes (i.e. assumes a specific sequence of dispersal events). This greatly reduced the number of possible states at most nodes.

3. Results

Lowest incongruence between length-variable and invariable gene regions was recovered using gap open penalties of 20 for both *rrnL* and 28S when gaps were treated as a fifth state (standard coding, Table 1). When gaps were coded as binary characters (simple coding), lowest incongruence was found for open penalties of 20 and 25 for *rrnL* and 28S, respectively (Table 1). Trees obtained from the length-invariable region alone were much shorter than those that included length-variable regions (Table 2), as expected when removing a large proportion (*ca.* 320 characters, 15% of the total) of the available sites. The CI of the length-variable and invariable regions was similar (0.10 vs. 0.11), indicating that invariable regions largely support the same phylogenetic signal as nucleotide changes (i.e., the alignment is a good reflection of homology) although there were topological differences that affected the number of Canthonini clades as well as some of the deeper-level relationships (Table 2).

The tree resulting from the 7-partition Bayesian analysis (standard gap matrix) provided a better fit to the data than either the single-partition (2ln Bayes factor = 7368.92) or 9-partition (coded gap matrix, 6114.94) searches. The latter was favored over the single-partition model (2ln Bayes factor = 627.38). A clear feature of these analyses was the difference in the gamma shape parameter for 3rd codon positions of *cox1* (Fig. 1a). The variation in the proportion of variable sites (Fig. 1b) and in rate matrices (data not shown) also suggested that multiple partitions were appropriate to characterize the data. Values for unpartitioned Bayesian as well as the maximum likelihood analyses were intermediate between both types, demonstrating the inferior model fit (Fig. 1).

Trees from model-based, parsimony, and direct optimization analyses were assessed in the light of the existing taxonomy, recent phylogenetic work, and biogeographical distributions. The 7-partition Bayesian tree exhibited the highest degree of taxonomic and geographical monophyly for the nodes we assessed, showing 8 of the 12 established tribes to be monophyletic, including the African Gymnopleurini, Onitini, Scarabaeini, and Sisyphini, the Neotropical Eurysternini, Eucraniini, Phanaeini, and the Old World Oniticellini (Table 2). Based on criteria of taxonomic and geographical monophyly as well as the superior model fit to the data described above, this topology is presented as the most favorable reconstruction (Fig. 2).

The likelihood of this topology was significantly higher than one for which tribal monophyly was constrained (Table 3). The Coprini and Onthophagini were separated into 3 and 4 independent lineages, respectively. These were closely related in Onthophagini but widely dispersed in the trees for Coprini (Fig. 2). Finally, the 'old' tribes Canthonini and Dichotomiini were highly polyphyletic, separating into 11 and 9 clades, respectively. Most of these consisted of small monophyletic groups of a genus or several genera (Table 2, see also Appendix B).

Table 1
Matrix size and parsimony tree-length for each of 6 different gap-opening costs used to align length-variable regions of *rrnL* and 28S

Gap treatment	Gap cost	Length-variable		Total		Length difference	Incongruence (%)
		Characters	Steps	Characters	Steps		
Fifth state							
rrnL							
	1	215	3839	1903	17,509	404	2.307
	5	176	3719	1864	17,384	399	2.295
	10	162	3582	1850	17,262	414	2.398
	15	155	3641	1843	17,321	414	2.390
	20	150	3538	1838	17,188	384	2.234*
	25	150	3532	1838	17,195	397	2.309
28S							
	1	190	1190	1878	14,743	287	1.947
	5	180	1193	1868	14,765	306	2.072
	10	179	1190	1867	14,748	292	1.980
	15	178	1189	1866	14,748	293	1.987
	20	180	1236	1868	14,779	277	1.874*
	25	182	1240	1870	14,785	279	1.887
Characters							
rrnL							
	1	414	4043	414	17,775	466	2.622
	5	318	3670	318	17,364	428	2.465
	10	246	3573	246	17,265	426	2.467
	15	230	3589	230	17,268	413	2.392
	20	197	3552	197	17,220	402	2.334*
	25	195	3543	195	17,217	408	2.370
28S							
	1	305	1260	305	14,976	450	3.005
	5	273	1229	273	14,910	415	2.783
	10	254	1182	254	14,785	337	2.279
	15	243	1178	243	14,790	346	2.339
	20	239	1242	239	14,856	348	2.342
	25	240	1235	240	14,787	286	1.934*

The length-invariable matrix had 1668 characters and 13,226 steps. * = matrices with lowest incongruence with the length-invariable tree that were combined for phylogenetic analysis. Phylogenetic analyses used fifth-state matrices for parsimony, 7-partition Bayesian and maximum likelihood searches. Character matrices were used for alternative parsimony searches and for the 9-partition Bayesian analysis.

The basal node of the Scarabaeinae ingroup was occupied by a small lineage ascribed to Dichotomiini (*Sarophorus* + *Coptorhina*) and this was a well supported sister to all other Scarabaeinae (posterior probability 0.94). The clade of *Odontoloma* + *Dicranocara* (Canthonini) also was near the base as were the dichotomines *Macroderes* and *Gromphas*. All remaining taxa were divided into two large clades of roughly equal size. The first was comprised of a large, well-supported group of Onthophagini + Oniticellini + Onitini + Sisyphini + Epirinus (Clade E), along with several other groups from the polyphyletic tribes (Fig. 2). The second major clade of Scarabaeinae was comprised of several smaller lineages, including a clade of Neotropical canthonine genera (Clade I), dichotomines, coprines, and the monophyletic groups Eucraniini + Phanaeini (Clade A), Eurysternini, Gymnopleurini + *Catharsius* + *Metacatharsius* (Clade G), and Scarabaeini (Fig. 2). This second major clade also included a large group of Australian lineages (including New Zealand and New Caledonian monophyletic lineages, Clade H), and a group of Neotropical, African and Malagasy groups.

The 9-partition (simple-coded matrix) tree revealed a similar topology except that the African tribe Oniticellini was made paraphyletic by the inclusion of four species of Onthophagini and increased numbers of canthonine and dichotomine lineages (Table 2). The maximum likelihood tree ($-\ln L = 73{,}679.71$) showed deep-level features similar to the Bayesian trees but with a different extent of monophyletic groups and genera. The large Australian and Neotropical clades were paraphyletic and the number of separate lineages for Canthonini, Coprini, and Dichotomiini increased to 20, 4, and 11, respectively (Table 2). Oniticellini was made paraphyletic by the inclusion of two species (*Digitonthophagus gazella*, *Onthophagus semiareus*) ascribed to the Onthophagini (Fig. 3).

Under maximum parsimony, 7 established tribes were monophyletic (Fig. 3, Table 2), whether based on length-invariable regions or the full data set (standard or simple coding). Oniticellini was paraphyletic with respect to the same two species as recovered by the maximum likelihood analysis. At deeper levels in the tree, relationships between lineages differed from the model-based analyses, although regular features included the basal position of several African lineages of primitive tunnelers and rollers (*Sarophorus*, *Coptodactyla*, *Odontoloma*, *Dicranocara*), the close association of tribes Onthophagini, Onitini, and (less clearly) Oniticellini,

Table 2
Tree score and topology summary for seven phylogenetic analyses conducted

	Model-based			Parsimony			
	Bayes-7	Bayes-9	ML	Length-invar	Gap-coding		POY
					Standard	Simple	
Tree score	−71,039.82	−74,096.89	−73,679.71	13,266	18,699	18,741	29,878
Tribal clades							
Gymnopleurini	M	M	M	M	M	M	M
Onitini	M	M	M	M	M	M	M
Scarabaeini	M	M	M	M	M	M	M
Sisyphini	M[a]	M[a]	M[a]	M	M	M	M
Eurysternini	M	M	M	M	M	M	M
Eucraniini	M	M	M	M	M	M	M
Phanaeini	M	M	M	M	M	M	M
Oniticellini	M	P[b]	P[c]	P[c]	P[c]	P[c]	P
Helictopleurus	M	P	P	P	P	P	P
Canthonini clades	*11*	*17*	*20*	*14*	*10*	*16*	*23*
Deltochilum	M	M	P	P	M	M	M
Epirinus	M	M	M	M	M	M	P
Monoplistes	M	P	M	P	M	P	P
Temnoplectron	M	M	M	P	M	M	P
Coprini clades	*3*	*3*	*4*	*3*	*3*	*4*	*4*
Coptodactyla	M	M	P	M	M	P	M
Catharsius + *Metacatharsius*	M	M	M	M	M	M	P
Coptodactyla + *Amphistomus*	M	M	P	M	M	P	P
Dichotomiini clades	*9*	*10*	*11*	*12*	*12*	*12*	*11*
Canthidium	P	P	P	P	M	P	P
Demarziella	M	M	P	M	M	M	P
Dichotomius	M	P	M	P	M	M	M
Canthidium + *Dichotomius*	M	P	P	P	P	P	P
Onthophagini clades	*4*	*4*	*4*	*3*	*4*	*4*	*4*
Digitonthophagus gazella + *Phalops ardea*	M	M	M	M	M	M	M
Deeper relationships							
A Eucraniini + Phanaeini	M	M	M	P	M	P	M
B Onthophagini + Oniticellini	P	M	P	P	M	M	P
C Clade B + Onitini	M	P	P	M	M[d]	P	M
D Clade C + Sisyphini	M	M	P	P	M[d]	P	P
E Clade D + *Epirinus*	M	M	P	P	P	P	P
F *Copris* + *Panelus* + *Heliocopris*	M	P	P	P	P	P	P
G Gymnopleurini + *Catharsius* + *Metacatharsius*	M	M	M	P	P	P	P
Biogeographical clades							
H Australian clade ($n = 29$)	M	M[e]	P	P	P	P	P
Australian clade ($n = 17$)	M	M	M	P	M	M	P
New Caledonia	M	M	M	P	M	M	M
I Neotropical Canthonini ($n = 14$)	M	M	P	P	M	M	P

Scores are expressed as ln likelihood for model-based searches and as tree length for parsimony searches. Tribes (Balthasar, 1963) as well as selected deep relationships and biogeographical nodes were scored for monophyly, where M, monophyletic and P, paraphyletic. The number of lineages is reported for the most polyphyletic clades.

[a] Excludes *Neosisyphus ruber* for which only 16S data were available.
[b] Includes *Digitonthophagus diabolicus*, *Onthophagus semiareus*, *Proagoderus bicallossus* and *P. schwaneri*.
[c] Includes *Digitonthophagus gazellae*, *Onthophagus semiareus*.
[d] Includes *Macroderes*.
[e] Includes *Neosisyphus ruber*.

and the sister relationship of Phanaeini and Eucraniini. The length-variable parsimony trees (standard or simple coding) recovered one Australian clade of 17 canthonine species as well as monophyletic groups from New Caledonia and New Zealand, but not the lineage of 29 species recovered in the Bayesian analysis (Fig. 3). The length-invariable tree recovered only one node of the deeper relationships and none of the biogeographical nodes (Table 2).

Reconstructions based on the alignment-generating tree search implemented in POY recovered the same 7 monophyletic tribes as in the parsimony searches, although the number of lineages ascribed to Canthonini increased to 23 and fewer genera were monophyletic (Table 2). *Saprophorus* + *Coptorhina* and *Odontoloma* + *Dicranocara* were basal lineages along with Neotropical *Deltochilum*. There were five major clades recovered in the POY analysis rather

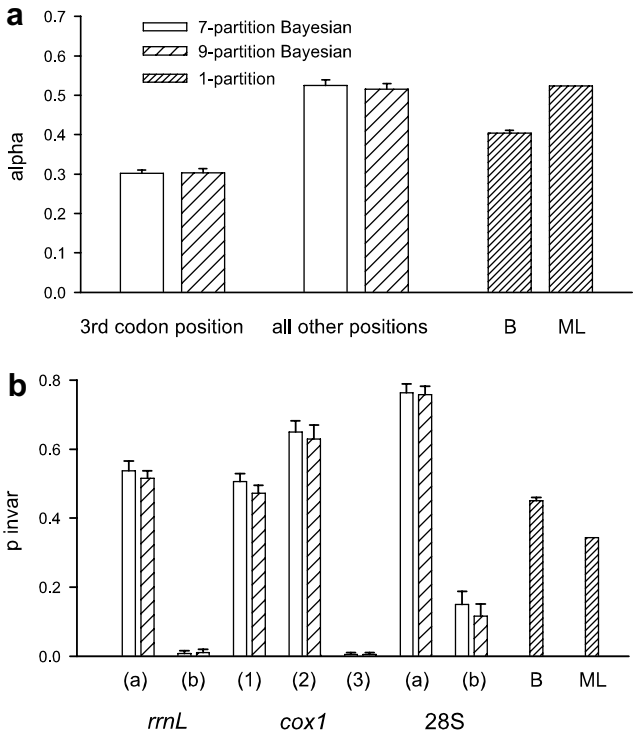

Fig. 1. Gamma shape distribution (upper) and proportion of invariable sites (lower) estimated from Bayesian and maximum likelihood analysis of the standard gap (fifth state) matrix (1-partition, 7-partition) and the Bayesian analysis of the simple-coded (9-partition) matrix. The proportion of invariable sites is reported separately for conserved (a) and length-variable (b) regions of *rrnl* and 28S and for each codon position of *cox1*. Single-partition Bayesian (B) and maximum likelihood (ML) model parameters are presented on the right of each panel. Estimates for the Bayesian analysis (mean + 1 SD) were based on values calculated for 1,000,000 generations after a burnin of 5,000,000 generations. Likelihood parameters were generated using PhyML (see Section 2).

than the two major clades in other reconstructions. The largest was a monophyletic group of Onthophagini + Oniticellini + Onitini, only the latter of which was monophyletic within the clade. Sister to this was a second clade of Eucraniini + Phanaeini, Gymnopleurini, and several members of the polyphyletic tribes. A third, smaller clade consisted of Sisyphini, Eurysternini, *Epirinus* and *Catharsius*, while a fourth contained the Scarabaeini, *Copris* + *Microcopris*, *Uroxys* and several Neotropical Dichotomiini, and Malagasy Canthonini. A fifth clade contained Malagasy *Aleiantus*, *Apotolamprus*, *Phacosomoides*, and *Sphaerocanthon* (ascribed to Canthonini) as well as many members of the Australian group described above.

Two notable groups were recovered consistently with specific data treatments. The group of Gymnopleurini and *Catharsius* + *Metacatharsius* (Clade G) was monophyletic in all model-based searches but was not recovered in any parsimony searches. Onthophagini + Oniticellini (Clade B) was monophyletic in all analyses that considered gaps as characters, whether with standard or simple coding. This was the case regardless of whether tree searches were model-based (9-partition Bayesian) or parsimony-based.

3.1. Biogeography and evolutionary ecology

Between 38 (7-partition Bayesian topology) and 48 (POY) character transformations were inferred when the six biogeographical regions were optimized as character states under parsimony (Table 4). These involved multiple changes for all biogeographical regions, but the majority (29–35 transitions) affected Africa. In the 7-partition Bayesian reconstruction (Fig. 2), African taxa constituted the inferred ancestral state when mapped on trees, as the basal clades of *Coptorhina* + *Sarophorus* (Dichotomiini) and *Dicranocara* + *Odontoloma* (Canthonini) are associated with a southern African distribution. Changes on deep nodes were exclusively forward changes, with reversals and a generally high rate of change between biogeographical regions observed in only two widespread groups: the Onthophagini and Coprini. Notable were a number of major clades confined to single biogeographical regions. First was the large Australian clade that included one subclade each from New Zealand and New Caledonia described above (Clade H, Fig. 2). Second was a clade of 7 genera attributed to Canthonini from South America (Clade I). Additional clades included the Neotropical Phanaeini + Eucraniini (Clade A) + *Dichotomius* + *Canthidium* (both ascribed to Dichotomiini), and a largely African clade composed of four tribes plus *Epirinus* (Clade E, Fig. 2). In contrast, the widespread genera *Copris* + *Microcopris* and *Onthophagus* were tip-level groups lacking geographic structure.

For DIVA analysis, only a single representative was retained from clades with uniform biogeographical distribution, for a total of 101 terminals. Mapped on a clock-constrained topology, dispersal–vicariance analysis showed that, originating from an African source area, links of Africa with all other biogeographical regions were inferred, several of them multiple times, and account for 13 of the 16 links between two areas (Fig. 4). The remaining connections were between the Neotropics and Madagascar in two cases, and the Neotropics and Australia. In addition, of three cases where the sequence of links between three areas could not be resolved, two involved Africa. A final 'global' link between all areas was inferred at the base of the Onthophagini, apparently marking a phase of wide dispersal of this group and leading to the geographically confined subclades in this tribe. While an absolute time scale for vicariance–dispersal scenarios was lacking, it was apparent from the relative dating on the clock-constrained tree that the connections between Africa and other areas were scattered throughout the time period, except for the Africa–Palearctic connections which were limited to the more recent portions of the phylogeny (Fig. 4).

The emerging picture of Scarabaeinae phylogeny suggests that the complex rolling behavior is highly homoplastic. Although there remain uncertainties in the tree, rolling lineages in the major continents are independent based on the topology of the 7-partition tree (S-H test, Table 3). In the African fauna, the rollers apparently consist of multiple independent lineages that have acquired this trait. This

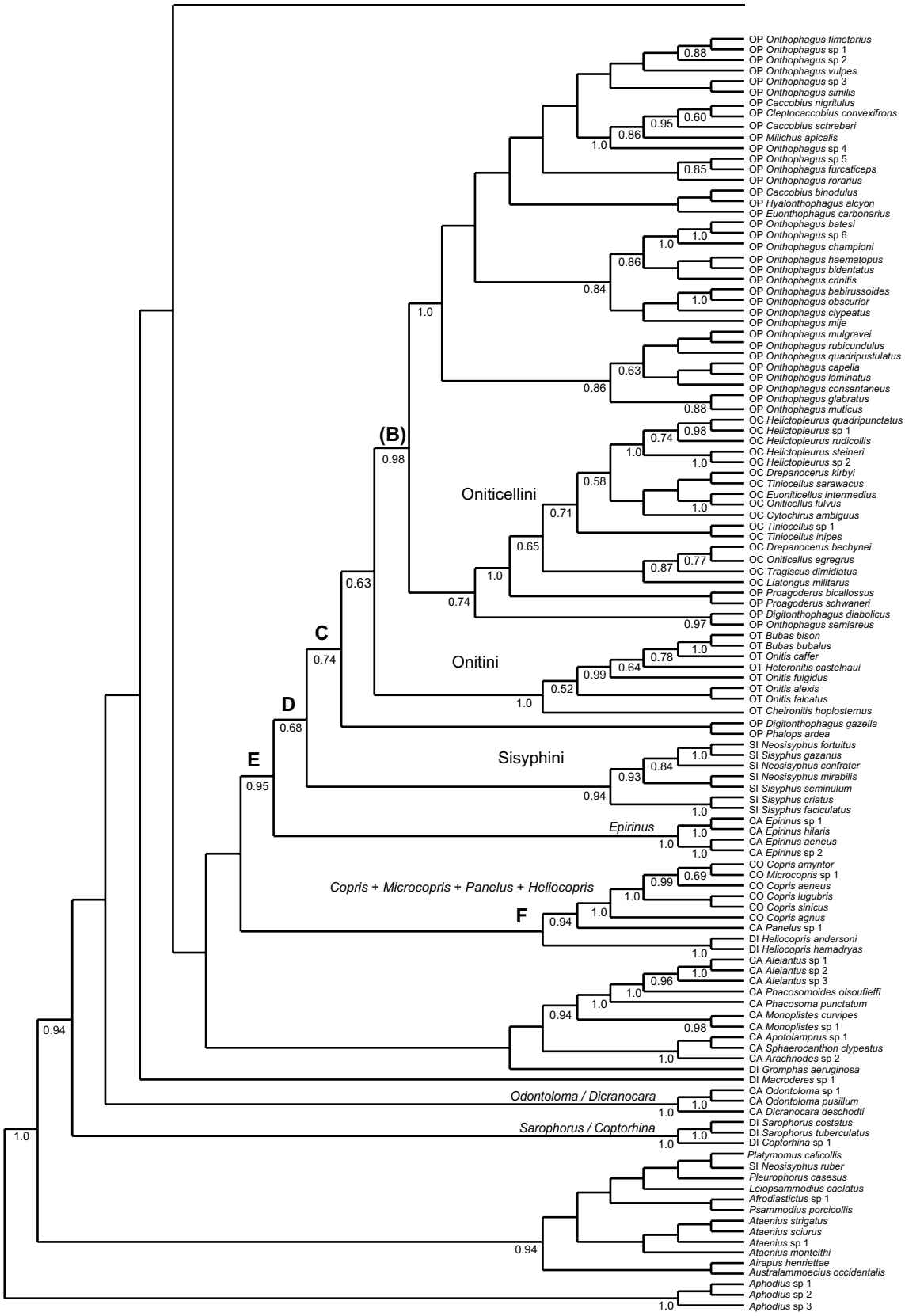

Fig. 2. Phylogenetic relationships among 214 Scarabaeinae and 11 Aphodiinae species based on the Bayesian 7-partition analysis. Nodes (A–I) are labelled as in Table 3 and posterior probability values are presented below branches leading to the node. Tribal classification of Balthasar (1963) is indicated by the 2-letter code preceding each binomial where CA, Canthonini; CO, Coprini; DI, Dichotomiini; EC, Eucraniini; ER, Eurysternini; GY, Gymnopleurini; OC, Oniticellini; OP, Onthophagini; OT, Onitini; PH, Phanaeini; SC, Scarabaeini; and SI, Sisyphini.

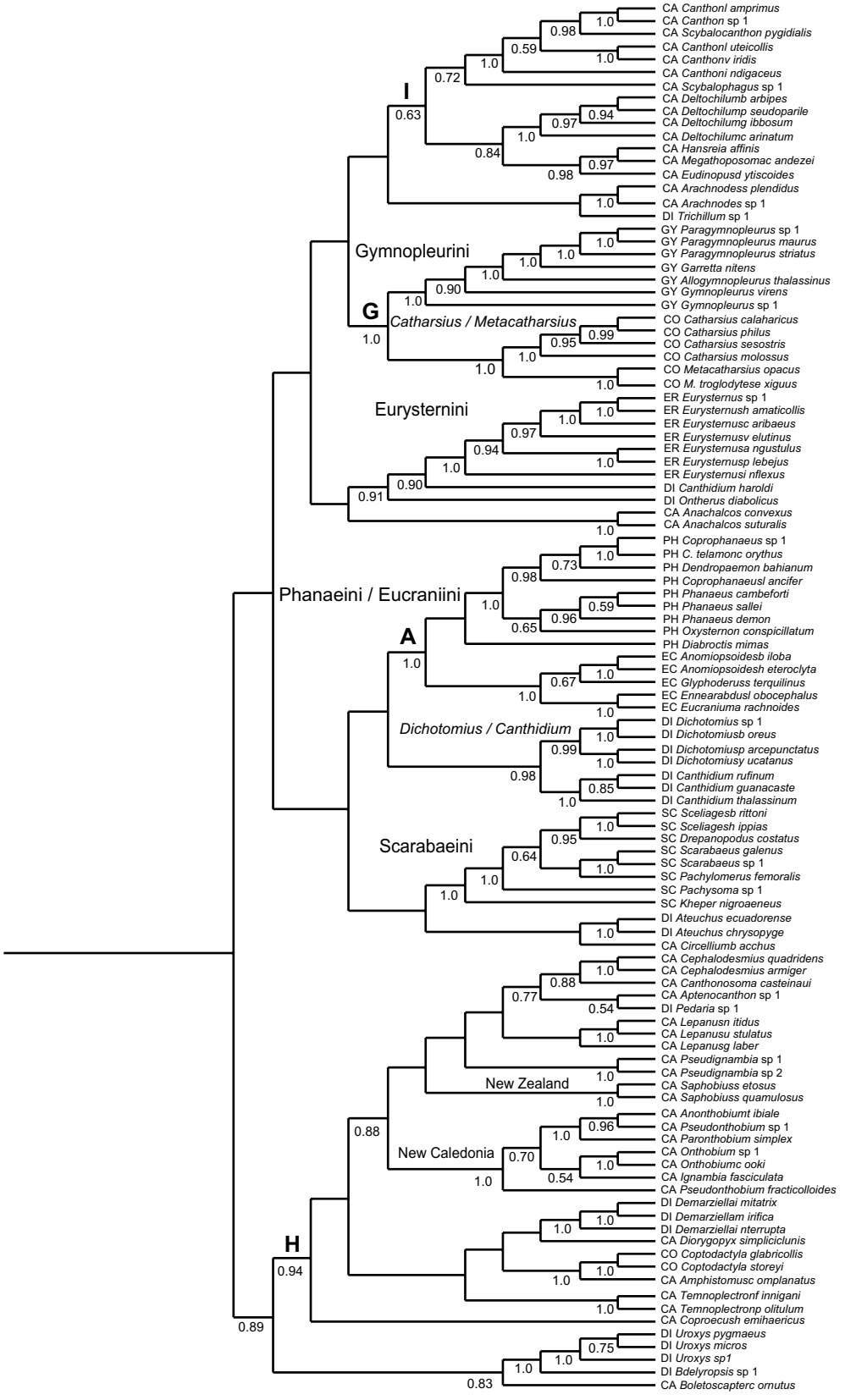

Fig. 2 (*continued*)

Table 3
Change in tree likelihood when the Bayesian topology (Fig. 3) was constrained for monophyly of tribes (see text) and nesting behaviors (tunneling, rolling)

Tree	−ln *L*	Difference −ln *L*	*p*
Bayes-7 tree	75661.57712	—	—
Tribes monophyletic	75973.33826	311.76114	0.00
Nesting monophyletic	75771.14088	109.56376	0.02

Significance was assessed using the Shimodaira–Hasegawa test using RELL bootstrap.

indicates a high rate of change in this syndrome despite its complexity, involving behavioral as well as morphological changes to hind legs, front legs, and overall body shape. The great evolutionary changeability is also reflected in the diversity of behaviors for dislocating food. For example, the Eurysternini and some Oniticellini (*Oniticellus* and *Tragiscus*) have been categorized as 'dwellers' because they nest in the droppings rather than translocate dung. The Eucraniini have been classified as 'carriers' and move dung by lifting it with their forelegs.

4. Discussion

The large body of work directed toward the study of species diversity, behavior, and biogeography in dung beetles has focused primarily on evolutionary scenarios of their origins and lineage diversification (Cambefort, 1991b; Davis et al., 2002). Here, we provide a thorough phylogenetic analysis of the group based on comprehensive sampling of species from areas with the greatest apparent phylogenetic diversity, mostly in the southern hemisphere where the diversity of those clades thought to be basal is concentrated. The aim was to provide a basis for explicit tests of some of the largely intuitive scenarios put forth to date. We conducted a rigorous exploration of data treatments, namely the influence of gap coding, alignment, and data-partitioning on the resulting topology. While the results from various strategies for alignment and tree search procedures cannot be compared directly because of the different optimality criteria (direct optimization, model-based, parsimony), it is of heuristic value to explore the effects of various properties of the data and their implications for alignment and tree searches.

The *rrnL* and 28S rRNA markers were affected by length variability and the resulting uncertainty of alignment. We used dynamic homology searches and static alignments, the latter were then subjected to two different coding schemes followed by model-based and parsimony tree searches. This provided an assessment of the sensitivity of the phylogenetic conclusions, and a test of the signal contained in length-variable sequences. Alignments generated from a range of search parameters showed a shallow optimum but no great effect on character conflict from slight changes in parameter values. Trees obtained under a simple coding scheme were slightly longer compared to the standard coding, indicating that multi-nucleotide indels could not be coded more parsimoniously with this procedure and thus that the greater weight assigned to longer indels in standard (fifth state) gap treatment was not likely to confound the tree topology. For model-based searches, likelihood scores were greatly improved after data partitioning, as has been observed in other recent studies (Brandley et al., 2005; Marshall et al., 2006). The example of 3rd versus non-3rd codon position characters demonstrated that the application of a single model uniformly to all characters resulted in intermediate rate estimates to very different categories of characters (Fig. 1), leading to incorrect estimates for characters in either category. Although the designation of partitions was intuitive using functional criteria (gene marker, codon position, length-variable and conserved regions), their validity was evident from the greatly improved model fit. The inclusion of two additional partitions for the simple-coded gap characters did not improve the likelihood estimates (9-partition Bayesian analysis). While this could be expected because the data matrix had more characters (Table 4), whether the decreased likelihood resulted from more characters or from inclusion of gaps (treated as missing data in the 7-partition analysis) is unknown. The increased level of polyphyly in the tree suggests the gap characters had an effect.

The multitude of approaches used here leaves us with the question of which trees obtained are the most defensible, although the various analyses did produce largely similar tree topologies. The main criterion used here for assessing tree topologies was a comparison with the existing classification and biogeography (Table 2). Despite its inadequacies, the tribal classification can serve as a useful scheme of grouping, as it is based on morphological similarities that seemingly represent evolutionary relationships at some hierarchical level. Seven tribal groups were found to be monophyletic under all procedures, demonstrating their stability to alignment strategy and method of tree search. An eighth was monophyletic in the preferred (7-partition) Bayesian tree. In contrast, the four tribes Coprini, Onthophagini, Canthonini, and Dichotomiini were never recovered, with the latter two groups breaking up into a large number of independent and often distantly related clades. Nonetheless, the break-up of these tribes does not negate the possible information content from recovery of subclades. Accordingly, the fact that the 7-partition Bayesian analysis recovered only 10 and 9 clades of Canthonini and Dichotomiini, compared to 23 and 11 subclades using POY, indicates that the Bayesian tree constitutes the preferred topology. Similar reasoning applies to the use of biogeographical patterns in determining the quality of tree topologies, where the number of inferred changes between regions varied between 38 (7-partition Bayesian) and 48 (POY).

4.1. Implications for dung beetle evolution and classification

Early classifications of Scarabaeinae were based on the assumption that the evolution of dung beetles involved a

Fig. 3. Maximum parsimony phylogram resulting from the analysis using a simple-coding matrix from GapCoder (see Section 2) . Nodes are labelled as in Table 3 and nodes in parentheses indicate paraphyly in comparison to the Bayesian topology (Fig. 2). Bremer support values are presented above branches only for those nodes for which support was calculated.

Table 4
Number of transitions between biogeographical regions optimized under parsimony for each tree topology

	Model-based			Parsimony			
	Bayes-7	Bayes-9	ML	Lgth-invar	Gap-coding		POY
					Standard	Simple	
Biogeographical steps	38	41	43	44	43	45	48
Number of lineages							
African	29	32	33	33	34	35	31
Neotropical	8	10	16	16	13	12	10
Malagasy	5	5	6	7	7	7	6
Australian	9	8	12	11	10	12	16
Oriental	12	13	13	12	13	13	15
Palearctic	8	9	8	10	10	10	10

single origin of the complex rolling behavior and associated morphological modifications. The non-monophyly of rolling groups has now been shown repeatedly (Ocampo and Hawks, 2006; Philips et al., 2004; Villalba et al., 2002; Zunino, 1983), while it is also clear that the classification of "rollers" includes a diversity of dung translocation behaviors, from ball rolling in the strict sense to dragging and carrying dung with the hind- or forelegs (Ocampo and Hawks, 2006; Philips et al., 2004). Although there is now broad agreement among recent studies that the deep sepa-

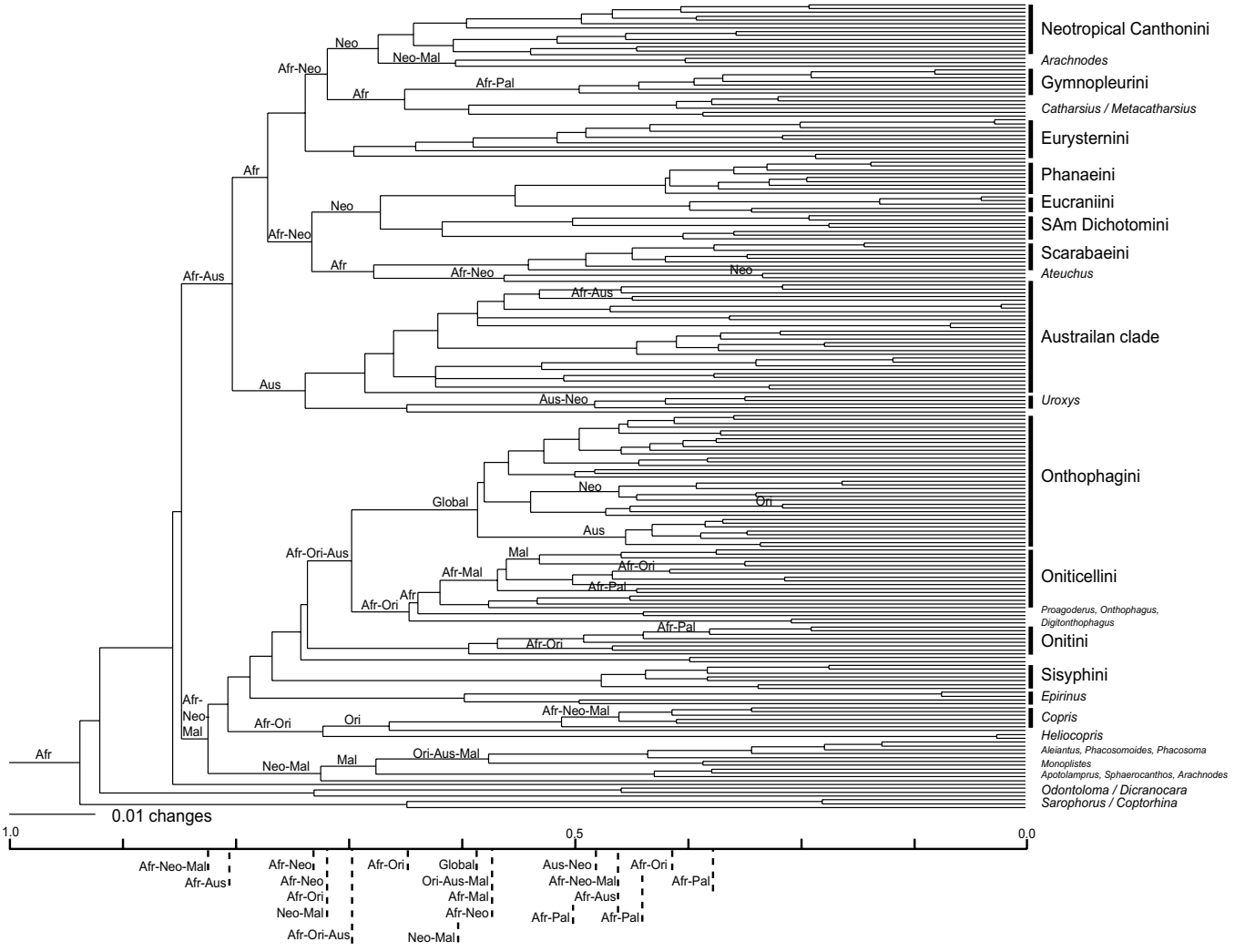

Fig. 4. Dispersal–vicariance analysis results for the 7-partition Bayesian topology (Fig. 2) with clock-constrained maximum likelihood branch lengths. The areas assigned to each node (Afr, African; Aus, Australian; Mal, Malagasy; Neo, Neotropical; Ori, Oriental; and Pal, Palearctic) were limited to those where a simple sequence of nodal assignments could be established upward toward the tips of the tree with a subset of two states assigned to the nodes (see Section 2).

ration of rolling and tunneling lineages is no longer tenable, here we used explicit tests of the strength of support (Table 4) and provide evidence based on extensive taxonomic and geographical sampling. Given the deep separation of most clades and their highly localized distribution in different continental areas, the difficulties faced by phylogenetic analyses of local assemblages (Villalba et al., 2002; Ocampo and Hawks, 2006) become clear. Insufficient sampling of major lineages is likely to produce phylogenetic uncertainty and a failure to place the unsampled lineages.

Using the present reconstruction, we can draw a number of broad conclusions about the validity of the existing classification and the role of biogeography, despite the data exploration exercise revealing some topological uncertainty. These provide a new synthesis of dung beetle evolution that does not focus on the highly plastic nesting and dung translocation behaviors. The twelve tribal groupings of the existing taxonomy obviously relate to very different hierarchical levels. Several of the smaller tribes were confirmed here as monophyletic terminal groups, just as most genera also were demonstrably monophyletic. In contrast, the 'old' tribes of rolling Canthonini and tunneling Dichotomiini each consisted of multiple lineages at hierarchical levels equivalent to the smaller tribes. Their original grouping, based on morphology, was probably due to plesiomorphies and convergence of characters associated with rolling and tunneling behavior. Small-bodied primitive lineages of rollers and tunnelers also occupy the basal nodes of the tree including *Coptorhina*, a fungus-feeder whose position is supported by morphological studies (Philips et al., 2004; Zunino, 1983). Its phylogenetic position lends support to the suggestion that the Scarabaeinae arose from mycetophagous ancestors (Scholtz and Chown, 1995). The other basal member recovered consistently was *Sarophorus*, thought to be a detritus feeder (old dung and carrion remains, see http://www.zin.ru/Animalia/Coleoptera/eng/sarophor.htm). Frolov (2004) also considered *Sarophorus* and *Coptorhina* to be sister taxa.

The position of *Odontoloma* + *Dicranocara*, recovered close to the basal clade *Sarophorus* + *Coptorhina* in all reconstructions, suggests a very early acquisition of rolling behavior in the Scarabaeinae. Like *Sarophorus* and *Coptorhina*, these genera currently have a very limited distribution in Southern Africa, but this basal plasticity observed in nesting behavior is likely to have been fundamental in generating the diversity observed in the group, with numerous subsequent functional switches apparent throughout the phylogeny.

The importance of this considerable plasticity in such a significant trait is evidenced by a number of critical reversals within clades. One such case is the recovery of *Coptodactyla* and *Demarziella* within the Australian clade of small rollers. These two genera were considered to be the sole Australian representatives of the Coprini and Dichotomiini tribes, respectively (Matthews, 1974), not least because both are functionally tunnelers. Indeed, the only other native Australian tunnelers are species of *Onthophagus*, making the Australian dung beetle assemblages distinctly 'tunneler-poor'. The recovery of *Coptodactyla* and *Demarziella* within a clade of rollers suggests that taxa from a rolling clade have taken advantage of an empty niche. *Coptodactyla* are among the largest tunnelers in the Australian fauna, with a mean body length of 13.5 mm, while the species of *Demarziella* are much smaller (4 mm; Cambefort, 1991b). Presumably, they are exploiting different niches, and do in fact appear to represent independent origins of tunneling in this geographical clade (Fig. 2).

The Neotropical Eurysternini are usually considered to be rollers, but they have never been observed to form balls for feeding. The female forms dung balls for reproduction, but produces them within the dung pat at the soil interface and these are not rolled away. Since the beetles remain within the main dung resource for both feeding and breeding purposes, they are regarded here as dwellers. Thus the 'dwellers' in Africa (*Oniticellus* and *Tragiscus* within the Oniticellini) and the Neotropics (the Eurysternini) appear to have independent origins. Feeding and breeding within the dung is an apparently 'primitive' behavior similar to the Aphodiinae outgroup; however, these examples reveal that shifts in breeding biology can occur in any direction, and that behaviors other than rolling can be highly derived. With such extensive and regular switching of methods of resource translocation, convergent morphology is probably widespread within the Scarabaeinae, and the problems with which morphological data have been faced can be more fully appreciated.

Entirely new clades that have not been recognized previously were defined by geography. The first was the Australian clade (H) that included nearly all species from this continent as well as two lineages from New Zealand and New Caledonia. There also was a Neotropical group of rollers (clade I) and the predominantly African and Oriental clade of Gymnopleurini + *Catharsius* + *Metacatharsius*. Finally there was a major, primarily African Clade E, comprising the "O-3" group (Clade C) plus Sisyphini and *Epirinus*. Another well supported geographical group, the Neotropical Phanaeini + Eucraniini, was detected previously (Ocampo and Hawks, 2006). The result was that only three lineages, Gymnopleurini, *Copris* + *Microcopris* and *Onthophagus*, were widespread and therefore dispersive at a global scale.

We suggest that future work in the systematics and taxonomy of the group should broadly consider the plasticity of traits evidenced here and the strong possibility of morphological convergence between distantly related lineages. One such example was the previous grouping of Eucraniini and Scarabaeini using morphological characters (Philips et al., 2004) that was not supported here. Another example is the morphological and behavioral similarity of the African genus *Pachysoma* (Scarabaeini) to the Neotropical eucraniines. The latter were recovered within a tunneling lineage of Phanaeini and two dichotomine genera, whereas *Pachysoma* was within a rolling lineage. The morphological

convergence may be the result of environmental pressures – both live in very similar arid habitats. A number of genera appeared to be polyphyletic and probably require taxonomic revision, including *Onitis*, *Onthophagus*, *Canthon*/*Scybalocanthon*, and *Sisyphus*/*Neosisyphus* (excluding *N. ruber* that was here affected by missing data).

Perhaps the most remarkable finding was the ancestral nature of the African fauna. Members of the great majority of clades were derived from African clades based on reconstruction of character transformations. These gave rise to the lineages on all other continents, with little apparent back-migration according to dispersal–vicariance analysis. This finding was unexpected under the proposed scenario of Gondwanan vicariant separation of major types (Cambefort, 1991b; Davis et al., 2002). The clock-constrained tree provided here is now open to an absolute calibration to further test these scenarios.

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Appendix A

Scarabaeinae ingroup and Aphodiinae outgroup species sequenced for the study

Tribe	Name	Origin	BMNH	*rrnL*	*cox1*	28S
CA	*Aleiantus* sp1	Madagascar	669891	EF656655	EF656746	EF656697
CA	*Aleiantus* sp2	Madagascar	669960	EF656663	EF656754	EF656705
CA	*Aleiantus* sp3	Madagascar	669910	EF656660	EF656751	EF656702
CA	*Amphistomus complanatus*	Australia	667354	AY131436	AY131808	–
CA	*Anachalcos convexus*	South Africa	679729	AY131437	AY131809	AY131628
CA	*Anachalcos suturalis*	Ivory Coast	679730	AY131438	AY131810	AY131629
CA	*Anonthobium tibiale*	New Caledonia	679731	AY131439	AY131811	AY131630
CA	*Apotolamprus* sp1	Madagascar	673991	EF656677	EF656768	EF656719
CA	*Aptenocanthon* sp1	Australia	667362	AY131440	AY131812	AY131631
CA	*Arachnodes splendidus*	Madagascar	673992	EF656678	EF656769	EF656720
CA	*Arachnodes* sp1	Madagascar	669969	EF656664	EF656755	EF656706
CA	*Arachnodes* sp2	Madagascar	669997	EF656665	EF656756	EF656707
CA	*Boletoscapter cornutus*	Australia	667363	AY131441	AY131813	AY131632
CA	*Canthon indigaceus*	Costa Rica	679733	AY131443	AY131814	AY131634
CA	*Canthon lamprimus*	Belize	668671	EF656648	EF656739	EF656690
CA	*Canthon luteicollis*	Ecuador	679734	AY131444	AY131815	AY131635
CA	*Canthon* sp1	Ecuador	670496	EF656668	EF656759	EF656710
CA	*Canthon viridis*	Costa Rica	679736	AY131446	AY131817	AY131637
CA	*Canthonosoma casteinaui*	Australia	667364	AY131447	AY131818	AY131638
CA	*Cephalodesmius armiger*	Australia	679737	AY131448	–	–
CA	*Cephalodesmius quadridens*	Australia	667365	AY131449	AY131819	AY131639
CA	*Circellium bacchus*	South Africa	679738	AY131450	AY131820	AY131640
CA	*Coproecus hemihaericus*	Australia	679739	AY131451	AY131821	AY131641
CA	*Deltochilum barbipes*	Ecuador	679741	–	AY131823	AY131643
CA	*Deltochilum carinatum*	Ecuador	679742	AY131453	AY131824	AY131644
CA	*Deltochilum gibbosum sublaeve*	Belize	679743	AY131454	AY131825	AY131645
CA	*Deltochilum pseudoparile*	Belize	679744	AY131455	AY131826	AY131646
CA	*Dicranocara deschodti*	Namibia	673982	EF656672	EF656763	EF656714
CA	*Diorygopyx simpliciclunis*	Australia	679745	AY131456	AY131827	AY131647
CA	*Epirinus aeneus*	South Africa	679747	AY131458	AY131829	AY131649
CA	*Epirinus hilaris*	South Africa	679748	AY131459	AY131830	AY131650
CA	*Epirinus* sp1	South Africa	679746	AY131457	AY131828	AY131648
CA	*Epirinus* sp2	South Africa	679749	AY131460	AY131831	AY131651
CA	*Eudinopus dytiscoides*	Argentina	679750	AY131461	AY131832	AY131652
CA	*Hansreia affinis*	French Guiana	679751	AY131462	AY131833	AY131653
CA	*Ignambia fasciculata*	New Caledonia	679752	AY131463	AY131834	AY131654
CA	*Lepanus glaber*	Australia	667379	EF656646	–	EF656688
CA	*Lepanus nitidus*	Australia	679753	AY131464	AY131835	AY131655

(*continued on next page*)

Appendix A (*continued*)

Tribe	Name	Origin	BMNH	*rrnL*	*cox1*	28S
CA	*Lepanus ustulatus*	Australia	679754	–	–	AY131656
CA	*Megathoposoma candezei*	Belize	679755	AY131465	AY131836	AY131657
CA	*Monoplistes curvipes*	Australia	667380	AY131467	–	AY131659
CA	*Monoplistes* sp1	Australia	667381	AY131466	AY131837	AY131658
CA	*Odontoloma pusillum*	South Africa	679757	AY131469	AY131839	AY131661
CA	*Odontoloma* sp1	South Africa	679756	AY131468	AY131838	AY131660
CA	*Onthobium cooki*	New Caledonia	679759	AY131471	AY131841	AY131663
CA	*Onthobium* sp1	New Caledonia	679758	AY131470	AY131840	AY131662
CA	*Panelus* sp1	Indonesia	679760	AY131472	AY131842	AY131664
CA	*Paronthobium simplex*	New Caledonia	679761	AY131473	AY131843	AY131665
CA	*Phacosoma punctatum*	Indonesia	679762	AY131474	AY131844	AY131666
CA	*Phacosomoides olsoufieffi*	Madagascar	673983	EF656673	EF656764	EF656715
CA	*Pseudignambia* sp1	Australia	679763	AY131475	AY131845	AY131667
CA	*Pseudignambia* sp2	Australia	679764	AY131476	AY131846	AY131668
CA	*Pseudonthobium fracticolloides*	New Caledonia	679765	AY131477	AY131847	AY131669
CA	*Pseudonthobium* sp1	New Caledonia	679766	AY131478	AY131848	AY131670
CA	*Saphobius setosus*	New Zealand	679767	AY131479	–	AY131671
CA	*Saphobius squamulosus*	New Zealand	679768	AY131480	–	AY131672
CA	*Scybalocanthon pygidialis*	French Guiana	679769	AY131481	AY131849	AY131673
CA	*Scybalophagus* sp1	Argentina	679770	AY131482	AY131850	AY131674
CA	*Sphaerocanthon clypeatus*	Madagascar	673987	EF656676	EF656767	EF656718
CA	*Temnoplectron finnigani*	Australia	667373	AY131483	AY131851	AY131675
CA	*Temnoplectron politulum*	Australia	667377	AY131484	–	AY131676
CO	*Catharsius calaharicus*	South Africa	679771	AY131485	AY131852	AY131677
CO	*Catharsius molossus*	Indonesia	679772	AY131486	AY131853	AY131678
CO	*Catharsius philus*	South Africa	679773	AY131487	AY131854	AY131679
CO	*Catharsius sesostris*	South Africa	679774	AY131488	AY131855	AY131680
CO	*Copris aeneus*	South Africa	679775	AY131489	AY131856	AY131681
CO	*Copris agnus*	Indonesia	679776	AY131490	AY131857	AY131682
CO	*Copris amyntor*	South Africa	679777	AY131491	AY131858	AY131683
CO	*Copris lugubris*	Costa Rica	679779	AY131493	AY131860	AY131684
CO	*Copris sinicus*	Hong Kong	679781	AY131495	AY131862	AY131686
CO	*Coptodactyla glabricollis*	Australia	667366	AY131496	AY131863	AY131687
CO	*Coptodactyla storeyi*	Australia	679782	AY131497	–	–
CO	*Metacatharsius opacus*	South Africa	679783	AY131498	AY131864	AY131688
CO	*M. troglodytes exiguus*	South Africa	679784	AY131499	AY131865	AY131689
CO	*Microcopris* sp 1	Indonesia	679780	AY131494	AY131861	AY131685
DI	*Ateuchus chrysopyge*	Belize	679788	AY131502	AY131866	AY131692
DI	*Ateuchus ecuadorense*	Ecuador	669100	EF656650	EF656741	EF656692
DI	*Bdelyropsis* sp1	Belize	669447	EF656654	EF656745	EF656696
DI	*Canthidium guanacaste*	Costa Rica	679791	AY131505	AY131867	AY131694
DI	*Canthidium haroldi*	Belize	679792	AY131506	AY131868	AY131695
DI	*Canthidium rufinum*	Ecuador	679793	AY131507	AY131869	AY131696
DI	*Canthidium thalassinum*	Ecuador	679794	AY131508	AY131870	AY131697
DI	*Coptorhina* sp1	South Africa	679795	AY131509	AY131871	AY131698
DI	*Demarziella imitatrix*	Australia	667371	EF656645	–	EF656687
DI	*Demarziella interrupta*	Australia	679796	AY131511	–	AY131700
DI	*Demarziella mirifica*	Australia	679797	AY131512	AY131872	AY131701
DI	*Dichotomius boreus*	Ecuador	679799	AY131514	AY131874	AY131703
DI	*Dichotomius parcepunctatus*	Ecuador	679800	AY131515	AY131875	AY131704
DI	*Dichotomius* sp2	Ecuador	679798	AY131513	AY131873	AY131702
DI	*Dichotomius yucatanus*	Costa Rica	679801	AY131516	AY131876	AY131705
DI	*Gromphas aeruginosa*	Ecuador	679802	AY131517	AY131877	AY131706
DI	*Heliocopris andersoni*	South Africa	679803	AY131518	AY131878	AY131707
DI	*Heliocopris hamadryas*	South Africa	679804	AY131519	AY131879	AY131708
DI	*Macroderes* sp1	South Africa	679805	AY131520	AY131880	AY131709
DI	*Ontherus diabolicus*	Ecuador	679806	AY131521	AY131881	AY131710
DI	*Pedaria* sp1	South Africa	679807	AY131522	AY131882	AY131711
DI	*Sarophorus costatus*	South Africa	679808	AY131523	AY131883	AY131712
DI	*Sarophorus tuberculatus*	South Africa	679809	AY131524	AY131884	AY131713
DI	*Trichillum* sp1	Costa Rica	679810	AY131525	–	AY131714
DI	*Uroxys micros*	Belize	679813	AY131528	AY131886	AY131717
DI	*Uroxys pygmaeus*	Ecuador	670512	EF656670	EF656761	EF656712
DI	*Uroxys* sp1	Costa Rica	669339	EF656652	EF656743	EF656694
EC	*Anomiopsoides biloba*	Argentina	679815	AY131530	AY131887	AY131719

Appendix A (*continued*)

Tribe	Name	Origin	BMNH	*rrnL*	*cox1*	28S
EC	*Anomiopsoides heteroclyta*	Argentina	679816	AY131531	AY131888	AY131720
EC	*Ennearabdus lobocephalus*	Argentina	679817	AY131532	AY131889	AY131721
EC	*Eucranium arachnoides*	Argentina	679818	AY131533	AY131890	AY131722
EC	*Glyphoderus sterquilinus*	Argentina	679819	AY131534	AY131891	AY131723
ER	*Eurysternus angustulus*	Belize	679820	AY131535	AY131892	AY131724
ER	*Eurysternus caribaeus*	Belize	679821	AY131536	AY131893	AY131725
ER	*Eurysternus hamaticollis*	Ecuador	670436	EF656666	EF656757	EF656708
ER	*Eurysternus inflexus*	Ecuador	679823	AY131538	AY131895	AY131726
ER	*Eurysternus plebejus*	Ecuador	679824	AY131539	AY131896	AY131727
ER	*Eurysternus* sp1	Ecuador	669090	EF656649	EF656740	EF656691
ER	*Eurysternus velutinus*	Belize	679825	AY131540	AY131897	AY131728
GY	*Allogymnopleurus thalassinus*	South Africa	679826	AY131541	AY131898	AY131729
GY	*Garretta nitens*	South Africa	679827	AY131542	AY131899	AY131730
GY	*Gymnopleurus* sp1	Turkey	676998	EF656682	EF656773	EF656724
GY	*Gymnopleurus virens*	South Africa	679828	AY131543	AY131900	AY131731
GY	*Paragymnopleurus maurus*	Indonesia	679830	AY131545	AY131902	AY131733
GY	*Paragymnopleurus* sp1	Malaysia	679829	AY131544	AY131901	AY131732
GY	*Paragymnopleurus striatus*	Indonesia	679831	AY131546	AY131903	AY131734
OC	*Cytochirus ambiguus*	South Africa	679832	AY131547	AY131904	AY131735
OC	*Drepanocerus bechynei*	South Africa	679833	AY131548	AY131905	AY131736
OC	*Drepanocerus kirbyi*	South Africa	679834	AY131549	AY131906	AY131737
OC	*Euoniticellus intermedius*	South Africa	679835	AY131550	–	AY131738
OC	*Helictopleurus quadripunctatus*	Madagascar	669892	EF656656	EF656747	EF656698
OC	*Helictopleurus rudicollis*	Madagascar	673985	EF656675	EF656766	EF656717
OC	*Helictopleurus* sp1	Madagascar	669899	EF656657	EF656748	EF656699
OC	*Helictopleurus* sp2	Madagascar	669916	EF656661	EF656752	EF656703
OC	*Helictopleurus steineri*	Madagascar	673984	EF656674	EF656765	EF656716
OC	*Liatongus militarus*	South Africa	679837	AY131552	AY131908	AY131739
OC	*Oniticellus egregrus*	South Africa	679838	AY131553	AY131909	AY131740
OC	*Oniticellus fulvus*	Spain	679839	AY131554	AY131910	AY131741
OC	*Tiniocellus inipes*	South Africa	679841	AY131556	AY131912	AY131743
OC	*Tiniocellus sarawacus*	Indonesia	679840	AY131555	AY131911	AY131742
OC	*Tiniocellus* sp1	Nepal	676999	EF656683	EF656774	EF656725
OC	*Tragiscus dimidiatus*	South Africa	679842	AY131557	AY131913	AY131744
OP	*Caccobius binodulus*	Indonesia	679843	AY131558	AY131914	AY131745
OP	*Caccobius nigritulus*	South Africa	679844	AY131559	AY131915	AY131746
OP	*Caccobius schreberi*	Spain	679845	AY131560	AY131916	AY131747
OP	*Cleptocaccobius convexifrons*	South Africa	679846	AY131561	AY131917	AY131748
OP	*Digitonthophagus diabolicus*	Indonesia	679847	AY131562	–	AY131749
OP	*Digitonthophagus gazella*	South Africa	679848	AY131563	AY131918	AY131750
OP	*Euonthophagus carbonarius*	South Africa	679849	AY131564	AY131919	AY131751
OP	*Hyalonthophagus alcyon*	South Africa	679850	AY131565	AY131920	AY131752
OP	*Milichus apicalis*	South Africa	679851	AY131566	AY131921	AY131753
OP	*Onthophagus babirussoides*	Indonesia	679853	AY131568	AY131922	AY131754
OP	*Onthophagus batesi*	Belize	668548	EF656647	EF656738	EF656689
OP	*Onthophagus bidentatus*	Ecuador	679854	AY131569	AY131923	AY131755
OP	*Onthophagus capella*	Australia	679855	AY131570	–	AY131756
OP	*Onthophagus championi*	Costa Rica	669324	EF656651	EF656742	EF656693
OP	*Onthophagus clypeatus*	Ecuador	670455	EF656667	EF656758	EF656709
OP	*Onthophagus consentaneus*	Australia	667394	AY131573	–	AY131758
OP	*Onthophagus crinitis panamensis*	Belize	679858	AY131574	AY131924	AY131759
OP	*Onthophagus fimetarius*	South Africa	679859	AY131575	AY131925	AY131760
OP	*Onthophagus furcaticeps*	Australia	679860	AY131576	–	AY131761
OP	*Onthophagus glabratus*	Australia	667398	AY131577	AY131926	AY131762
OP	*Onthophagus haematopus*	Ecuador	670502	EF656669	EF656760	EF656711
OP	*Onthophagus laminatus*	Australia	679863	AY131580	–	AY131764
OP	*Onthophagus mije*	Australia	679864	AY131581	–	AY131765
OP	*Onthophagus mulgravei*	Australia	667385	AY131582	AY131927	AY131766
OP	*Onthophagus muticus*	Australia	679865	AY131583	–	AY131767
OP	*Onthophagus obscurior*	Indonesia	679866	AY131584	AY131928	AY131768
OP	*Onthophagus quadripustulatus*	Australia	679867	AY131585	–	AY131769
OP	*Onthophagus rorarius*	Indonesia	679868	AY131586	AY131929	AY131770
OP	*Onthophagus rubicundulus*	Australia	667386	AY131587	AY131930	AY131771
OP	*Onthophagus semiareus*	Malaysia	679870	AY131589	AY131932	AY131773

(*continued on next page*)

Appendix A (*continued*)

Tribe	Name	Origin	BMNH	*rrnL*	*cox1*	28S
OP	*Onthophagus similis*	Spain	679871	AY131590	AY131933	AY131774
OP	*Onthophagus* sp1	South Africa	679869	AY131588	AY131931	AY131772
OP	*Onthophagus* sp2	Turkey	676985	EF656679	EF656770	EF656721
OP	*Onthophagus* sp3	Turkey	676993	EF656681	EF656772	EF656723
OP	*Onthophagus* sp4	Turkey	676988	EF656680	EF656771	EF656722
OP	*Onthophagus* sp5	Nepal	677008	EF656684	EF656775	EF656726
OP	*Onthophagus* sp6	Costa Rica	669344	EF656653	EF656744	EF656695
OP	*Onthophagus vulpes*	Indonesia	679872	AY131591	AY131934	AY131775
OP	*Phalops ardea*	South Africa	679873	AY131592	AY131935	AY131776
OP	*Proagoderus bicallossus*	South Africa	679874	AY131593	AY131936	AY131777
OP	*Proagoderus schwaneri*	Indonesia	679875	AY131594	AY131937	AY131778
OT	*Bubas bison*	Spain	679876	AY131595	AY131938	AY131779
OT	*Bubas bubalus*	Spain	679877	AY131596	AY131939	AY131780
OT	*Cheironitis hoplosternus*	South Africa	679878	AY131597	AY131940	AY131781
OT	*Heteronitis castelnaui*	South Africa	679879	AY131598	AY131941	AY131782
OT	*Onitis alexis*	South Africa	679880	AY131599	AY131942	AY131783
OT	*Onitis caffer*	South Africa	670526	EF656671	EF656762	EF656713
OT	*Onitis falcatus*	Hong Kong	679882	AY131601	AY131943	AY131785
OT	*Onitis fulgidus*	South Africa	679883	AY131602	–	AY131786
PH	*Coprophanaeus lancifer*	French Guiana	679885	AY131604	AY131945	AY131788
PH	*C. telamon corythus*	Belize	679886	AY131605	AY131946	AY131789
PH	*Coprophanaeus* sp1	Ecuador	679884	AY131603	AY131944	AY131787
PH	*Dendropaemon bahianum*	Ecuador	679887	AY131606	AY131947	AY131790
PH	*Diabroctis mimas*	Brazil	679888	AY131607	–	AY131791
PH	*Oxysternon conspicillatum*	Ecuador	679889	AY131608	AY131948	AY131792
PH	*Phanaeus cambeforti*	Ecuador	679890	AY131609	AY131949	–
PH	*Phanaeus demon*	Costa Rica	679891	AY131610	AY131950	–
PH	*Phanaeus sallei*	Belize	679892	AY131611	AY131951	AY131793
SC	*Drepanopodus costatus*	Namibia	679893	AY131612	AY131952	AY131794
SC	*Kheper nigroaeneus*	South Africa	679894	AY131613	AY131953	AY131795
SC	*Pachylomerus femoralis*	South Africa	679895	AY131614	AY131954	AY131796
SC	*Pachysoma* sp1	South Africa	679896	AY131615	AY131955	AY131797
SC	*Scarabaeus galenus*	South Africa	679897	AY131616	AY131956	AY131798
SC	*Scarabaeus* sp1	South Africa	679898	AY131617	AY131957	AY131799
SC	*Sceliages brittoni*	South Africa	679899	AY131618	AY131958	AY131800
SC	*Sceliages hippias*	South Africa	679900	AY131619	AY131959	AY131801
SI	*Neosisyphus confrater*	South Africa	679901	AY131620	AY131960	AY131802
SI	*Neosisyphus fortuitus*	South Africa	679902	AY131621	AY131961	AY131803
SI	*Neosisyphus mirabilis*	South Africa	679903	AY131622	AY131962	AY131804
SI	*Neosisyphus ruber*	South Africa	679904	AY131623	–	–
SI	*Sisyphus criatus*	South Africa	679905	AY131624	AY131963	AY131805
SI	*Sisyphus faciculatus*	South Africa	679906	AY131625	AY131964	AY131806
SI	*Sisyphus gazanus*	South Africa	679907	AY131626	AY131965	AY131807
SI	*Sisyphus seminulum*	South Africa	679908	AY131627	AY131966	–
	Afrodiastictus sp	Namibia	703538	EF656686	EF656778	EF656729
	Airapus henriettae	Australia	694800	–	EF656777	EF656728
	Aphodius sp1	Madagascar	669919	EF656662	EF656753	EF656704
	Aphodius sp2	Madagascar	669906	EF656659	EF656750	EF656701
	Aphodius sp3	Madagascar	669904	EF656658	EF656749	EF656700
	Ataenius monteithi	Ecuador	792207	–	EF656783	EF656734
	Ataenius sciurus	USA	703547	–	EF656779	EF656730
	Ataenius sp1	USA	792206	–	EF656782	EF656733
	Ataenius strigatus	USA	792208	–	EF656784	EF656735
	Australammoecius occidentalis	Australia	703639	–	EF656781	EF656732
	Leiopsammodius caelatus	USA	703561	–	EF656780	EF656731
	Platymomus calicollis	USA	792209	–	EF656785	EF656736
	Pleurophorus casesus	Spain	792210	–	EF656786	EF656737
	Psammodius porcicollis	England	679909	EF656685	EF656776	EF656727

Tribe membership is based on Balthasar (1963) where CA = Canthonini, CO = Coprini, DI = Dichotomiini, EC = Eucraniini, ER = Eurysternini, GY = Gymnopleurini, OC = Oniticellini, OP = Onthophagini, OT = Onitini, PH = Phanaeini, SC = Scarabaeini, and SI = Sisyphini. BMNH (British Museum - Natural History) frozen collection database catalogue number and NCBI/GenBank accession numbers are provided for each individual. – = sequence data not available.

Appendix B

Tree score and topology summary for each of the seven phylogenetic analyses conducted

	Model-based			Parsimony			
	Bayes-7	Bayes-9	ML	Lgth-invar	Gap-coding		POY
					Standard	Simple	
Clade	−71,039.82	−74,096.89	−73,679.71	13,266	18,699	18,741	29,878
Canthonini							
Aleiantus	M	M	M	M	M	M	M
Anachalcos	M	M	M	M	M	M	M
Arachnodes	P	P	P	P	P	P	P
Canthon	P	P	P	P	P	P	P
Cephalodesmius	M	M	P	M	M	M	P
Deltochilum	M	M	P	P	M	M	M
Epirinus	M	M	M	M	M	M	P
Lepanus	M	M	P	P	P	M	P
Monoplistes	M	P	M	P	M	P	P
Odontoloma	M	M	M	M	M	M	M
Onthobium	M	M	M	M	M	M	M
Pseudignambia	M	M	M	M	M	M	M
Pseudonthobium	P	P	P	P	P	P	P
Saphobius	M	M	M	M	M	M	M
Sphaerocanthon	M	M	M	M	M	M	M
Temnoplectron	M	M	M	P	M	M	P
Coprini							
Catharsius	M	M	M	M	M	M	M
Copris + Microcopris	M	M	M	M	M	M	M
Coptodactyla	M	M	P	M	M	P	M
Metacatharsius	M	M	M	M	M	M	P
Dichotomiini							
Ateuchus	M	M	M	M	M	M	M
Canthidium	P	P	P	P	M	P	P
Demarziella	M	M	P	M	M	M	P
Dichotomius	M	P	M	P	M	M	M
Heliocopris	M	M	M	M	M	M	M
Sarophorus	M	M	M	M	M	M	M
Uroxys	M	M	M	M	M	M	M
Oniticellini							
Helictopleurus	M	P	P	P	P	P	P
Tiniocellus	P	P	P	P	P	P	P
Onthophagini							
Caccobius	P	P	P	M	P	P	P
Digitonthophagus	P	P	P	P	P	P	P
Onthophagus	P	P	P	P	P	P	P
Proagoderus	M	M	M	M	M	M	M
Onitini							
Bubas	M	M	M	M	M	M	M
Onitis	P	P	P	P	P	P	P
No. monophyletic genera	26	23	20	21	25	23	18

Scores are expressed as ln likelihood for model-based searches and as tree length for parsimony searches. Genera within the larger tribes (Balthasar, 1963) and were scored for monophyly, where M, monophyletic and P, paraphyletic.

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