Chapter III

Testing for congruence between morphological and molecular data partitions of *Scarabaeus (Pachysoma)* (Scarabaeidae: Scarabaeinae).

Catherine L. Sole[1], Armanda D. S. Bastos[1, 2] and Clarke H. Scholtz[1]

[1] *Department of Zoology & Entomology, University of Pretoria, Pretoria, 0002, South Africa*

[2] *Mammal Research Institute (MRI), Department of Zoology & Entomology, University of Pretoria, Pretoria, 0002, South Africa*

Running Title: Congruence between data partitions of *Scarabaeus (Pachysoma)* (Scarabaeidae: Scarabaeinae).

Abstract

Scarabaeus (Pachysoma) comprises 13 species endemic to the west coast of southern Africa. A species level phylogenetic analysis was conducted using 64 morphological characters and 1197 bp of the Cytochrome Oxidase I (COI) gene. All 13 in-group and eight out-group species were included in the analyses. Morphological and molecular data sets were analysed both separately and combined, using the total evidence approach. Strong support is shown for all 13 species within *Scarabaeus (Pachysoma)* and its monophyly within *Scarabaeus* is confirmed. The COI sequence data had high inter- and intra-specific sequence divergence as well as a high A/T bias. All trees generated using Parsimony, Maximum Likelihood, Neighbor-Joining and Bayesian methods exhibited similar topologies. The morphological and molecular data partition phylogenies showed congruence with the combined phylogeny, lending strong support for combining datasets using total evidence. Phylogenetic trees based on combined data partitions were relatively more resolved than those based on the individual data analyses. The relative contribution of each data partition to individual nodes was assessed using Bremer and Partitioned Bremer Support. The morphological dataset, though small, was not overshadowed by the large molecular dataset in the combined analysis. A strong association between the phylogenies and geographic distribution over the total *Scarabaeus (Pachysoma)* distribution was demonstrated. This study was contrasted with other phylogenetic studies done on *Scarabaeus (Pachysoma)* as well as other insect orders. Lastly *Scarabaeus (Pachysoma)* mtDNA variation was compared within and between the orders Coleoptera, Lepidoptera, Hymenoptera and Diptera.

Keywords -Scarabaeidae, *Scarabaeus*, total evidence, cytochrome oxidase I, morphology, congruence

Introduction

Scarabaeus (Pachysoma) MacLeay 1821 is a group of the tribe Scarabaeini (Scarabaeidae: Scarabaeinae). Members of the Scarabaeini are found in moist savanna through drier regions to very hot dry deserts (Scholtz, 1989) of the Afrotropics and southern latitudes of the Palaearctic. The Scarabaeini comprise some 146 species of ball-rolling dung beetles belonging to two genera (Forgie *et al*., 2005). Diversification of scarabaeines was thought to coincide with the diversification of angiosperms and mammalian herbivores resulting in a shift of their feeding habits from saprophagy and mycetophagy to coprophagy (Cambefort, 1991; Scholtz & Chown, 1995). Scarabaeines predominantly feed on dung, but have also been known to feed on humus, carrion and fungi (Scholtz & Chown, 1995). Most scarabaeine species are adapted to open habitats and feed on resources that are usually patchy and ephemeral. Although true food specialisation is uncommon, it does exist. *Scarabaeus* (*Sceliages)* (Forgie *et al*., 2005), are specialist necrophages where both adults and larvae feed only on dead millipedes (Forgie *et al*., 2002) while the flightless *Scarabaeus* (*Pachysoma)* utilise dry dung pellets or detritus (Holm & Scholtz, 1979; Scholtz, 1989). In contrast to feeding specialisation, generalist - *Scarabaeus (Scarabaeolus)* contains species that will utilise dung or carrion - and opportunistic - *Scarabaeus rubripennis* has been observed rolling pieces of millipede along as it would a dung ball (Mostert & Scholtz, 1986) - feeders also exist within this tribe (Forgie *et al*., 2005).

MacLeay (1821) described the genera *Pachysoma* and *Mnematium* for all flightless species of the Scarabaeini that occur in south-west and north Africa, respectively. The genus *Neopachysoma* was created by Ferreira (1953) for the species of *Pachysoma* inhabiting the central Namib Desert. *Pachysoma* was defined by aptery, absence of humeral calli, semi-contiguous mesocoxae and short mesosterna (Ferreira, 1953). An evaluation by Holm & Scholtz (1979) concluded that these characteristics were either due to convergence or were too variable and inconsistent to use as the justification for a genus. They also found no justification for the separation of *Neopachysoma* and *Mnematium* and consequently synonomised both with *Pachysoma*. *Pachysoma* was tentatively maintained as a genus due to its unique biology. However, the genus was later synonomised with the genus *Scarabaeus* Linnaeus (1758) by Mostert & Holm (1982) an act that was questioned by Endrödy-Younga (1989) and Scholtz (1989) because of *Pachysoma's* unique set of morphological and behavioural apomorphies.

Scarabaeus (Pachysoma) is represented by 13 species, endemic to the west coast of southern Africa. Their southerly distribution begins near Cape Town, in South Africa (S33°56'-E18°28'), with their northerly distribution being halted at the Kuiseb River (S22°58'-E14°30'), in Namibia, which marks the end of the central Namib dune sea. Southern and eastern expansion by *Scarabaeus* (*Pachysoma)* is confined by the Cape Fold Mountains and escarpments, which act as topographical and climatic barriers (Harrison *et al*., 2003). *Scarabaeus (Pachysoma)* species are, therefore, restricted to the arid or semi-arid sandy regions of south-western Africa and psammophily is readily apparent as seen by the long setal hairs on the middle and hind limbs. Little is known about their biology (Scholtz *et al*., 2004), but they are unique in their food relocation strategy. They utilize dry dung pellets or detritus, which they randomly search for, and bury in a pre-constructed burrow. Dry dung pellets or detritus are gathered up in the setal fringes of their hind limbs and, dragged forward to be buried below the moisture line, in the pre-constructed holding chamber (Holm & Scholtz, 1979; Scholtz, 1989; Harrison *et al*., 2003).

In a recent revision of *Scarabaeus* (*Pachysoma*) by Harrison & Philips (2003) the phylogenetic validity of *Pachysoma* was evaluated using cladistic methods. Harrison & Philips (2003) maintained the synonymy of *Neopachysoma* Ferreira with *Pachysoma* while *Mnematium* MacLeay was regarded as a synonym of *Scarabaeus.* *Pachysoma* was confirmed as being a distinct monophyletic clade within *Scarabaeus* and was therefore classified as a derived subgenus thereof (Harrison *et al*., 2003; Forgie *et al*., 2005). Based on Harrison & Philips's (2003) phylogeny, Sole *et al*. (2005) re-examined *Scarabaeus (Pachysoma)* at a molecular level using Cytochrome Oxidase I (COI) mitochondrial sequence data. Eleven of the 13 species were supported at a molecular level with *S. (P.) hippocrates* and *S. (P.) glentoni* forming a species complex. *Scarabaeus (Pachysoma)* was confirmed as being monophyletic within *Scarabaeus*. The synonymy of *Neopachysoma* with *Pachysoma* was supported even though it is clearly a distinct lineage within *Scarabaeus (Pachysoma)* (Sole *et al*., 2005; Forgie *et al*., 2005).

In this study we firstly, re-construct the phylogeny of *Scarabaeus (Pachysoma)* using both morphological (Harrison *et al*., 2003) and molecular (Sole *et al*., 2005) data partitions and secondly, by using the total evidence approach we test for congruence between the two data partitions. In this way the relative overall contribution of these character sets to the combined phylogeny could be assessed.

Material and Methods

Taxa

In-group taxa - All 13 species of the subgenus *Pachysoma* were used to infer the phylogeny. These are: *S. (P.) aesculapius* (Olivier), *S. (P.) bennigseni* (Felsche), *S. (P.) denticollis* (Péringuey), *S. (P.) endroedyi* Harrison, Scholtz & Chown, *S. (P.) fitzsimonsi* (Ferreira), *S. (P.) gariepinus* (Ferreira), *S. (P.) glentoni* Harrison, Scholtz & Chown, *S. (P.) hippocrates* (MacLeay), *S. (P.) rodriguesi* (Ferreira), *S. (P.) rotundigenus* (Felsche), *S. (P.) schinzi* (Fairmaire), *S. (P.) striatus* (Castelnau) and *S. (P.) valeflorae* (Ferreira).

Out-group taxa - The following species were included as they are atypical (see Table 1) and their taxonomy was controversial in the past (Forgie *et al*., 2005): *Scarabaeus* [*Drepanopodus*] *proximus* Janssens, *Scarabaeus rugosus* (Hausman), *Scarabaeus* [*Neateuchus*] *proboscideus* (Guérin), *Scarabaeus galenus* (Westwood), *Scarabaeus* (*Scarabaeolus*) *rubripennis* (Boheman), *Scarabaeus* (*Sceliages*) *brittoni* zur Strassen, *Scarabaeus rusticus* (Boheman) and *Scarabaeus westwoodi* Harold all from the tribe Scarabaeini. The out-group representatives were chosen based on relationships indicated by recent phylogenetic studies (Harrison *et al*., 2003; Forgie *et al*., 2005) and taking into account selection criteria of Nixon & Carpenter (1993).

All species mentioned above were included in the molecular, morphological and combined data analyses. Synonyms of *Scarabaeus* used in this study are indicated in square brackets and include *Neateuchus* Gillet (synonomised by Mostert & Scholtz, 1986), *Neopachysoma* Ferreira (synonomised by Holm & Scholtz, 1979) and *Drepanopodus* Jannsens (synonomised by Forgie *et al*., 2005). Table 2 includes all the species used in this study, the data partitions used, the source of the data and accession numbers.

Phylogenetic Analysis

Statistics

The molecular data were subjected to preliminary sequence analyses prior to phylogenetic analysis. The best model of sequence evolution, the proportion of invariable sites and the α parameter of the distribution of rate variation among sites (Yang *et al*., 1994) were estimated in Modeltest 3.0 (Posada & Crandall, 1998). The average nucleotide and amino acid p-distances

were calculated in MEGA version 2.1 (Kumar *et al*., 2001), within and between both out - and in - group taxa. MEGA was also used to calculate sequence divergence values.

Molecular data

The total aligned molecular matrix consists of 1197 base pairs (bp), corresponds to bases 1713 to 2910 of the Cytochrome Oxidase I gene of *Locusta migratoria* Linneaus (Genbank Accession No. NC_001712). A total of 54 individuals were used for this study, of which 46 (accession numbers on GenBank AY258214 – AY258258) where in-group taxa and 8 identified as out-group taxa. The laboratory procedures for amplifying and sequencing followed standard protocols described previously (Sole *et al*., 2005). Sequences were aligned in Clustal X (Thompson *et al*., 1997) and subsequent analyses were performed in PAUP*4.0b1 (Swofford, 1998)**.**

Both Maximum Likelihood (ML) and Maximum Parsimony (MP) methods were used to infer phylogenetic relationships between species. The robustness of the results was assessed by means of bootstrap analysis (Felsenstein, 1985), using 1 000 pseudoreplicates and branch-and-bound searching (nucleotides treated as unordered characters). A single representative from each species was included in the ML analysis, except for *S. (P.) glentoni* for which two specimens were included one of which no resolution had been previously obtained (see results below and Chapter 2 (Sole *et al*., 2005) for details). The parameters estimated by Modeltest were used in a Maximum Likelihood heuristic analysis with 1000 pseudoreplicates.

Table 1. Summary of the wing status, feeding specialisation and modes of dung removal for the species used in this study.

Taxa	Distribution	Wing Status	Feeding Specialisation	Modes
S. [*Neateuchus*] *proboscideus* (Guérin)	Afrotropical (W South Africa, Kalahari)	Macropterous	wet dung	Rolling
S. [*Neopachysoma*] *denticollis* (Péringuey)	Afrotropical (Namib desert)	Apterous	dry dung pellets/detritus	Dragging
S. [*Neopachysoma*] *rodriguesi* (Ferreira)	Afrotropical (Namib desert)	Apterous	dry dung pellets/detritus	Dragging
S. [*Neopachysoma*] *rotundigenus* (Felsche)	Afrotropical (Namib desert)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *aesculapius* Olivier	Afrotropical (W South Africa)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *hippocrates* (MacLeay)	Afrotropical (W South Africa)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *glentoni* Harrison, Scholtz & Chown	Afrotropical (W Africa; south Olifants River)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *endroedyi* Harrison, Scholtz & Chown	Afrotropical (W Africa; north Olifants River)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *striatus* (Castelnau)	Afrotropical (W South Africa)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *gariepinus* (Ferreira)	Afrotropical (W Africa)	Apterous	dry dung pellets/detritus	Dragging, Rolling
S. (*Pachysoma*) *bennigseni* (Felsche)	Afrotropical (W Africa)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *schinzi* (Fairmaire)	Afrotropical (W Namibia)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *valeflorae* (Ferreira)	Afrotropical (W Namibia)	Apterous	dry dung pellets/detritus	Dragging
S. (*Pachysoma*) *fitzsimonzi* (Ferreira)	Afrotropical (W Namibia)	Apterous	dry dung pellets/detritus	Dragging
S. (*Scarabaeolus*) *rubripennis* (Boheman)	Afrotropical (Namib desert)	Macropterous	opportunistic	Rolling
Scarabaeus galenus (Westwood)	Afrotropical (Southern Africa)	Macropterous	wet dung pellets	Carrying, Tunnelling, Pushing
Scarabaeus rusticus (Boheman)	Afrotropical (South Africa)	Macropterous	wet dung	Rolling
Scarabaeus westwoodi Harold	Afrotropical (Southern + East Africa)	Macropterous	wet dung	Rolling
Scarabaeus rugosus (Hausman)	Afrotropical (SW South Africa)	Macropterous	wet dung	Rolling
Scarabaeus (*Sceliages*) *brittoni* zur Strassen	Afrotropical (W South Africa)	Macropterous	obligate necrophage	Pushing
Scarabaeus [*Drepanopodus*] *proximus* Jannsens	Afrotropical (South Africa)	Macropterous	wet dung	Rolling

*Most species of Scarabaeini are adapted to open habitats and feed on resources that are patchy. True food specialisation in the tribe is uncommon but does occur, listed above.

Table 2. Summary of the species used in this study including where the data were obtained.

Taxa	Tribe	Morphology	Molecular	Accession Numbers
S. [*Neopachysoma*] *denticollis* (Péringuey)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. [*Neopachysoma*] *rodriguesi* Ferreira	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. [*Neopachysoma*] *rotundigenus* (Felsche)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *aesculapius* Olivier	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *hippocrates* (MacLeay)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *glentoni* Harrison, Scholtz & Chown	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *endroedyi* Harrison, Scholtz & Chown	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *striatus* (Castelnau)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *gariepinus* (Ferreira)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *bennigseni* (Felsche)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *schinzi* (Fairmaire)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *valeflorae* (Ferreira)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Pachysoma*) *fitzsimonzi* (Ferreira)	Scarabaeini	Harrison, 1999	Sole et al., 2005	See Text
S. (*Scarabaeolus*) *rubripennis* (Boheman)	Scarabaeini	Harrison, 1999	Sole et al., 2005	AF499763
S. [*Neateuchus*] *proboscideus* (Guérin)	Scarabaeini	Harrison, 1999	Sole et al., 2005	AF499757
Scarabaeus rusticus (Boheman)	Scarabaeini	Harrison, 1999	Forgie, 2003	AF499767
Scarabaeus westwoodi Harold	Scarabaeini	Harrison, 1999	Forgie, 2003	AF499769
Scarabaeus galenus (Westwood)	Scarabaeini	Harrison, 1999	Forgie, 2003	AF499764
Scarabaeus rugosus (Hausman)	Scarabaeini	Harrison, 1999	Forgie, 2003	AF499766
Scarabaeus (*Sceliages*) *brittoni* zur Strassen	Scarabaeini	Harrison, 1999	Forgie, 2003	AF499772
Scarabaeus [*Drepanopodus*] *proximus* Jannsens	Scarabaeini	Harrison, 1999	Sole, unpubl.	AY965239

* The columns entitled molecular and morphology are data types that were used and the references in these columns indicate the source of the data

Morphological data analysis

The raw morphological data for the analyses were obtained from Harrison & Philips (2003). The morphological dataset comprised 64 characters of which 39 were external and 25 internal characters; 16 were bipolar and 48 multi-state (see Appendix 1 for characters) (For details of the morphological characters see Harrison & Philips, 2003). This morphological dataset which was originally analysed in NONA v 2.0 (Goloboff, 1997) was re-analysed in PAUP* using Parsimony analysis to determine the phylogenetic relationships between the species. The parsimony analysis was re-weighted using the re-scaled consistency index (Farris, 1969) and bootstrap analysis was used to assess the robustness of the results, using 1 000 pseudoreplicates and branch-and-bound searching. All trees were rooted and characters were coded as unordered.

Combined data analysis

A total of 21 species, comprising a single individual from each taxon, was used for the combined analysis. To compare the similarity of phylogenetic signal between different data partitions, the partition homogeneity test was calculated across and between both data partitions in PAUP*, with 1,000 replications (Farris *et al*., 1995; Creer *et al*., 2003). A Parsimony analysis was done, in PAUP*, and re-weighted using the re-scaled consistency index after which 1000 bootstrap replicates were performed (Felsenstein, 1985) with branch-and-bound searching.

TreeRot.v2 (Sorensen, 1999) was used to calculate total Bremer support (BS) values at each node (Bremer, 1988) and to determine partitioned Bremer support (PBS) (Baker & DeSalle, 1997; Baker *et al*., 1998) values for each data partition in the combined parsimony tree. Different datasets provide different amounts of support when combined. PBS, therefore, calculates the amount of support each dataset contributes towards the complete combined phylogeny. PBS values can be positive, negative or zero and their sum equals the value of the Bremer support for that node. Positive values indicate that, within a combined data framework, a given partition supports that particular node over any alternative relationships specified by the most parsimonious tree(s) without that node. Negative values indicate that, again in a combined analysis framework, the length of a partition is shorter on the topology of alternate tree(s) not containing a given node and therefore contains contradictory evidence for that node (Baker *et al*., 1998). Bremer support values were calculated using 20 unrestricted random addition sequences per node.

Bayesian Analysis

A Bayesian phylogenetic analysis for the combined and molecular datasets was performed with MrBayes 3 (Huelsenbeck & Ronquist, 2001). The Bayesian analysis approximates the posterior probability (Huelsenbeck *et al*., 2001) for a phylogenetic tree by successively altering the model parameter values in a Markov Chain Monte Carlo (MCMC) procedure. A random tree and parameter values are initially chosen and for each step in the chain a new combination of topology and parameter values are either accepted or rejected according to the Metropolis-Hastings-Green algorithm. Log-likelihood values are calculated for each topology combination and recorded, once these have reached a plateau i.e. stabilised, the frequency at which a clade appears among the sampled trees is then deemed an approximation of the posterior probability. In order to efficiently traverse the parameter space, several chains are run simultaneously at different designated theoretical temperatures. A heated chain moves more easily across a valley and thereby prevents the chain being trapped at a local optimum.

The model for Bayesian analysis was selected with the likelihood-ratio test in Modeltest. Four different analyses were run, beginning with random starting trees. For every analysis five Markov Chains (four heated (temperature = 0.05) and one cold (temperature = 1)) where run for 3 000 000 generations with trees being sampled every 100th generation. Of the four analyses, 25 000 trees were used to determine a consensus phylogeny and posterior probability of the nodes (Warren *et al*., 2003).

Results

Molecular dataset statistics

Modeltest selected the GTR model (Rodriguez *et al*., 1990) with proportion of invariable sites and gamma distribution shape parameter estimated at 0.57 and 0.88, respectively. The within-species sequence divergence ranged from as low as 0.8 % in *S. (P.) schinzi* to 5.7 %, 5.8 % and 6.3 % in *S. (P.) hippocrates*, *S. (P.) glentoni* and *S. (P.) valeflorae*, respectively (Table 3). The average nucleotide pairwise distances within *Scarabaeus (Pachysoma)* ranged from 8 % to 15.3 %, while the average amino acid pairwise distance ranged from 1.3 % to 5.5 % (Table 4).

Table 3. Average intra-specific sequence divergences for the species of *Scarabaeus (Pachysoma)*.

Species	Divergence	Std Error
S. (P.) aesculapius	0.011	0.003
S. (P.) hippocrates	0.057	0.005
S. (P.) endroedyi	0.041	0.004
S. (P.) glentoni	0.058	0.006
S. (P.) fitzsimonzi	0.016	0.003
S. (P.) gariepinus	0.027	0.003
S. (P.) bennigseni	0.024	0.004
S. (P.) rotundigenus	0.018	0.003
S. (P.) rodriguesi	0.012	0.003
S. (P.) schinzi	0.008	0.002
S. (P.) striatus	0.009	0.002
S. (P.) denticollis	0.022	0.003
S. (P.) valeflorae	0.063	0.007
Outgroups	0.118	0.006

Table 4. Average uncorrected nucleotide- (p) and amino acid- distances over all 54 individuals of *S. (Pachysoma)* analysed. The average nucleotide p-distances are indicated in the bottom left of the table while the amino acid p-distances can be found at the top right hand corner.

Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14
S. (P.) aesculapius	**1**		0.029	0.039	0.027	0.033	0.034	0.033	0.044	0.041	0.030	0.041	0.037	0.043	0.051
S. (P.) hippocrates	**2**	0.123		0.043	0.013	0.040	0.037	0.042	0.043	0.043	0.039	0.051	0.040	0.049	0.053
S. (P.) endroedyi	**3**	0.123	0.110		0.043	0.041	0.039	0.041	0.045	0.055	0.044	0.049	0.042	0.046	0.056
S. (P.) glentoni	**4**	0.120	0.080	0.105		0.038	0.035	0.039	0.040	0.042	0.038	0.049	0.037	0.047	0.051
S. (P.) fitzsimonzi	**5**	0.138	0.128	0.118	0.125		0.017	0.020	0.032	0.036	0.020	0.034	0.022	0.028	0.040
S. (P.) gariepinus	**6**	0.131	0.121	0.120	0.118	0.104		0.025	0.026	0.030	0.025	0.040	0.018	0.030	0.031
S. (P.) bennigseni	**7**	0.134	0.135	0.132	0.132	0.111	0.116		0.032	0.035	0.025	0.034	0.029	0.036	0.040
S. (P.) rotundigenus	**8**	0.153	0.139	0.136	0.144	0.124	0.126	0.144		0.026	0.036	0.041	0.022	0.039	0.038
S. (P.) rodriguesi	**9**	0.140	0.127	0.128	0.137	0.124	0.125	0.137	0.110		0.035	0.048	0.031	0.045	0.044
S. (P.) schinzi	**10**	0.137	0.123	0.120	0.127	0.114	0.130	0.134	0.136	0.127		0.033	0.028	0.031	0.043
S. (P.) striatus	**11**	0.141	0.142	0.133	0.141	0.127	0.122	0.138	0.145	0.151	0.133		0.045	0.044	0.054
S. (P.) denticollis	**12**	0.151	0.140	0.134	0.139	0.129	0.129	0.144	0.111	0.109	0.135	0.149		0.032	0.036
S. (P.) valeflorae	**13**	0.127	0.109	0.109	0.119	0.117	0.116	0.132	0.137	0.121	0.095	0.131	0.132		0.046
Outgroups	**14**	0.153	0.144	0.137	0.144	0.126	0.136	0.148	0.146	0.139	0.137	0.158	0.142	0.126	

Molecular data set

Of the 474 variable sites identified, 421 sites were parsimoniously informative and 53 were singletons. The ratio of parsimoniously informative characters (421) to the number of OTU's/haplotypes (42) was very high and would have contributed to the good resolution of the MP tree. The proportion of nucleotide mutations at first, second and third base positions was 19 %, 5 % and 76 % respectively and base composition over the 1 197 base pairs was 39.2 %, 16.1 %, 30.5 % and 14.2 % for T, C, A and G respectively.

The un-weighted parsimony analysis resulted in a single tree with a length of 2177, a consistency index (CI) of 0.314, a retention index (RI) of 0.691, and a re-scaled consistency index (RC) of 0.217 (Fig 1). A single Maximum Likelihood (ML) tree was obtained assuming the GTR model with 57.2% invariant sites, a transition-transversion ratio of 1.2 and a gamma distribution shape parameter of 0.88. The un-weighted MP tree had a similar topology to those trees obtained following Neighbor Joining (NJ), Minimum Evolution (ME), ML and Bayesian analyses (results not shown) confirming that the data were not sensitive to the underlying assumptions of the different analysis methods.

The COI gene phylogeny (Fig. 1) reveals the presence of three distinct clades (labelled A, B and C). Clade A comprises 21 individuals, representing six morphological species, namely *S. (P.) hippocrates, S. (P.) glentoni, S. (P.) aesculapius*, *S. (P.) endroedyi, S. (P.) valeflorae and S. (P.) schinzi*. There is high bootstrap support (between 85 % and 100 %) for four of the six morphological species in this clade with a single individual, *S. (P.) glentoniLEIPV03,* not grouping with the other two representatives of this morphological species. Instead, a species complex comprising 11 individuals of *S. (P.) glentoni* and *S. (P.) hippocrates* (henceforth referred to as the hippocrates/glentoni complex) was recovered. Clade B supports four species, *S. (P.) fitzsimonsi, S. (P.) bennigseni, S. (P.) striatus* and *S. (P.) gariepinus* each with 100% support. Clade C (100 % support) supports three species each with 100% bootstrap support, namely *S. (P.) denticollis, S. (P.) rotundigenus* and *S. (P.) rodriguesi*.

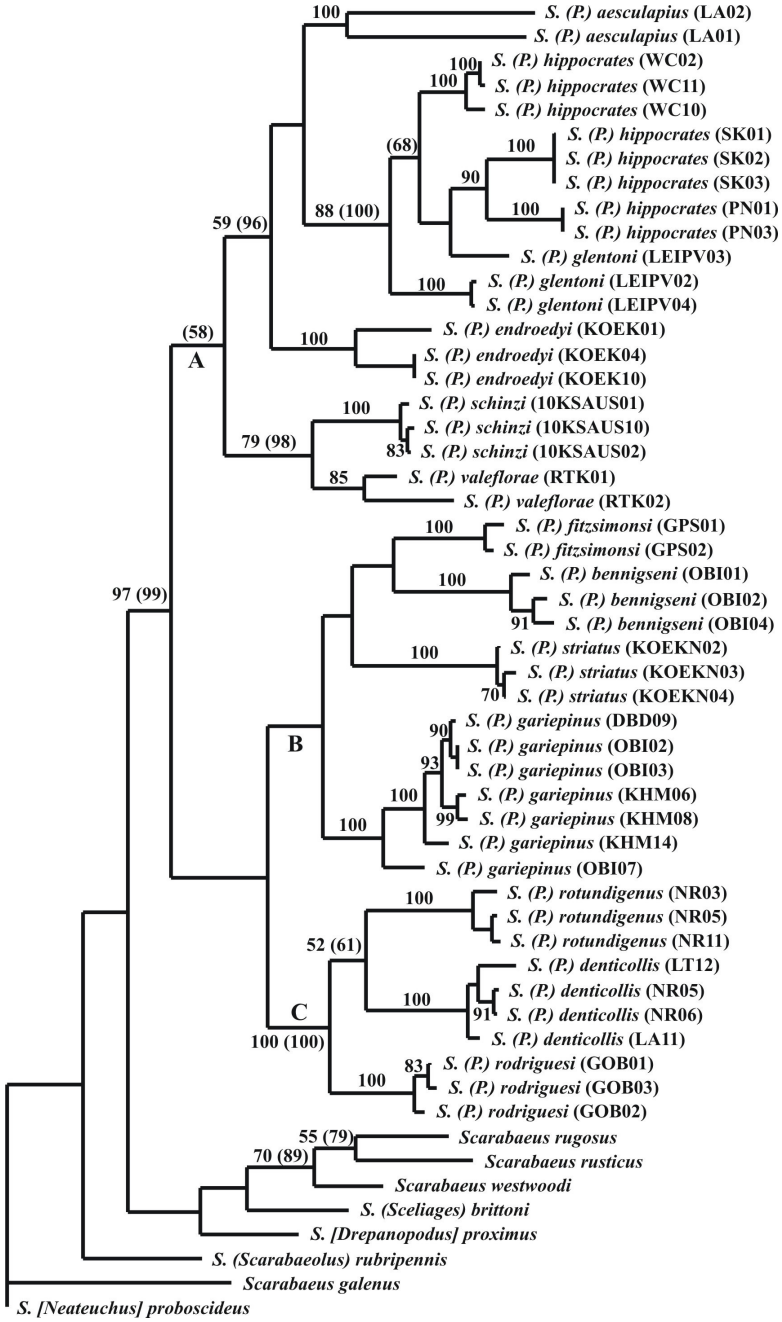

Figure 1. The single most Parsimonious tree of the COI gene phylogeny of *Scarabaeus (Pachysoma)* with bootstrap values greater than 50 % indicated next to the relevant nodes. A, B and C indicate three distinct clades within *Scarabaeus (Pachysoma)*. Maximum Likelihood bootstrap values are in brackets.

Morphological data set

Un-weighted analysis of the 64 characters (Appendix 1), of which 61 were informative, resulted in 20 most parsimonious trees (length = 244, CI = 0.51, RI = 0.74, RC = 0.38). The re-weighted parsimony analysis resulted in two most parsimonious trees (length = 77.447, CI = 0.696, RI = 0.888 and RC = 0.618), of which the strict consensus tree is shown in Figure 2. *Scarabaeus (Pachysoma)* appears monophyletic within *Scarabaeus* with 100 % bootstrap support. All the species within *Scarabaeus (Pachysoma)* appear monophyletic with relatively good bootstrap support (between 50 % and 95 %) for all 13 species. *S. (P.) schinzi* and *S. (P.) valeflorae* are sister species, with 60 % bootstrap support and appear as outliers to the other 11 species. *S. (P.) hippocrates, S. (P.) glentoni*, *S. (P.) endroedyi* and *S. (P.) aesculapius* form a distinct group (91 % bootstrap support) within the *Scarabaeus (Pachysoma)* lineage. *S. (P.) hippocrates* and *S. (P.) glentoni* form sister species, with 85 % bootstrap support.

Figure 3 shows a scanned copy of the tree taken directly out of Harrison & Philips (2003). Harrison & Philips (2003) constructed the Parsimony tree in NONA v. 2.0 (Goloboff, 1997) and for details thereof see Harrison & Philips (2003). *Scarabaeus (Pachysoma)* is clearly monophyletic within *Scarabaeus*. *S. (P.) hippocrates* and *S. (P.) endroedyi* are sister taxa *S. (P.) schinzi* and *S. (P.) valeflorae* are sister species and do not fall as outliers as in the molecular analysis. *S. (P.) fitzsimonsi*, *S. (P.) bennigseni*, *S. (P.) striatus* and *S. (P.) gariepinus* group together and are central within the *Scarabaeus (Pachysoma)* lineage. *S. (P.) denticollis*, *S. (P.) rotundigenus* and *S. (P.) rodriguesi* are sister to each other and form a distinct clade within *Scarabaeus (Pachysoma)*.

Combined Analysis

The partition homogeneity test (Farris *et al*., 1995) on the combined data (two partitions: COI 1197 bp and 64 morphological characters) indicated that the data partitions did not differ significantly (p = 0.187 at $p \geq 0.05$) and could therefore be combined. A heuristic search produced two most parsimonious trees (length = 2058, CI = 0.34, RI = 0.40 and RC = 0.14). A single most parsimonious tree was obtained by successive weighting using the re-scaled consistency index (length = 237.107, CI = 0.541, RI = 0.742 and RC = 0.402) and is presented in Figure 4. Bootstrap, Bremer and partitioned Bremer support values are also shown on Figure 4.

The combined data analysis supports both the morphological and molecular analysis by showing that *Scarabaeus (Pachysoma)* is a monophyletic lineage within *Scarabaeus* supported by both high bootstrap and Bremer support (100 % bootstrap support and BS = 30) with both the molecular and morphological data partitions contributing. The *Scarabaeus (Pachysoma)* lineage shows three distinct clades (labelled A, B and C) as in the molecular data set analysis (Fig. 1). Clade A supports six morphological species (80 % bootstrap support), namely *S. (P.) hippocrates, S. (P.) glentoni, S. (P.) aesculapius*, *S. (P.) endroedyi, S. (P.) valeflorae* and *S. (P.) schinzi*. *S. (P.) hippocrates, S. (P.) glentoni, S. (P.) aesculapius* and *S. (P.) endroedyi* form a distinct lineage (100 % bootstrap) within this clade as in both the morphological and molecular data partition analyses. *S. (P.) hippocrates* and *S. (P.) glentoni* form sister species (100 % bootstrap and BS = 12), as do *S. (P.) valeflorae* and *S. (P.) schinzi* (99 % bootstrap and BS = 6). *S. (P.) aesculapius* and *S. (P.) endroedyi* form sister species with 93 % bootstrap support. Group B supports four monophyletic species, *S. (P.) fitzsimonsi, S. (P.) gariepinus, S. (P.) bennigseni* and *S. (P.) striatus* (85 % bootstrap support, BS = 2). Group C supports three species, *S. (P.) denticollis, S. (P.) rotundigenus* and *S. (P.) rodriguesi*, with 100% bootstrap support (BS = 17). The molecular and morphological data partitions, according to the PBS, appear in most instances to contribute equally to the whole phylogeny. There are however two instances where very large support is obtained from the molecular dataset, Clade C (PBS = 16) and for *S. (P.) hippocrates* and *S. (P.) glentoni* (PBS = 11) as sister species.

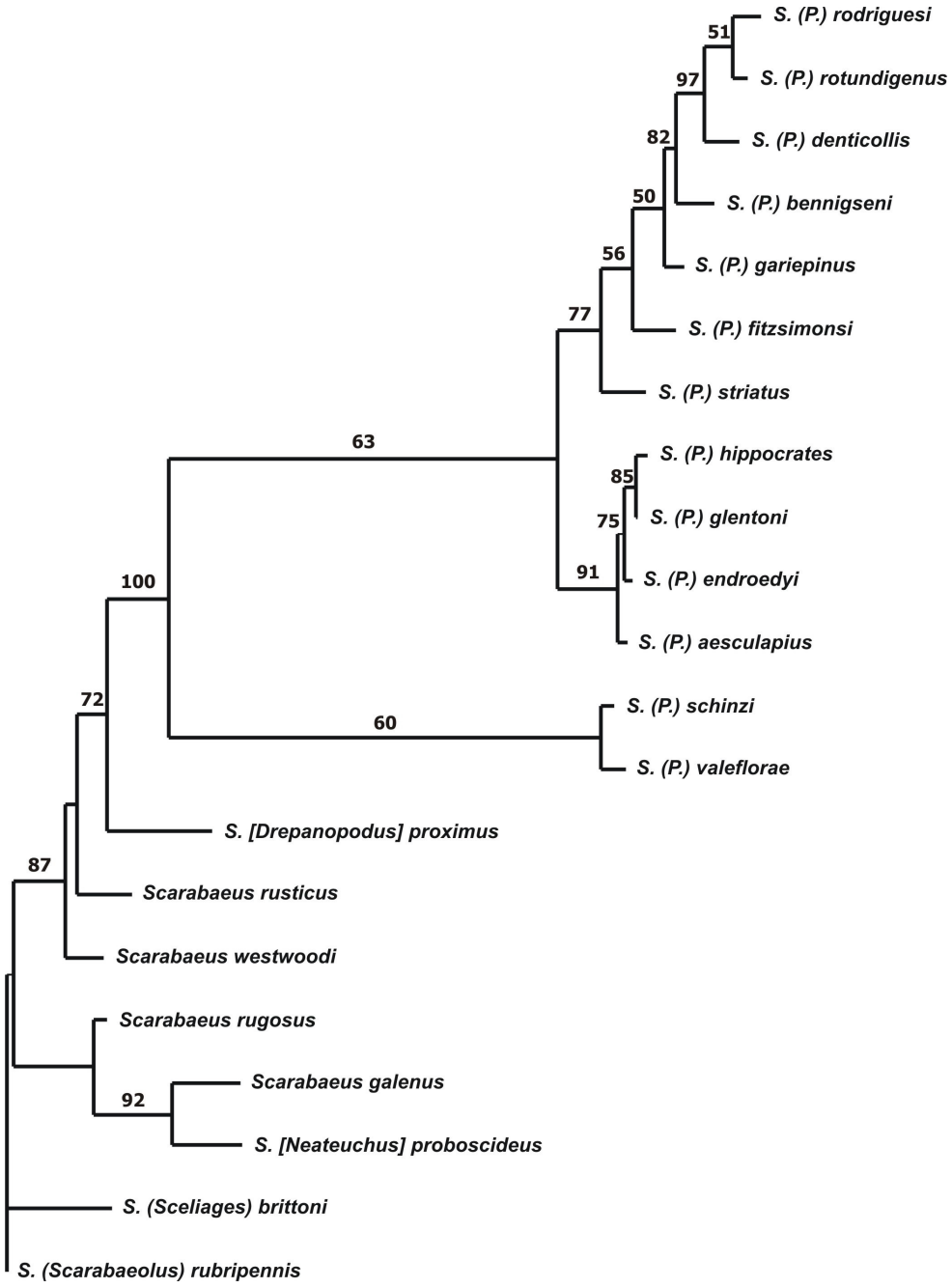

Figure. 2. The Strict consensus Parsimony tree of the morphological data partition of *Scarabaeus (Pachysoma)* with bootstrap values indicated.

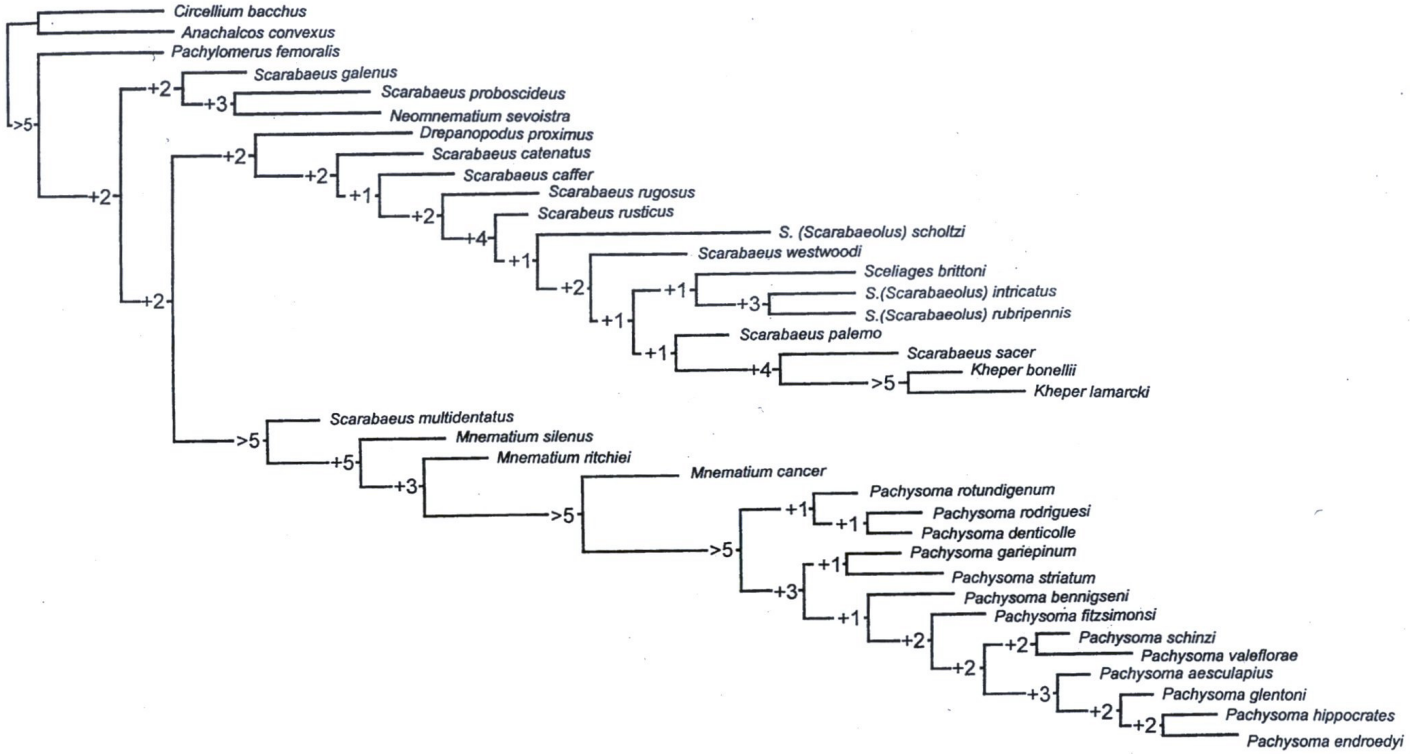

Figure 3. Cladogram depicting the relationships between '*Pachysoma*' and other winged and wingless taxa (obtained from Harrison & Philips (2003) for comparative purposes). The 823-step cladogram (CI = 0.52; RI = 0.85) was obtained after successive weighting of 37 taxa and 64 characters with NONA v 2.0. Numbers indicate decay indices i.e. number of steps needed to collapse a node.

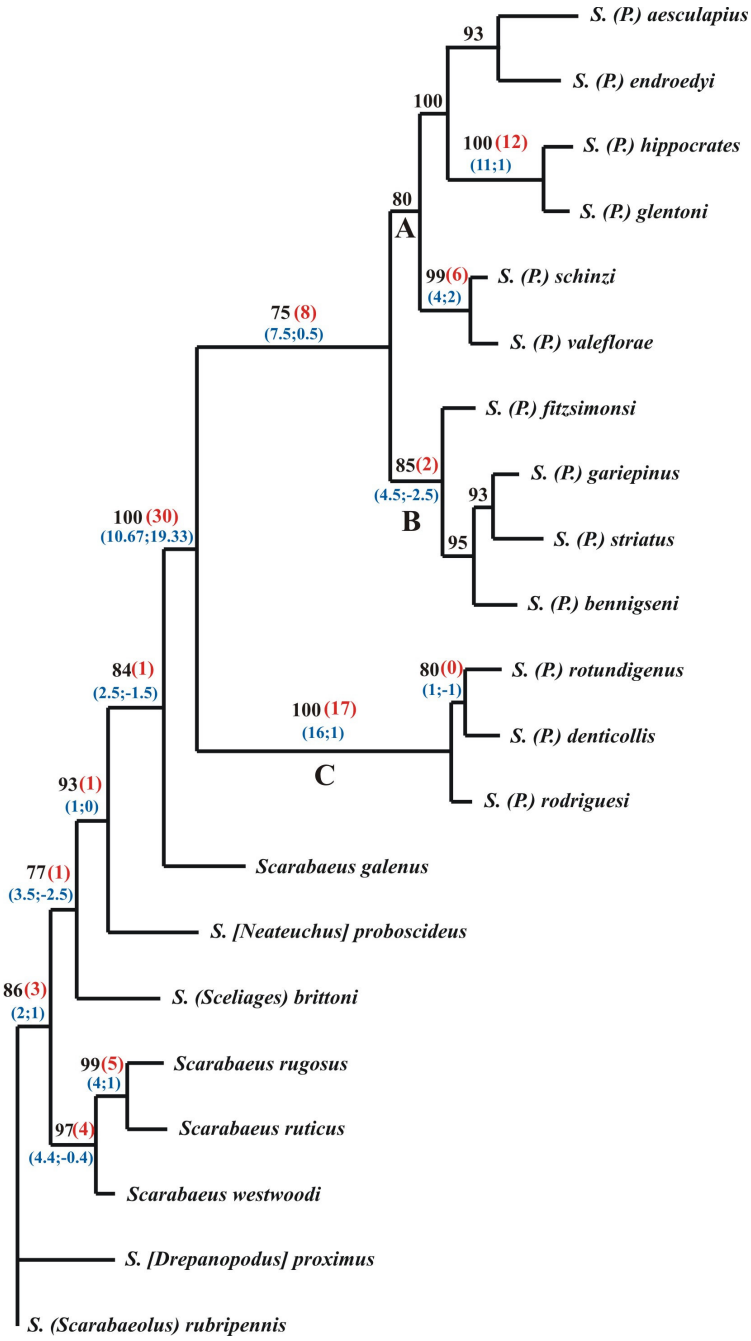

Figure 4. Parsimony tree of combined data partitions. Bootstrap values presented in bold black, Bremer support values are in red and partitioned Bremer support (PBS) values in blue. For PBS values the first value is that for the COI data partition and the second value that for the morphological data partition. (A, B and C represent three clades within the *Scarabaeus (Pachysoma)* lineage, as seen in the molecular phylogeny).

Discussion

Data analysis

Phylogenetic relationships of *Scarabaeus (Pachysoma)* were reconstructed using both molecular and morphological datasets. The expression of differences within a clade is related to its history and to the environmental parameters within which it develops. Both the morphological and COI data partitions display similar patterns between the relationships of the species and have significant phylogenetic structure. It is interesting that such different datasets provide strong phylogenetic signal as individual data partitions as well as when combined. In addition, congruence among datasets is a strong indicator of support for phylogenies based on individual datasets (Wheeler, 1995). Partitioned Bremer Support (Baker & DeSalle, 1997) provides a means of assessing the contribution of molecular and morphological data to the total support of the simultaneous analysis tree. It appeared that the COI dataset lent more support to the overall tree topology of the combined dataset analysis. However, despite the potential differences, consistent compatible trees were recovered which suggest that the models used to analyse the data were adequate for recovering the correct phylogenetic signal (Miyamoto & Fitch, 1995; Clark *et al*., 2001). Considering that the partition homogeneity test was not significant, it would indicate that the combined dataset maximises the amount of information gained by revealing the correct tree (Vogler & Pearson, 1997; Clark *et al*., 2001). Combining of datasets is under debate and a contentious issue (Bull *et al*., 1993; de Queiroz *et al*., 1995; Miyamoto & Fitch, 1995; Funk *et al*., 1995b; Huelsenbeck *et al*., 1996; Yoder *et al*., 2001). However the decision to combine datasets in this study was conservative as similar trees were obtained from both the morphological and molecular phylogenies, and the combined dataset improved resolution, lending strong support for combining good datasets. This was reflected by the robust support for clades in both the molecular and morphological data partition analyses, which was upheld by combining the data partitions.

Differences between phylogenies based on different datasets

Even though it can clearly be seen that increased or better resolution is obtained by combining datasets in this study certain differences do occur between the phylogenies. The morphological phylogeny shows *S. (P.) schinzi* and *S. (P.) valeflorae* group almost as a totally separate clade, which is a major difference between the phylogenies. The other difference noted was the relationships of the central four species, *S. (P.) fitzsimonsi, S. (P.) gariepinus, S. (P.) bennigseni* and *S. (P.) striatus,* to each other. *S. (P.) hippocrates* and *S.*

(P.) glentoni appear to be sister taxa in all our analyses but not in the phylogeny of Harrison & Philips (2003). These differences do not, however, detract from the fact that 13 good species can be identified and *Scarabaeus (Pachysoma)* is a monophyletic lineage within *Scarabaeus*. As morphological and genetic distinctiveness are not strictly correlated discrepancies are often encountered between gene trees and species trees (Vink & Paterson, 2003).

Many studies to date have included combining of datasets, some combining only different genes (i.e. mitochondrial and nuclear) while others combine genes with morphology. Both the genes and morphology of a single individual are exposed to the same environmental parameters but may respond differently in the way that these parameters are dealt with. Different data types are independent indicators of a phylogeny and by combining the unlinked data partitions one would hope to attain an overall similar picture of the relationships relating to the relevant studied taxa. Different studies based on the total evidence approach have shown that by combining different datasets as well as different combinations of the overall available data better resolved trees are more often than not obtained. For examples see Notothenioidei: Channichthyidae (Near *et al*., 2003), Aranae: Lycosidae (Vink & Paterson, 2003), Diptera: Muscidae (Savage *et al*., 2004), Coleoptera: Scarabaeidae (Cabrero-Sañudo & Zardoya, 2004) and Rodentia: Bathyergidae (Ingram *et al*., 2004). Combining data partitions provides a means to discriminate amongst alternate hypotheses posed within the group of interest.

Comparison with prior phylogenetic studies

a) Pachysoma vs. Pachysoma

The inferred trees provide robust evidence for the monophyly of *Scarabaeus (Pachysoma)*, supporting previous studies by Harrison *&* Philips (2003) and Forgie *et al*. (2005). All phylogenetic estimates in the study support the traditional morphological phylogeny by Harrison & Philips (2003). This is not surprising as *Scarabaeus (Pachysoma)* is a well-studied group of dung beetles from a taxonomic point of view (MacLeay, 1821; Ferreira, 1953; Holm & Scholtz, 1979; Mostert & Holm, 1982; Endrödy-Younga, 1989; Harrison *et al*., 2003; Sole *et al*., 2005).

Our results also generally concur with Davis's (1990) phenogram which shows three distinct groupings on the phenogram that correspond to the three clades revealed by the molecular and combined analyses within *Scarabaeus (Pachysoma),* indicated by 'A, B and C'

(Figures 1 & 4, respectively). The species groups delineated by Davis (1990) were based on 28 coded characters described by Holm & Scholtz (1979) and are as follows:

I) *S. (P.) aesculapius, S. (P.) hippocrates* and *S. (P.) schinzi* = clades labelled A on the combined and morphological phylogenies respectively

II) *S. (P.) bennigseni, S. (P.) gariepinus, S. (P.) striatus* and *S. (P.) fitzsimonsi* = clades labelled B on the combined and morphological phylogenies respectively

III) *S. (P.) rodriguesi, S. (P.) denticollis* and *S. (P.) rotundigenus* = clades labelled C on the combined and morphological phylogenies respectively.

b) mtDNA variation in Pachysoma vs. other insect orders

Intra-genic variability in evolutionary rate, at lower level taxonomy, has received little attention, but it appears that the evolutionary rates among portions of COI have remained similar throughout much of insects' evolutionary history (Lunt *et al*., 1996; Langor & Sperling, 1997). The overall A-T content of the 1 197 bp region of the partial COI gene in *Scarabaeus (Pachysoma)* is 69.7 %, which is at the lower end of the 68-76 % range reported for other insects (reviewed by Lunt *et al*., 1996). The average intra-specific COI divergences for *Scarabaeus (Pachysoma)* range between 0.8 and 6.3 % which are comparable to other species of Coleoptera for example *Pissodes* species complex (Curculionidae) (0.5 - 7.5 %; Langor & Sperling, 1997); *Ophraella* (Chrysomelidae) (3.8%; Funk *et al*., 1995a; Funk *et al*., 1995b); *Hypera postica* (Gyllenhal) (Curculionidae) (3.1 %; Erney *et al*., 1996) and *Prodontria* Broun (Scarabaeidae: Melolonthinae) (1.47 %; Emerson & Wallis, 1995). The figures are also similar in other insect orders for example *Papilio* (Lepidoptera: Papilionidae) (0 - 9 %; Sperling, 1993; Sperling & Harrison, 1994), *Apis* (Hymenoptera: Apidae) 0.15 - 1.70 %; Sittipraneed *et al*., 2001), *Drosophila* (Diptera: Drosophilidae) (1.5 - 10 %; Solignac *et al*., 1986) and *Anopheles* (Diptera: Culicidae) (0.005 - 1.2 %; Sedaghat *et al*., 2003). Comparison with these studies is cautioned, however, as the portions of mtDNA, the assessment methods used (nucleotide data/RFLP), and the degree of relatedness of the clades examined may all have differed between studies (Langor & Sperling, 1997). Recent population bottlenecks, selective sweeps of favoured haplotypes or high variance among-family reproductive success may tend to reduce mtDNA diversity within a species. Intra-specific mtDNA variation and geographic distribution of genetic variation within a species depend on both current and historical population structure as well as directional selection. Species thought to have large population sizes and/or a subdivided population structure tend

to maintain greater amounts of mtDNA variability either as nucleotide diversity within populations or as sequence divergence between populations. All of the species of *Scarabaeus (Pachysoma)*, except *S. (P.) schinzi* and *S. (P.) striatus*, show relatively large intra-specific sequence divergences. The species of *Scarabaeus (Pachysoma)* are clearly closely related and exhibit both subdivided population structure, in that some of the populations of species occur in isolated pockets within their distributional range, and others occur along continuous dune fields in relatively large population sizes.

Inter-specific divergences for *Scarabaeus (Pachysoma)* range from 8 to 16 %, which appear to be high compared with other families of Coleoptera (*Cicindela* (0.36 – 1.09 %; Cicindelidae) (Vogler et al., 1993); *Pissodes* (6.0 – 7.5 %; Curculionidae) (Langor & Sperling, 1997)) as well as when compared with other insect orders (*Heliconius erato* (3.4 %; Lepidoptera) (Brower, 1994); *Papilio* (2 - 7.7 %; Lepidoptera) (Sperling & Harrison, 1994); *Feltia* (0.5 - 4.8 %; Lepidoptera) (Sperling *et al*., 1996) and *Drosophila* (7.1 %; Diptera) (Solignac *et al*., 1986). The mtDNA lineages appear, therefore, to have diverged to a level comparable to that beyond where most sister species have attained reproductive isolation. Consequently at the inter-species level the COI gene proved to be a strong phylogenetically informative marker for distinguishing between species within *Scarabaeus (Pachysoma)* (Jones & Gibbs, 1997).

mtDNA vs. ecological divergence in the hippocrates/glentoni complex

S. (P.) hippocrates and *S. (P.) glentoni* are morphologically very similar species and can only be reliably identified based on their male genitalia and habitat preference (for details see Harrison *et al*., 2003). The two species occur sympatrically with *S. (P.) hippocrates* having wider habitat tolerance and geographic distribution - preferring vegetated soft to firm sand of coastal hummocks and hillocks, the periphery of dune systems, and river beds and banks - while *S. (P.) glentoni* is more localised and more of a habitat specialist - preferring firm vegetated sand of river banks and coastal hummocks (Harrison *et al*., 2003). These two species provide an interesting example of ecological divergence without mtDNA sequence divergence. Two reasons can be given for mtDNA showing poor resolution at the phylogenetic level. Firstly, ecological differentiation could be occurring at a faster rate than mtDNA evolution. Secondly, the flow of mtDNA is relatively free, whereas alleles for genes coding for ecological differences are anchored to local conditions. Evidence exists that indicates that relatively fast ecological divergence contributes to poor mtDNA divergence (Shapiro & Masuda, 1980; Sims, 1980; Sperling & Harrison, 1994), suggestive of the

hippocrates/glentoni complex being a recent divergent event that has yet to show distinct mtDNA divergence.

Insight into evolutionary hypotheses of Scarabaeus (Pachysoma)

The phylogenies show a strong geographic association in that the species that group together within a clade have similar distributions. *S. (P.) hippocrates, S. (P.) glentoni, S. (P.) endroedyi* and *S. (P.) aesculapius* have the most southerly distribution (Namaqualand based) within the total *Scarabaeus (Pachysoma)* distribution as well as exhibit the most plesiomorphic characters. *S. (P.) fitzsimonsi, S. (P.) bennigseni, S. (P.) striatus* and *S. (P.) gariepinus* occur in the centre of the total *Scarabaeus (Pachysoma)* distribution. The most derived species – *S. (P.) denticollis, S. (P.) rotundigenus* and *S. (P.) rodriguesi* – have the most northerly distribution and are ultra-psammophilous and therefore well adapted to the loose sand of the Namib Dune Sea.

Aridification would have placed high selection pressure on the xeric adapted winged wet dung feeders. Three main solutions can be used to deal with aridity: increasing diurnal flying efficiency by flying less and foraging faster, reducing body size and feeding on both dung and carrion (Klok, 1994, Harrison & Philips, 2003). *Scarabaeus (Pachysoma)* are flightless which would reduce water loss and energy costs and also exhibit the strategy of feeding on dry dung/detritus (Scholtz, 1989; Klok, 1994; Harrison & Philips, 2003). Dryness of the environment would result in slow rates of decay, hence insects feeding on detritus, carcasses or on persistent plant parts would find that they would persist over long periods of time (Roff, 1990; Scholtz, 2000). The combination of various factors, such as low or no competition for dry dung/detritus, the stable environment and the morphological and physiological constraints of surviving in a sandy xeric habitat may have resulted in the evolution of the present *Scarabaeus (Pachysoma)* lineage (Scholtz, 1989; Klok, 1994; Chown *et al*., 1998; Harrison & Philips, 2003).

Conclusion

Species richness, relative abundance of taxa, genetic and morphological diversity, and ecological diversity are various concepts encompassed by biodiversity. Biodiversity is a result of historical processes; therefore to study biodiversity, access to these patterns and processes is needed. A start to understanding the history behind the diversity is a good phylogeny, which was the reasoning behind this study. The major conclusion is that the combined phylogeny obtained in this study supports both the morphological and molecular

phylogenies. Results are dependant upon both the taxa sampled and the resolving ability of the datasets. In combining the information the two datasets complement each other, and the deficiencies thought to affect the overall result i.e. the small number of morphological characters being overshadowed by the large molecular dataset, are compensated for by each other. It would be advantageous in future studies to expand phylogenetic examination to include nuclear ribosomal genes like 18S or nuclear protein coding genes like elongation factor-1 α (Clark *et al*., 2001) as well as additional individuals from the hippocrates/glentoni complex. We interpret the general agreement of reconstructions between data partitions as an indicator of the validity for the combination of datasets.

Acknowledgements

NAMDEB, in Namibia, and De Beers, in South Africa, are thanked for allowing CS and CHS to complete field work in restricted areas. James du G Harrison is thanked for making his morphological data available. Shaun Forgie is thanked for certain out-group sequences. CS was partially funded by the National Research Foundation (NRF). Bursaries from the University of Pretoria and NRF are gratefully acknowledged.

References

Baker RH, DeSalle R (1997) Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. *Systematic Biology*, **46**, 654-673.

Baker RH, Yu X, DeSalle R (1998) Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. *Molecular Phylogenetics and Evolution*, **9**, 427-436.

Bremer K (1988) The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. *Evolution*, **42**, 795-803.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Science of the United States of America*, **91**, 6491-6495.

Bull JJ, Huelsenbeck JP, Cunningham CW, Swofford DL, Waddell PJ (1993) Partitioning and combining data in phylogenetic analysis. *Systematic Biology*, **42**, 384-397.

Cabrero-Sañudo F-J, Zardoya R (2004) Phylogenetic relationships of Iberian Aphodiini (Coleoptera: Scarabaeidae) based on morphological and molecular data. *Molecular Phylogenetics and Evolution*, **31**, 1084-1100.

Cambefort Y (1991) From Saprophagy to Coprophagy. In: *Dung Beetle Ecology* (eds. Hanski I, Cambefort Y), pp. 22-35. Princeton University Press, Princeton.

Chown SL, Pistorius P, Scholtz CH (1998) Morphological correlates of flightlessness in southern African Scarabaeinae (Coleoptera: Scarabaeidae): testing a condition of the water conservation hypothesis. *Canadian Journal of Zoology*, **76**, 1123-1133.

Clark TL, Meinke LJ, Foster JE (2001) Molecular phylogeny of *Diabrotica* beetles (Coleoptera: Chrysomelidae) inferred from analysis of combined mitochondrial and nuclear DNA sequences. *Insect Molecular Biology*, **10**, 303-314.

Creer S, Malhotra A, Thorpe RS (2003) Assessing the phylogenetic utility of four mitochondrial genes and a nuclear intron in the Asian pit viper genus *Trimeresurus*: separate, simultaneous and conditional data combination analyses. *Molecular Biology and Evolution,* **20**, 1240-1251.

Davis ALV (1990) Climatic change, habitat modification and relative age of dung beetle taxa (Coleoptera: Scarabaeidae, Hydrophilidae, Histeridae, Staphylinidae) in the southwestern Cape. PhD Thesis, University of Cape Town.

De Queiroz AM, Donoghue J, Kim J (1995) Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecological Systematics,* **26**, 657-681.

Emerson BC, Wallis GP (1995) Phylogenetic relationships of the *Prodontria* (Coleoptera; Scarabaeidae; subfamily Melolonthinae), derived from sequence variation in the mitochondrial cytochrome oxidase II gene. *Molecular Phylogenetics and Evolution,* **4,** 433-447.

Endrödy-Younga S (1989) The evolution of alternative life styles in Coleoptera. In: *Alternative Life-history Styles of Animals* (ed. Bruton MN), pp. 317-327. Dordrecht: Kluwer Academic Publishers.

Erney SJ, Pruess KP, Danielson SD, Powers TO (1996) Molecular differentiation of Alfalfa weevil strains (Coleoptera: Curculionidae). *Annals of the Entomological Society of America,* **89**, 804-811.

Farris JS (1969) A successive approximations approach to character weighting. *Systematic Zoology,* **18**, 374-385.

Farris JS, Källersjö M, Kluge AG, Bult C (1995) Testing significance of congruence. *Cladistics,* **10**, 315-319.

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

Ferreira MC (1953) Monografia dos Escarabaeídeos da África do Sul. Tribo-Scarabaeini. I Parte Sub-tribo Pachysomides. *Boletím da Sociedade de Estudos da Província de Moçambique*, **23**, 1-85.

Forgie SA (2003) Phylogeny of the Scarabaeini (Coleoptera: Scarabaeidae). PhD Thesis, University of Pretoria.

Forgie SA, Grebennikov VV, Scholtz CH (2002) Revision of *Sceliages* Westwood, a millipede-eating genus of southern African dung beetles (Coleoptera: Scarabaeidae). *Invertebrate Systematics,* **16**, 931-955.

Forgie SA, Philips TK, Scholtz CH (2005) Evolution of the Scarabaeini (Scarabaeidae: Scarabaeinae). *Systematic Entomology*, **30**, 60-97.

Funk DJ, Futuyma DJ, Orti G, Meyer A (1995a) A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): new evidence from mitochondrial DNA. *Evolution*, **49**, 1008-1017.

Funk DJ, Futuyma DJ, Orti G, Meyer A (1995b) Mitochondrial DNA sequences and multiple data sets: a phylogenetic study of phytophagous beetles (Chrysomelidae: *Ophraella*). *Molecular Biology and Evolution,* **12**, 627-640.

Goloboff PA (1997) NONA Version 2.0 (For Windows). Computer software and documentation. Published by the author, Instituto Miguel Lillo, Miguel Lillo 205, 400 Sierra Madre de Tucuman, Argentina.

Hanski I, Cambefort Y (eds) (1991) *Dung Beetle Ecology*. Princeton University Press, Princeton.

Harrison JduG (1999) Systematics of the endemic south-west African dung beetle genus *Pachysoma* MacLeay (Scarabaeidae: Scarabaeinae). MSc Thesis, University of Pretoria.

Harrison JduG, Phillips TK (2003) Phylogeny of Scarabaeus (Pachysoma MacLeay) sta. nov., and related flightless Scarabaeini (Scarabaeidae: Scarabaeinae). *Annals of the Transvaal Museum*, **40**, 47-71.

Harrison JduG, Scholtz CH, Chown SL (2003) A revision of the endemic south-western African dung beetle subgenus *Scarabaeus (Pachysoma)* MacLeay, including notes on other flightless Scarabaeini (Scarabaeidae: Scarabaeinae). *Journal of Natural History*, **37**, 305-355.

Holm E, Scholtz CH. (1979) A revision of the genus *Pachysoma* M'Leay with an evaluation of the subtribe Pachysomina Ferreira and its genera (Coleoptera: Scarabaeidae). *Journal of the Entomological Society of South Africa***, 42**, 225-244.

Huelsenbeck JP, Bull JJ, Cunningham CW (1996) Combining data in phylogenetic studies. *Trends in Ecology and Evolution*, **11**, 152-158.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754 – 755.

Huelsenbeck JP, Ronquist F, Nielsen R, Rollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science,* **294**, 2310-2314.

Ingram CM, Burda H, Honeycut RL (2004) Molecular phylogenetics and taxonomy of the African mole-rats, genus *Cryptomys* and the new genus *Coetomys* Gray, 1864. *Molecular Phylogenetics and Evolution*, **31**, 997-1014.

Jones DA, Gibbs HL (1997) Intra- and interspecific sequence variation in a portion of the mitochondrial ND6 gene in Cuckoos. *The Condor*, **99**, 815-818.

Klok JC (1994) Desiccation resistance in dung-feeding Scarabaeinae. MSc Thesis, University of Pretoria.

Kumar S, Tamura K, Jacobsen IB, Nei M (2001) Molecular Evolutionary Genetic Analysis Software. *Bioinformatics*, **17**, 1244-1245.

Langor DW, Sperling FAH (1997) Mitochondrial DNA sequence divergence in weevils of the *Pissodes strobi* species complex (Coleoptera: Curculionidae). *Insect Molecular Biology,* **6**, 255-265.

Linnaeus C (1758) *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonimus, locis*. Ed. Decima, reformata, vol **1**. L. Salvii. Holmiae, 824 + iii p.

Lunt DH, Zhang D-X, Szymura JM, Hewitt G.M (1996) The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology*, **5**, 153-165.

MacLeay WS (1821) *Horae Entomologicae: or essays on The Annulose Animals*, Vol 1(2) (London Bagster), 524pp + 3 pls.

Miyamoto MM, Fitch WM (1995) Testing the covarion hypothesis of molecular evolution. *Molecular Biology and Evolution,* **12**, 503-513.

Mostert LE, Holm E (1982) Notes on the flightless Scarabaeina (Coleoptera: Scarabaeidae) with a description of a new species. *Cimbebasia (A)*, **5**, 273-284.

Mostert LE, Scholtz CH (1986) Systematics of the subtribe Scarabaeina (Coleoptera: Scarabaeidae). *Entomology Memoir, Department of Agriculture and Water Supply, Republic of South Africa*, **65**, 1-25.

Near TJ, Pesavento JJ, Cheng CHC (2003) Mitochondrial DNA, morphology, and the phylogenetic relationships of Antarctic icefishes (Notothenioidei: Channichthyidae). *Molecular Phylogenetics and Evolution*, **28**, 87-98.

Nixon KC, Carpenter JM (1993) On simultaneous analysis. *Cladistics*, **12**, 221-241.

Posada D, Crandall KA (1998) Modeltest 3.0: testing the model of DNA substitution. *Bioinformatics,* **14**, 817-818.

Rodriguez F, Oliver JF, Medina JR (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, **142**, 485-501.

Roff DA (1990) The evolution of flightlessness in insects. *Ecological Monographs*, **60**, 389-421.

Savage J, Wheeler TA, Wiegman BM (2004) Phylogenetic analysis of the genus *Thricops* Rondani (Diptera: Muscidae) based on molecular and morphological characters. *Systematic Entomology*, **29**, 395-414.

Scholtz CH (1989) Unique foraging behaviour in *Pachysoma* (=*Scarabaeus*) *striatum* Castelnau (Coleoptera: Scarabaeidae): an adaptation to arid conditions? *Journal of Arid Environments,* **16**, 305-313.

Scholtz CH (2000) Evolution of flightlessness in Scarabaeoidea (Insecta, Coleoptera). *Mitteilungen aus dem Museum füer Naturkunde Berlin, Deutsche Entomologische Zeitschrift*, **47**, 5-28.

Scholtz CH, Chown SL (1995) The evolution of habitat use and diet in the Scarabaeoidea: A phylogenetic approach. In: *Biology, Phylogeny and Classification of Coleoptera: Papers celebrating the 80th birthday of Roy A. Crowson* (eds. Pakaluk J, Slipinski SA), pp.354-374. Museum I Instytut Zoologii PAN, Warszawa.

Scholtz CH, Harrison JduG, Grebennikov VV (2004) Dung beetle (*Scarabaeus (Pachysoma)*) biology and immature stages: reversal to ancestral states under desert conditions (Coleoptera: Scarabaeidae)? *Biolgical Journal of the Linnean Society*, **83**, 453-460.

Sedaghat MM, Linton Y-M, Nicolescu G, Smith L, Koliopoulos G, Zounos AK, Oshagi MA, Vatandoost H, Harbach RE (2003) Morphological and molecular characterization of *Anopheles* (*Anopheles*) *sacharovi* Favre, a primary vector of malaria in the middle east. *Systematic Entomology*, **28**, 241-256.

Shapiro AM, Masuda KK (1980) The opportunistic origin of a new citrus pest. *California Agriculture*, **36**, 4-5.

Sims SR (1980) Diapause dynamics and host plant suitability of *Papilio zelicaon* (Lepidoptera: Papilionidae). *American Midland Naturalist*, **103**, 375-384.

Sittipraneed S, Sihanuntavong D, Klinbunga S (2001) Genetic differentiation of the honey bee (Apis cerana) in Thailand revealed by polymorphism of a large subunit of mitochondrial ribosomal DNA. *Insect Sociaux*, **48**, 266-272.

Sole CL, Scholtz CH, Bastos ADS (2005) Phylogeography of the Namib Desert dung beetles *Scarabaeus (Pachysoma)* MacLeay (Coleoptera: Scarabaeidae). *Journal of Biogeography*, **32**, 75-84.

Solignac M, Monnerot M, Mounolou J-C (1986) Mitochondrial DNA evolution in the *Melanogaster* species subgroup of *Drosophila*. *Journal of Molecular Evolution*, **23**, 31-40.

Sorenson MD (1999) TreeRot, version 2. Boston University, Boston, MA.

Sperling FAH (1993) Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucas* and *P. troilus* species groups. *Heredity*, **70**, 227-233.

Sperling FAH, Harrison RG (1994) Mitochondrial DNA variation within and between species of the *Papilio machaon* group of swallowtail butterflies. *Evolution*, **48**, 408-422.

Sperling F, Byers R, Hickey D (1996) Mitochondrial DNA sequence variation among pheromotypes of the dingy cutworm, *Feltia jaculifera* (Gn.) (Lepidoptera: Noctuidae). *Canadian Journal of Zoology*, **74**, 2109-2117.

Swofford DL (1998) PAUP*. *Phylogenetic Analysis using Parsimony, Beta version 4.0b1*. Computer program distributed by the Illinois Natural History Survey, Champaign, IL.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24**, 4876-4882.

Vink CJ, Paterson AM (2003) Combined molecular and morphological phylogenetic analyses of the New Zealand wolf spider genus *Anoteropsis* (Aranae: Lycosidae). *Molecular Phylogenetics and Evolution*, **28**, 576-587.

Vogler AP, DeSalle R, Assman T, Knisley CB, Schultz TD (1993) Molecular population genetics of the endangered tiger beetle Cincindela dorsalis (Coleoptera: Cicindelidae). *Annals of the Entomogical Society of America*, **86**, 142-152.

Vogler AP, Pearson DL (1996) A molecular phylogeny of the Tiger Beetles (Cicindelidae): congruence of mitochondrial and nuclear rDNA data sets. *Molecular Phylogenetics and Evolution*, **6**, 321-338.

Warren BH, Bermingham E, Bowie RCK, Prys-Jones RP, Thébaud C (2003) Molecular phylogeography reveals island colonisation history and diversification of western Indian Ocean sunbirds (*Nectarinia*: Nectariniidae). *Molecular Phylogenetics and Evolution,* **29**, 67-85.

Wheeler WC (1995) Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. *Systematic Biology*, **44**, 321-331.

Yang Z, Goldman N, Friday A (1994) Comparison of models from nucleotide substitution used in maximum-likelihood phylogenetic estimation. *Molecular Biology and Evolution*, **11**, 316-324.

Yoder AD, Irwin JA, Payseur BA (2001) Failure of the ILD to determine data combinability for slow Loris phylogeny. *Systematic Biology*, **50**, 408-424.