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Chapter II

Phylogeography of the Namib Desert dung beetles *Scarabaeus (Pachysoma)* MacLeay (Coleoptera: Scarabaeidae)

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Abstract

Aim Namib Biogeography in many instances remains reliant on advanced and detailed systematic studies. This study attempts to combine molecular phylogenetic data, geology and palaeo-climatic data to, firstly, resolve the relationships of the 13 morphological species of *Scarabaeus (Pachysoma)* and, secondly, to relate their evolution to past climatic and geological events.

Location South Africa and Namibia

Methods Sequencing of an 1197 bp segment of the mitochondrial cytochrome oxidase I (COI) gene of the 13 species within *Scarabaeus (Pachysoma)* was undertaken. Analyses performed included Parsimony and Maximum Likelihood as well as imposing a molecular clock.

Results The molecular phylogeny showed strong support for 11 of the 13 morphological species. The remaining two species, *S. (P.) glentoni* and *S. (P.) hippocrates,* formed a complex and could not be assigned specific status on the basis of the COI gene phylogeny. Strong support for the three species formerly classified within the genus *Neopachysoma* was consistently obtained. The subgenus appears to have arisen approximately 2.9 million years ago. Species within the subgenus arose at different times, with the common ancestor to *Neopachysoma* and the hippocrates complex having evolved 2.65 and 2.4 million years ago respectively. *S. (P.) denticollis*, *S. (P.) rotundigenus*, *S. (P.) rodriguesi* and *S. (P.) schinzi* are some of the youngest species having diverged between 2 million and 600 000 years ago.

Main conclusions *Scarabaeus (Pachysoma)* is a derived monophyletic clade within the Scarabaeini. The subgenus appears to be young in comparison with the age of the Namib Desert, which dates back to the Miocene (*ca* 15 Ma). The psammophilous taxa are shown to disperse with their substratum and habitat, barchan dunes. Clear south/north evolutionary gradients can be seen within the species of this subgenus, which are consistent with the unidirectional wind regime. Species with a suite of mostly plesiomorphic characters have a southerly distribution while their derived psammophilous relatives have central to northern Namib distributions. Major rivers such as the Orange, Buffels and Holgat appear to be gene barriers to certain species as well as areas of origin of speciation events.

Keywords Coleoptera, Scarabaeidae, *Scarabaeus (Pachysoma),* Aptery, Endemic, Namib Desert, Biogeography, Phylogeny, Mitochondrial DNA, Cytochrome Oxidase I (COI).

Introduction

Scarabaeus (Pachysoma) MacLeay (1821) represents a group of 13 atypical flightless dung beetle species belonging to the ball-rolling Scarabaeini (Scarabaeidae: Scarabaeinae) that are distributed along the west coast of southern Africa from Cape Town in South Africa (S33°56'-E18°28') to the Kuiseb River (S22°58'-E14°30') in Namibia (Harrison *et al*., 2003). Individual species, however, usually have very restricted distributions. Flightlessness has resulted in atypical morphology in these species such as the absence of humeral calli, semi-contiguous mesocoxae and short mesosterna (Harrison *et al*., 2003). Their biology is also highly unusual as they feed on dry herbivore dung pellets and detritus that they drag forwards (Scholtz, 1989) whereas their *Scarabaeus* relatives form balls from wet herbivore dung, which they roll backwards. Their dung-burial activity also differs from other ball-rolling dung beetles. *Scarabaeus (Pachysoma)* first locate food, dig a burrow, then forage repeatedly using polarized light for orientation (Dacke *et al.,* 2002), until they have collected sufficient dung fragments or bits of detritus. Related rollers locate dung, form a ball at the source and roll it away to be buried in a suitable place. *Pachysoma* species are restricted to sandy coastal habitats whereas *Scarabaeus* species have a much wider habitat tolerance (Harrison & Philips, 2003). These morphological and biological differences have led to contention about *Pachysoma/Scarabaeus* taxonomy over the years. *Pachysoma* has been treated as a separate genus (Ferreira, 1953), as a synonym of *Scarabaeus* (Mostert & Holm, 1982) and more recently, as a result of a morphology based phylogenetic analysis of the tribe Scarabaeini, it has been accorded subgeneric status (Harrison & Philips, 2003). It is hypothesized to be a monophyletic group and sister to the main *Scarabaeus sensu stricto* lineage that radiated in the Namib Desert after the onset of hyper-aridity in the region.

The narrow, low-lying, coastal strip between the Atlantic Ocean and the Great Escarpment of southern Africa (Fig. 1) stretching from Cape Town in the south to the Carunjamba River in Angola (S15°10'00" – E12°15'00") extends over roughly 2000 km of arid, sandy regions and encompasses three distinct biomes (Rutherford & Westfall, 1994). The southern tip of this area comprises the western extreme of the Fynbos Biome and the enormously species-rich Cape Floristic Region. The area up to the Orange River (S28°40' – E16°30'), which divides South Africa and Namibia, comprises elements of the Succulent Karoo Biome, and is geographically considered to be Namaqualand. The area north of the Orange River and stretching

into Angola is treated as Desert Biome and comprises the Namib Desert. Geologically, however, the region from the Olifants River (S31°42' – E18°11') to the Carunjamba River is considered to be the Namib Desert (Pickford & Senut, 1999). All three regions are characterized by a sandy substrate and aridity, which has been maintained by the cold Benguela Current flowing up the west coast of the continent since the Miocene, 15 million years ago (Mya) (Pickford & Senut, 1999). Aridity increases from south to north. The southern half falls in a winter rainfall regime whilst the northern half receives rain in summer. Rainfall, however, is very low throughout the region but moisture is available to plants and animals in the form of regular dense fogs (Seely & Louw, 1980). The whole area is biologically characterized by exceptionally high plant and animal endemicity. Many of the adaptations seen in animals and plants can be attributed to the harsh conditions to which they are exposed.

Namib Desert beetles are amongst the animal groups with high endemicity and with a suite of morphological, behavioural and physiological characters that adapt them to these conditions (Endrödy-Younga, 1982; Crawford *et al*., 1990; Hanrahan & Seely, 1990; Nicolson 1990). Amongst these are several groups of Scarabaeoidea, including *Scarabaeus (Pachysoma)* (Holm & Scholtz, 1979; Scholtz, 1989; Dacke *et al*., 2002; Harrison *et al*., 2003).

The Namib Desert has been an evolutionary hotspot since the Miocene because of dramatic geological and climatic changes that have selected for taxa capable of withstanding hyper-aridity and barren, mostly sandy, landscapes. The area is currently characterized by barren, sand and gravel plains, extensive dune seas and rocky outcrops interspersed by wide beds of ancient rivers. These westward-directed rivers cut deep courses across the Namib, apparently in response to epeirogenic uplift in the Late Tertiary, possibly during the Pliocene 3-5 Mya (Ward & Corbett, 1990). This resulted in the availability of considerable sediment for transporting back onshore under the influence of the southerly palaeo-wind regime and arid climate. Since at least Late Miocene times, southerly winds have dominated the climate of the near shore parts of the southern Namib. Currently these winds are still some of the most persistent on earth (Pickford & Senut, 1999). They have contributed significantly to depositing the massive sea of mobile sands of the Central Namib, the 40 000 km^2 Sossus Sand Formation or, as it is colloquially known, the Namib Sand Sea.

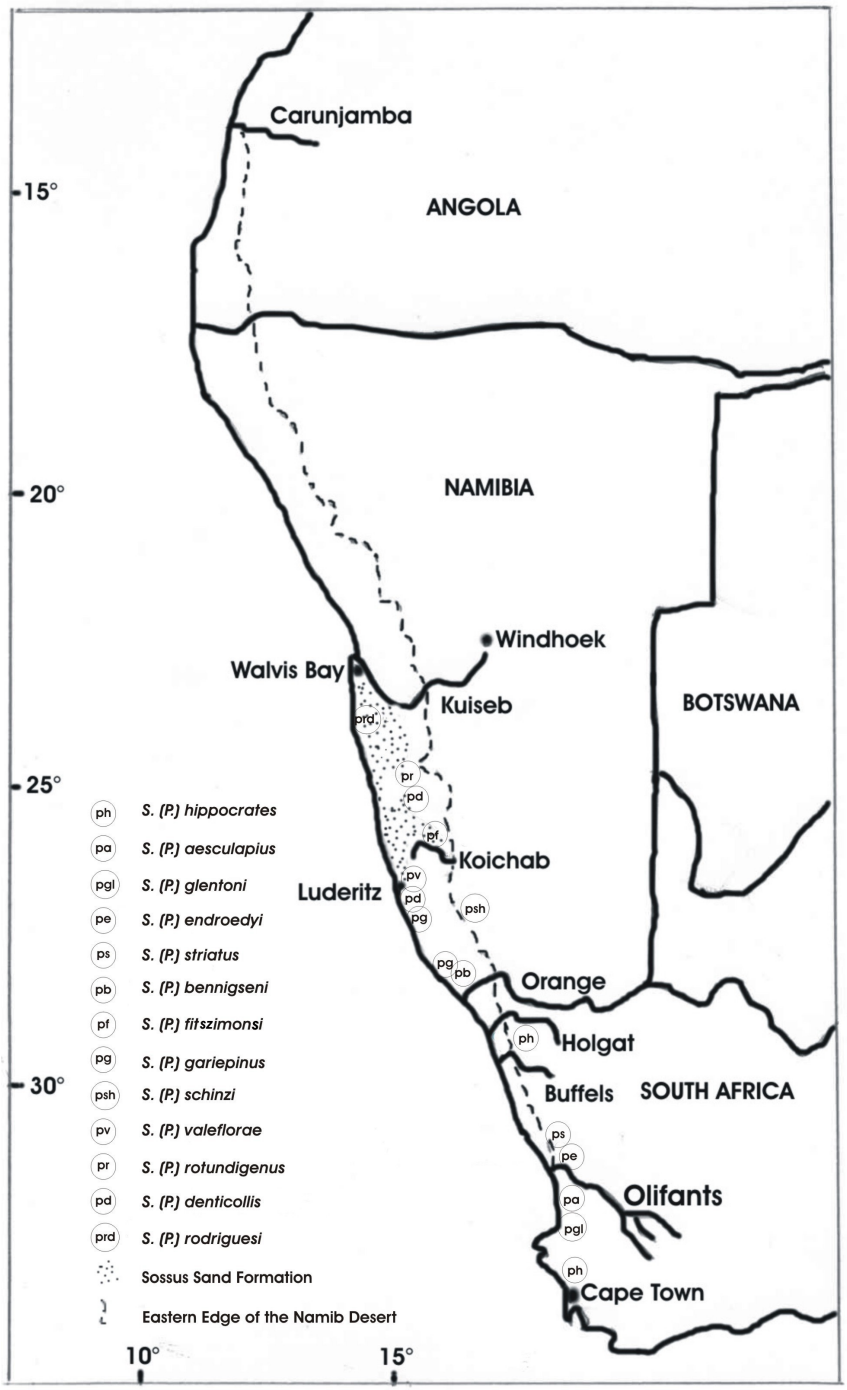

Figure 1. The Namib Desert, extending from the Olifants River, in South Africa, to the Carunjamba River, in Angola, indicating specimen collection sites for this study.

Although a recent morphological phylogeny of *Scarabaeus (Pachysoma)* exists (Harrison & Philips, 2003) it is unable to answer questions regarding the age of lineages or speciation events. However, radiation of the species and their biogeographical history may now be inferred because a comprehensive history of the geology and palaeo-climate of the Namib Desert is available (Pickford & Senut, 1999). In addition, molecular analyses allow estimates of lineage ages by applying a molecular clock (Zuckerkandl & Pauling, 1965; Tajima, 1993). Consequently, this study was aimed at resolving relationships between the 13 morphological species of *Scarabaeus* (*Pachysoma*) at a molecular level and at estimating the divergence times and ages of the species within the subgenus in relation to past geological and climatic events.

Methods

Representative 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 – Two flighted *Scarabaeus* species, *S. proboscideus* and *S. rugosus*, characterized in a separate study (Forgie, 2003), that occur sympatrically with *Pachysoma*, were used. The phylogenetic relatedness of these taxa falls within the selection criteria discussed by Nixon and Carpenter (1993) and by Wheeler (1990) to effectively polarize the in-group character sets.

Sampling and nucleic acid extraction

Twelve of the 13 species of *Scarabaeus (Pachysoma)* were collected along the west coast of southern Africa from the West Coast National Park in the Cape Province to the Kuiseb River just south of Walvis Bay (Fig. 1), in Namibia (Summarized in Table 1). For each species, individual's representative of diverse localities, were collected, and preserved in absolute ethanol. Two museum specimens of *S. (P.) valeflorae* were obtained from the National

Collection of Insects (NCI) at the Agricultural Research Council (ARC) in Pretoria, South Africa. Identification of three morphologically similar species, *S. (P.) hippocrates, S. (P.) endroedyi* and *S. (P.) glentoni*, was confirmed by James du G Harrison of the Transvaal Museum using male genitalia.

Where possible, at least three individuals per locality and per species were selected for genetic characterization of the mitochondrial Cytochrome Oxidase subunit I (COI) gene (Avise *et al*., 1987; Simon *et al*., 1994). For the specimens preserved in ethanol muscle tissue from the thorax was used for DNA extraction whilst DNA from dried specimens was extracted from the tarsus of one leg. DNA was ultimately extracted from 46 individuals representing the 13 species (Table 1) using the Dneasy Tissue Kit (Qaigen).

Genomic amplification and nucleic sequence determination

Primers used for amplification of contemporary DNA were TL2–N-3014 and C1–J–1718 (Simon *et al*., 1994), which target a 1345-bp fragment. For the dried museum material, *Scarabaeus* (*Pachysoma)* specific primers were designed to amplify regions of between 300 and 600-bp. Two forward primers - C-301-F and C-526-F - and two complimentary reverse primers - C-409-R and C-602-R - were designed on the basis of aligned *Scarabaeus* (*Pachysoma)* sequences generated in this study (all primers are summarized in Table 2).

PCR was performed using a Perkin Elmer Gene Amp 2400 in a final volume of 50µl containing 20pmol of each primer, 10mM dNTP's and 1 X buffer in the presence of 1 unit of *Taq* DNA polymerase (Takara). BSA was added to improve the sensitivity of the reaction when the dried material was amplified (Higuchi, 1991). Thermal cycling parameters comprised an initial denaturation for 90 seconds at 94°C followed by 35 cycles of 94°C for 22 seconds, 48°C for 30 seconds and 72°C for 90 seconds with a final elongation step at 72°C for 1 min. The amplified COI gene products were purified from the tube using the High Pure PCR Product Purification kit (Roche) according to manufacturer specifications.

Sequencing reactions were performed at an annealing temperature of 48°C with versions 2.0 and 3.0 of the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer). Each amplicon was sequenced with the external PCR primers plus two internal primers, C1-J-2183 and a modified version of C1-N-2329 (Simon *et al*., 1994; Table 2).

Table 1. Summary of the 46 *Scarabaeus* (*Pachysoma)* individuals characterized in this study.

Species	Specimen ID	Locality	Co-ordinates	GenBank Accession No.
S. (P.) aesculapius	LA01	10km W Leipoldtville	S32°13'06.3" - E18°26'06.8"	AY258214
S. (P.) aesculapius	LA02	10km W Leipoldtville	S32°13'06.3" - E18°26'06.8"	AY258213
S. (P.) glentoni	LEIP02	10km W Leipoldtville	S32°13'06.3" - E18°26'06.8"	AY258226
S. (P.) glentoni	LEIP03	10km W Leipoldtville	S32°13'06.3" - E18°26'06.8"	AY258227
S. (P.) glentoni	LEIP04	10km W Leipoldtville	S32°13'06.3" - E18°26'06.8"	AY258228
S. (P.) hippocrates	WC02	West Coast National Park	S33°48' - E18°27'	AY258215
S. (P.) hippocrates	WC10	West Coast National Park	S33°48' - E18°27'	AY258216
S. (P.) hippocrates	WC11	West Coast National Park	S33°48' - E18°27'	AY258217
S. (P.) hippocrates	PN01	Port Nolloth	S29°14'12.9" - E16°52'01.1"	AY258221
S. (P.) hippocrates	PN03	Port Nolloth	S29°14'12.9" - E16°52'01.1"	AY258222
S. (P.) hippocrates	SK01	Kleinsee - Sandkop	S29°40'03" - E17°12'13.2"	AY258218
S. (P.) hippocrates	SK02	Kleinsee - Sandkop	S29°40'03" - E17°12'13.2"	AY258219
S. (P.) hippocrates	SK03	Kleinsee - Sandkop	S29°40'03" - E17°12'13.2"	AY258220
S. (P.) endroedyi	KOEK01	Koekenaap	S31°30'32.7" - E18°12'29.2"	AY258223
S. (P.) endroedyi	KOEK04	Koekenaap	S31°30'32.7" - E18°12'29.2"	AY258224
S. (P.) endroedyi	KOEK10	Koekenaap	S31°30'32.7" - E18°12'29.2"	AY258225
S. (P.) striatus	KOEKN02	Koekenaap	S31°30'32.7" - E18°12'29.2"	AY258250
S. (P.) striatus	KOEKN03	Koekenaap	S31°30'32.7" - E18°12'29.2"	AY258251
S. (P.) striatus	KOEKN04	Koekenaap	S31°30'32.7" - E18°12'29.2"	AY258252
S. (P.) gariepinus	OBI02	Obib Dune Fields	S28°01'03.5" - E16°39'03.8"	AY258235
S. (P.) gariepinus	OBI03	Obib Dune Fields	S28°01'03.5" - E16°39'03.8"	AY258236
S. (P.) gariepinus	OBI07	Obib Dune Fields	S28°01'03.5" - E16°39'03.8"	AY258237
S. (P.) gariepinus	KHM06	Klingharts Mountains	S27°24'18" - E15°37'25.6"	AY258232
S. (P.) gariepinus	KHM08	Klingharts Mountains	S27°24'18" - E15°37'25.6"	AY258233
S. (P.) gariepinus	KHM14	Klingharts Mountains	S27°24'18" - E15°37'25.6"	AY258234
S. (P.) gariepinus	DBD09	Daberas Dune Fields	S28°11'20.6" - E16°46'59.9"	AY258231
S. (P.) schinzi	10KSAUS01	10km S Aus	S26°47'14.2" - E16°17'46.6"	AY258247
S. (P.) schinzi	10KSAUS02	10km S Aus	S26°47'14.2" - E16°17'46.6"	AY258248
S. (P.) schinzi	10KSAUS10	10km S Aus	S26°47'14.2" - E16°17'46.6"	AY258249

S. (P.) fitzsimonzi	GPS01	Namib Rand Road	S25°32'19.4" - E16°16'29.9"	AY258229
S. (P.) fitzsimonzi	GPS02	Namib Rand Road	S25°32'19.4" - E16°16'29.9"	AY258230
S. (P.) denticollis	NR05	Namib Rand	S25°12'52.5" - E16°01'10"	AY258255
S. (P.) denticollis	NR06	Namib Rand	S25°12'52.5" - E16°01'10"	AY258256
S. (P.) denticollis	LT12	Luderitz - Agate Beach	S26°41'17.1" - E15°15'50.1"	AY258253
S. (P.) denticollis	LA11	Luderitz - Agate Beach	S26°41'17.1" - E15°15'50.1"	AY258254
S. (P.) rotundigenus	NR03	Namib Rand	S25°12'52.5" - E16°01'10"	AY258241
S. (P.) rotundigenus	NR05	Namib Rand	S25°12'52.5" - E16°01'10"	AY258242
S. (P.) rotundigenus	NR11	Namib Rand	S25°12'52.5" - E16°01'10"	AY258243
S. (P.) bennigseni	DBD01	Daberas Dune Fields	S28°11'13.4" - E16°47'03.2"	AY258238
S. (P.) bennigseni	DBD02	Daberas Dune Fields	S28°11'13.4" - E16°47'03.2"	AY258239
S. (P.) bennigseni	DBD04	Daberas Dune Fields	S28°11'13.4" - E16°47'03.2"	AY258240
S. (P.) rodriguesi	GOB01	Gobabeb	S23°39'53.1" - E15°12'48.1"	AY258244
S. (P.) rodriguesi	GOB02	Gobabeb	S23°39'53.1" - E15°12'48.1"	AY258245
S. (P.) rodriguesi	GOB03	Gobabeb	S23°39'53.1" - E15°12'48.1"	AY258246
S. (P.) valeflorae	RT01	Rotkop	S26°43' - E15°23'	AY258257
S. (P.) valeflorae	RT02	Rotkop	S26°43' - E15°23'	AY258258

Table 2. Summary of oligonucleotide primers used in this study.

Primer	Primer sequence	Length	Position (§)	Reference
C1-J-1718	5' GGAGGATTTGGAAATTGATTAGTTCC 3'	26mer	1651-1676	Simon et al., 1994
C1-J-2183	5' CAACATTTATTTTGATTTTTTGG 3'	23mer	2219-2241	Simon et al., 1994
C1-N-2329	5' ACTGTA AATAT**G**TGATGAGCTCA 3'	23mer	2287-2309	Simon et al., 1994 modified by Forgie and Bloomer (unpubl.)
TL2-N-3014	5' TCCAATGCACTAATCTGCCATATTA 3'	25mer	3323-3302	Simon et al., 1994
C-301-F£	5' CAACAGGAATAACTTTTGATCGTA 3'	25mer	2014-2039	Sole and Bastos, unpubl.
C-409-R£	5' GATGTATTTAAR(A/G)TTTCGATCTGT 3'	25mer	2122-2147	Sole and Bastos, unpubl.
C-526-F£	5' GGATTTGGR(A/G)ATAATTTCTCATAT 3'	23mer	2239-2262	Sole and Bastos, unpubl.
C-602-R£	5' CCAATAGTTATTATAGCATAAAT 3'	23mer	2315-2338	Sole and Bastos, unpubl.

£ Denotes the *Pachysoma* specific primers. § Refers to the corresponding position in *Locusta migratoria* (Genbank accession no. NC_001712).

For the dried museum material up to six primers were used for amplification and sequencing purposes. Both the external amplification primers and the three additional internal forward and reverse primers, C1-J-2183 (Simon *et al*., 1994), C-301-F and C-409-R and, where necessary, C-526-F, were used.

Phylogenetic analysis

Sequence chromatograms were visualized and edited in Chromas (Version 1.43) and were subsequently aligned using Clustal X (Thompson *et al*., 1997). A homologous region of 1197 base pairs (bp) corresponding to nucleotide positions 1713-2910 of *Locusta migratoria* Linneaus (Flook *et al*., 1995) was used for phylogenetic analysis. Both Maximum Parsimony (MP) and Maximum Likelihood (ML) were used to infer the phylogenetic relationships between the species of *Scarabaeus* (*Pachysoma*) (PAUP*4.08b; Swofford, 1998). An initial un-weighted parsimony analysis of the sequences from all individuals was performed, employing branch and bound searches and heuristic searches with 10 random addition sequences for each of 1000 bootstrap replicates (Farrell, 2001).

A posteriori and *a priori* weighting schemes such as the successive approximations weighting method (Farris, 1969; Park & Backlund, 2002) and positional weighting (Huelsenbeck *et al*., 1994; Krajewski & King, 1996) were investigated. In the former approach weights were applied according to the rescaled consistency index (RC), consistency index (CI) and the retention index (RI), whilst with the latter, first, second and third base positions were assigned weights of 4, 1 and 15.7, respectively.

In order to determine the model of sequence evolution, which best fits the COI data at hand, hierarchical likelihood ratio tests were performed using Model Test 3.0 (Posada & Crandall, 1998). Parameters from Model Test were used in a ML heuristic search in PAUP* and nodal support was estimated following 500 bootstrap pseudoreplications.

To use genetic data to infer evolutionary rates the data needs to meet two criteria: firstly, rates of genetic evolution among organismal lineages need to be consistent with a molecular clock model and secondly, the availability of a reliable fossil record (Yoder *et al*., 2000). Equality of evolutionary rates between lineages was assessed with Phyltest 2.0 (Kumar, 1996). In addition rate heterogeneity was investigated by comparing branch lengths and log-likelihood ratios estimated in PAUP* on the most parsimonious tree using the HKY85 model of sequence evolution, with and without the constraint of a molecular clock (Hasegawa & Kishino, 1994). Divergence times were estimated from uncorrected pairwise -

distances in MEGA version 2.1 (Kumar *et al*., 2001) and calibrated on arthropod mtDNA where a 2.3% pair-wise divergence per million years is postulated (Brower, 1994).

Results

Of the 434 variable sites identified across the 46 taxa used in this study, 408 sites were informative and 26 were singletons. The proportion of nucleotide mutations at first, second and third base positions was 19 %, 5 % and 76 % respectively and base composition over the 1197 base pairs was 39.2 %, 16.1 %, 30.5 % and 14.2 % for T, C, A and G respectively

Maximum Likelihood and Maximum Parsimony Analyses

The un-weighted parsimony analysis resulted in three trees with a length of 1711, consistency index (CI) of 0.381, a retention index (RI) of 0.742 and rescaled consistency index (RC) of 0.283. Weighted parsimony searches using CI, RI and RC resulted in the recovery of a single most parsimonious tree, whereas, positional re-weighting did not improve resolution despite accounting for saturation at the third base position. A single ML tree was obtained assuming the GTR model (Rodriguez *et al*., 1990) with 52.4% invariant sites, a transition-transversion ratio of 1.2 and a gamma distribution shape parameter of 0.77. Weighted parsimony analysis using the rescaled consistency index gave a single tree of length 490.52, CI of 0.54, RI of 0.82 and RC of 0.45 (Fig. 2). This MP tree had a similar topology to those trees obtained following Neighbour Joining (NJ), Minimum Evolution (ME) and ML analyses (results not shown).

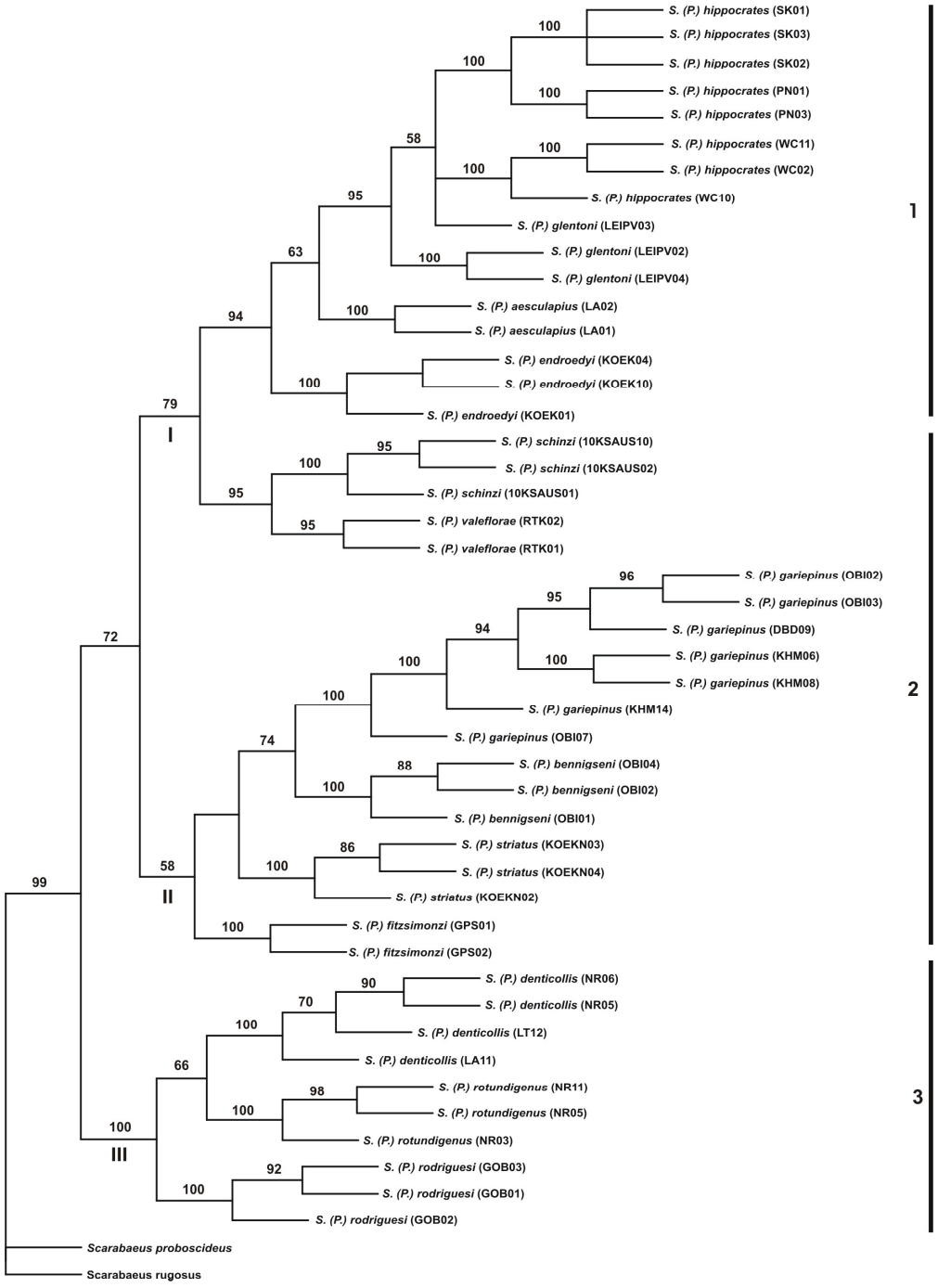

Figure 2. Phylogram of COI gene phylogeny of *Scarabaeus (Pachysoma).* Parsimony tree obtained following successive weighting using RC (tree length = 490.52, CI = 0.54 and RI = 0.82).

The COI gene phylogeny (Fig. 2) reveals the presence of three distinct clades (labelled I, II and III, respectively). Clade I, which has 79% bootstrap support, comprises 21 individuals, representative of six morphological species, namely *S. (P.) hippocrates, S. (P.) glentoni, S. (P.) aesculapius*, *S. (P.) endroedyi, S. (P.) valeflorae and S. (P.) schinzi*. Although there is high bootstrap support (between 94 % - and 100 %) for four of the six morphological species in this clade, a single individual *S. (P.) glentoniLEIPV03* does not group 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 (95% bootstrap support). Clade II supports four species (58% bootstrap support), *S. (P.) fitzsimonzi, S. (P.) gariepinus, S. (P.) bennigseni and S. (P.) striatus,* each with 100% bootstrap support. Clade III supports three species each with 100% bootstrap support, namely *S. (P.) denticollis, S. (P.) rotundigenus* and *S. (P.) rodriguesi*, which were formerly placed in the genus *Neopachysoma.*

Numbers 1 through 3 (right hand side of Fig.2) correspond to the species occurring in three areas differing in aridity as follows: Number 1; *S. (P.) hippocrates*, *S. (P.) glentoni, S. (P.) endroedyi* and *S. (P.) aesculapius* occur within the Fynbos and Namaqualand south and have the most southerly distribution of *Scarabaeus (Pachysoma)*. Number 2; *S. (P.) fitzsimonzi, S. (P.) gariepinus, S. (P.) bennigseni, S. (P.) striatus, S. (P.) valeflorae* and *S. (P.) schinzi* corresponds to those species that occur across two biomes and occupy the central part of the *Scarabaeus (Pachysoma)* distributional range, *S. (P.) striatus* occurs only in the Namaqualand while *S. (P.) fitzsimonzi, S. (P.) gariepinus, S. (P.) bennigseni, S. (P.) valeflorae* and *S. (P.) schinzi* can be found in the southern part of the Desert biome. Number 3; *S. (P.) denticollis, S. (P.) rotundigenus* and *S. (P.) rodriguesi* have the most northerly Desert Biome distribution and are the three ultrapsammophilous species, and also those species formerly classified as *Neopachysoma* (Clade III, Fig. 2).

Imposing a Molecular Clock

The likelihood of the tree with and without enforcing a molecular clock was -log 7205.1461 and –log 7167.44184 respectively. The difference was not significant according to the likelihood ratio test ($p<0.05$). In addition, rate constancy could also not be rejected using PHYLTEST ($p < 0.05$). As both results indicate that the molecular clock hypothesis cannot be rejected, a rate of 2.3% sequence divergence per million years was used to infer a molecular clock (Brower, 1994).

The subgenus is estimated to have arisen about 2.9 million years ago. The hippocrates complex (consisting of *S. (P.) hippocrates, S. (P.) glentoni* and *S. (P.) endroedyi* (Harrison *et al*., 2003)), and *S. (P.) aesculapius* appear to have diverged approximately 2.66 Mya and species of the former genus *Neopachysoma* appear to have diverged approximately 2.4 Mya. The youngest species of *Scarabaeus (Pachysoma)* include *S. (P.) schinzi, S. (P.) rodriguesi, S. (P.) rotundigenus, S. (P.) denticollis*, *S. (P.) bennigseni, S. (P.) aesculapius* and *S. (P.) fitzsimonzi*, and are estimated to have arisen between 200 000 and 600 000 Ya.

Discussion

Palaeontological History

The ball-rolling dung beetles of the tribe Scarabaeini comprise 146 species belonging to five genera and three subgenera. Their distribution extends throughout the Afrotropical region (including Madagascar) and southern latitudes of the Palaearctic (Forgie, 2003). Diversification of the Scarabaeini was thought to coincide with the radiation of both Angiosperms (Eocene: 50 Mya) and mammalian herbivores (lower Oligocene: 35 Mya), with a shift from saprophagy to mycetophagy to coprophagy by adults and larvae (Cambefort, 1991b; Scholtz & Chown, 1995). The Scarabaeini appear to have evolved during the Cenozoic from ancient scarabaeoid lineages dating back to the lower Jurassic ca. 180 – 200 Mya (Crowson, 1981; Cambefort, 1991a; Scholtz & Chown, 1995). The flightless Scarabaeini are monophyletic and contain the most derived members within the tribe with *Scarabaeus (Pachysoma)* representing the most highly evolved of the lineages (Forgie, 2003).

Ideas about rates of evolution of the rich, endemic Namib fauna and flora fall broadly into two schools of thought. Some authors argue that the desert must be very ancient (Cretaceous) in order for the specialized fauna and flora to have had time to evolve. For these scientists, the rates of evolution envisaged are extremely slow. For the second group who consider that the desert is appreciably younger (Miocene), rates of evolution are postulated to have been much more rapid (Pickford & Senut, 1999). However, the various authors have essentially been arguing about different taxa and different hierarchical levels. Some ancient lineages of Late Cretaceous proto-Namib desert ancestry are identifiable amongst insects, for example Lepismatidae (Thysanura: Insecta) (Irish, 1990), but the fauna associated with the post-Miocene Namib Desert Phase (Ward & Corbett, 1990) is logically much younger. Now that we know the hyperaridity of the Namib is no older than the Middle

Miocene (*ca* 15 Mya) (Pickford & Senut, 1999) it is evident that rates of evolution have been orders of magnitude more rapid. This could therefore imply more severe selection pressures and perhaps enhanced generation of genetic variability in desert environments, or a combination of both (Pickford & Senut, 1999).

Biogeographical Inferences

Endrödy-Younga (1978) coined the term "pocket speciation" to describe processes resulting from the numerous small dunes and dune fields of Namib or Kalahari sand origins which have been isolated from the main sand systems and occur throughout southern Namibia and the northern Cape (Koch, 1962). Most of these are alluvial sands that originate at the mouths of the large Tertiary rivers. Any separation of sand dunes from a major system could constitute a vicariance event (Prendini, 2001). These isolated sand dunes are often encountered in unlikely places on the flats and as deposits against mountain slopes. This sand is clearly wind-blown from major dune fields, so the possibility exists that psammophilous taxa may extend their distribution, following pockets of sand to their eventual destinations and thus becoming completely isolated from main populations in time. Endrödy-Younga (1982) provided evidence for this process by demonstrating that, over 11 years, barchan dunes in the southern Namib moved considerable distances across gravel plains together with their associated Tenebrionidae fauna. Dispersal of these species could be attributed not to the movement of individuals but to the movement of their substratum and habitat, the dune. Clear south to north evolutionary gradients in the majority of ultrapsammophilous taxa can be adequately explained in terms of sand movement of barchan dunes, which have been shown to move 10-100 $m.yr^{-1}$ within historical time (Penrith, 1979; Prendini, 2001).

Due to the low, unpredictable rainfall in the Namib since the advent of hyperaridity in the Miocene the fauna is and probably always has been, dependent on the regular, dense fogs that represent virtually the only free water available to it (Seely & Louw, 1980). The fogs have become frequent along the Namib coast since the Early Pleistocene (1.8.Mya) when cold upwellings from the Benguela Current caused cold air that condenses to form fog in contact with the warm air off the land (Pickford & Senut, 1999). This may have been the main environmental parameter that permitted dispersal into, and subsequent radiation, in areas that may have been inhospitable until then.

Speciation Events

It is around the riverbeds and in the deep loose sand of the Sossus Sand Formation, that speciation in *Scarabaeus (Pachysoma)* seems principally to have occurred. The rivers probably presented barriers to the spread of some of the species during the Plio-Pleistocene, and may have vicariously split populations of some others that lead to speciation events. The areas around these riverbeds have high species numbers, and some still appear to be barriers to further range expansion. Isolated populations that occur on sandy plains and in dune fields interspersed by dry riverbeds, gravel plains and rocky outcrops represent the current distribution of most species. Exceptions to this are the ultra-psammophilous species that occur throughout much of the Namib Sand Sea (Harrison *et al*., 2003). As the dune fields shifted and became more continuous through the southern and central Namib, so this allowed for the movement of these isolated populations in a northerly direction. Psammophilous taxa evolved subsequent to establishment of these systems, speciating after initial dispersal events into an environment that had previously constituted a barrier. The older species seem to have inhabited the Karoo (interior Cape Province of South Africa), the southern parts of Namibia and/or the Kaokoveld (north-western Namibia). These are areas of rocky, not excessively sandy substrates indicating that these conditions probably prevailed in much of the Gondwana Desert (Irish, 1990).

The Olifants, Buffels, Holgat, Orange and Kuiseb Rivers (see Fig. 1), which still flow, all affect *Scarabaeus (Pachysoma)* in some way. The Orange River appears to have been of lesser or sporadic importance as a gene barrier, since many psammophilous southern Namib species, for example *S. (P.) gariepinus* and *S. (P.) bennigseni*, occur on both sides of the river. The boundary between related Namib and Namaqualand species lies further south at the Holgat and Buffels Rivers (Irish, 1990). The Buffels River appears to be the southern limit for *S. (P.) gariepinus.* The Holgat River appears to be the barrier to *S. (P.) striatus* from extending its distribution northwards and *S. (P.) bennigseni* from moving southwards. *S. (P.) striatus, S. (P.) gariepinus* and *S. (P.) bennigseni* probably speciated around the Olifants, Buffels and Holgat Rivers, respectively and then moved northwards with the sand. The evolution of *S. (P.) endroedyi* could have resulted from a vicariance event caused by the Olifants River splitting the *S. (P.) aesculapius* population into two and thereby allowing for the speciation of *S. (P.) endroedyi* (For detailed distribution maps of *Scarabaeus (Pachysoma)* see Harrison *et al*., 2003).

Regarding the hippocrates/glentoni complex, *S. (P.) glentoni* is distributed along the Olifants River, from Lambert's Bay, inland to Clanwilliam as opposed to the wider

distribution of *S. (P.) hippocrates.* In some localities they occur sympatrically. *S. (P.) glentoni* prefer the firm vegetated sand of riverbanks and coastal hummocks while *S. (P.) hippocrates* prefer soft to firm sand of coastal hummocks and hillocks on the periphery of dune systems, and river beds and banks. *S. (P.) hippocrates* shows south/north morphological clinal variation implying that the species might be undergoing speciation (Harrison *et al*., 2003). Distances between populations of these two species can range from a few metres to about 40km. The overall small distance between populations and the young age of *S. (P.) glentoni* may underlie the lack of resolution of these two species with the molecular data. Increasing the number of individuals from different localities of the two species and use of an alternative gene marker may help resolve the species complex.

Inferences from the Molecular Clock

Phylogenetic analysis indicates that the psammophilous and ultrapsammophilous species of *Scarabaeus (Pachysoma)*, formerly placed in the genus *Neopachysoma*, are the most derived and have the most northerly distribution in the Sossus Sand Formation which is consistent with the findings of Irish (1990). One may therefore safely assume that psammophilous taxa evolved from an older non-psammophilous ancestor (Irish, 1990). Three of the species of *Scarabaeus (Pachysoma)* show distinct morphological south/north clinal variation, *S. (P.) hippocrates, S. (P.) gariepinus* and *S. (P.) denticollis* (Harrison *et al*., 2003). The clear south/north morphological clinal variation shows strong support for the movement of taxa with the wind blown sand from the barchan dunes. The distribution of *Scarabaeus (Pachysoma)* is halted at the Kuiseb River.

Rapid radiation of most of the species and/or their ancestors, between 2.35 Mya and 2.66 Mya, can clearly be seen within the subgenus and may be linked to the reliability of regular fog in the Pleistocene. Formation of regular fogs would constitute a consistent and reliable form of water. All of the species of *Scarabaeus (Pachysoma)* occur within the fog belt except for *S. (P.) schinzi,* which is confined to the areas around Aus on the Huib-Hoch Plateau, indicating it must be dependent on rainfall. This area is approximately 100km inland from the coast. Rainfall increases while the fog decreases as one moves inland. As seen here and in other insect groups (for examples see Irish, 1990), the distinction between coastal and inland fauna is not absolute as coastal species penetrate inland due to the shared similarities between the slips face/dune-crest habitats of the inland and coastal dunes. The reverse is not true (inland species are absent from the coast). Historical separation appears to be the primary cause of this east/west distributional gradient. One can clearly see the importance of coastal

dunes as a species reservoir and a dispersal vessel, since wherever this sand and its associated fauna have been blown inland, new taxa have evolved.

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