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Did dung beetles arise in Africa? A phylogenetic hypothesis based on five gene regions

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ABSTRACT

Scarabaeinae dung beetle phylogenetics are poorly understood, although recent phylogenetic reconstruction based on morphology and molecular analyses are congruent on the hypothesis that the oldest Gondwana tribes are polyphyletic and that representatives of the ancestral groups are found in Africa. We present a molecular phylogeny of the African representatives of the two oldest tribes, Canthonini (the putative ancestor of all "rollers") and Dichotomiini (thought to represent the ancestral "tunnellers"), based on partial sequence data from two mitochondrial and three nuclear genes, extracted from eight of the nine dichotomiine genera and 17 of the 23 genera of Canthonini. Three well-supported lineages were consistently obtained. Divergence times estimated the origin of the two tribes at around 56 million years ago (MYA) with the splits of the three dung beetle lineages being estimated to have taken place between 40 and 34 MYA. The ages of these splits and subsequent radiation of the modern dung beetle groups concur with those predicted by the fossil record and coincide with the proposed age of radiation of the large mammal groups with whose dung most African dung beetles are still associated. Dispersal of dung beetle groups from Africa is proposed as a biogeographic model, and evidence is presented that dung beetles disperse quickly and widely across continents, and even oceans.

1. Introduction

Although dung beetles represent a relatively small group of insects and appear to live similar lives in an apparently homogenous environment, they have a set of very complex morphological, ecological and behavioural attributes (Hanski and Cambefort, 1991) and their importance in ecosystem services has been shown to be profound (Nichols et al., 2008). Most species are associated with moist mammalian herbivore dung and they have evolved an array of traits to utilise this patchy and ephemeral resource. These traits would appear to be the driving force behind the diversity seen in morphology, behaviour and ecology of the beetles (Philips et al., 2004). On the basis of some of these they have traditionally been divided into two groups, ones that either bury dung directly beneath the dung source (tunnellers) or those that sequester a piece, form it into a ball, and roll it some distance from the source before burying it (rollers) (Hanski and Cambefort, 1991). The buried dung is the food supply for the larvae.

Two schools of thought exist and are represented in the current classification system of dung beetles. Balthasar (1963) recognised two groups, the Scarabaeinae – comprising six tribes of rollers – and the Coprinae – comprising six tribes of tunnellers. Lawrence and Newton (1995), on the other hand place the 12 tribes into the subfamily Scarabaeinae with which the Coprinae were considered synonymous. Most studies undertaken since 1995 have used the latter classification system (e.g. Philips et al., 2004) Two of the 12 tribes, the virtually cosmopolitan rolling Canthonini and tunnelling Dichotomiini are considered ancient lineages pre-dating the break-up of Gondwana (Cambefort, 1991a; Davis et al., 2002, 2008). The biogeographically more localised tribes are considered to have been derived from these widespread and ancient lineages to form 'intermediate' and 'modern' tribes (Cambefort, 1991a; Monaghan et al., 2007).

These groups and their possible origins, however, are entirely intuitive and although dung beetles have been intensively studied for the past 100-odd years (Fabre, 1918), attempts at reconstructing phylogenetic hypotheses amongst large numbers of taxa are recent (morphology – Zunino, 1983; Montreuil, 1998; Philips et al., 2004 – and molecular – Villalba et al., 2002; Forgie et al., 2006; Ocampo and Hawks, 2006; Monaghan et al., 2007; Orsini et al., 2007; Wirta et al., 2008). Although these studies used different datasets and different taxa and were across different biogeographical regions several common trends emerged from them. Some of the more profound ones were that: the composition of the ancestral groups proved difficult to resolve satisfactorily although the most basal group (Zunino, 1983; Philips et al., 2004; Monaghan et al., 2007) was consistently found to be the African tunnelling genus *Coptorhina*; the tunnelling and rolling groups are largely

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poly- or paraphyletic and that treating them as intermediate or modern, although intuitively appealing, is phylogenetically untenable (Philips et al., 2004; Monaghan et al., 2007).

Evidence from recent morphological studies indicates that the dung beetles evolved in Africa (Philips et al., 2004; Monaghan et al., 2007) and that relicts of most 'ancient' groups are still found in southern Africa. Furthermore, at a global scale, although each of the tribes may be polyphyletic (Philips et al., 2004; Monaghan et al., 2007), studies indicate that the African lineages of the tribes are monophyletic. However, the lack of a well-supported phylogenetic hypothesis in the Philips et al. (2004) and Monaghan et al. (2007) studies – which are based on limited taxa, morphology and gene regions – makes attempts at reconstructing the evolutionary history of the group somewhat speculative.

Since the number and diversity of molecular characters are increasing rapidly many recent phylogenetic studies are based on multi-gene or multiple dataset approaches (Brooks et al., 2007; Aduse-Poku et al., 2009). Mitochondrial DNA (mtDNA) genes are mostly used to resolve species level relationships while nuclear genes, being more conserved, provide resolution at higher taxonomic levels (Cummings et al., 1995). With the advancement of molecular markers come improved technologies in estimating the precision of confidence limits and thereby allowing us to test specific hypotheses of monophyly or species group relationships (Brooks et al., 2007; Aduse-Poku et al., 2009).

Only 23 fossils have conclusively been attributed to members of the Scarabaeinae, 20 to extant genera and three to extinct ones (Krell, 2006). The oldest of these are from the Oligocene (about 35–25 MYA) while more than half of the rest are from the Miocene (about 25–5 MYA). The late Miocene was when the ancestral lines to modern day mammal groups, on which dung beetles now largely depend for food, radiated (Janis, 1993). During this period, progressive aridification and the spread of grasslands reached a peak in Africa, and dung beetles are hypothesised to have radiated explosively in relation to the increasing amounts of dung produced by the mammalian richness evolving on the continent at the time (Cambefort, 1991a). Speciation on the African continent has continued up to the present, resulting in the richest fauna of any region with between 40% and 50% of the world's extant genera (~250) and species (~5000) (Davis et al., 2008). The available molecular evidence on various dung beetle groups, albeit fragmentary, supports this radiation time frame (Forgie et al., 2006; Ocampo and Hawks, 2006; Orsini et al., 2007; Wirta et al., 2008).

Although there is well-supported evidence that the basal taxa of both the tunnelling Dichotomiini and rolling Canthonini are from Africa (Philips et al., 2004; Monaghan et al., 2007), the lineages and relationships amongst their components are poorly resolved. The aim of this study was, therefore, to test the relatedness of the African genera of the Canthonini and Dichotomiini. Consequently, we undertook a phylogenetic analyses using partial gene sequences from two mitochondrial and three nuclear genes. As the fossil record tells us little about the possible time of origin of dung beetles we used the proposed phylogenetic hypothesis to estimate times of major splits in the Canthonini and Dichotomiini and to relate these divergence times to factors that may have contributed to the diversification and radiation of the tribes.

2. Materials and methods

2.1. Taxa

Eight of the nine Afrotropical Dichotomiini genera were included in this phylogenetic study: *Coptorhina* Hope 1830, *Delopleurus* Erichson 1847, *Frankenbergerius* Balthasar 1938, *Heliocopris* Hope 1837, *Macroderes* Westwood 1876, *Pedaria* Castelnau 1832, *Sarophorus* Erichson 1847, and *Xinidium* Harold 1869. The only genus not included was *Paraphytus* Harold 1877.

Seventeen of the 23 Canthonini genera known to occur in Africa were included in this phylogenetic study: *Anachalcos* Hope 1837, *Aphengoecus* Péringuey 1901, *Bohepilissus* Paulian 1975, *Byrrhidium* Harold 1869, *Canthodimorpha* Davis, Scholtz and Harrison 1999, *Circellium* Latreille 1825, *Dicranocara* Frolov and Scholtz 2003, *Dwesasilvasedis* Deschodt and Scholtz 2008, *Endroedyolus* Scholtz and Howden 1987, *Epirinus* Reiche 1841, *Gyronotus* van Lansberge 1847, *Hammondantus* Cambefort 1978, *Namakwanus* Scholtz and Howden 1987, *Odontoloma* Boheman 1857, *Outenikwanus* Scholtz and Howden 1987, *Peckolus* Scholtz and Howden 1987 and *Pycnopanelus* Arrow 1931. African genera not included: *Aliuscanthoniola* Deschodt and Scholtz 2008, *Janssensantus* Paulian 1976, *Madaphacosoma* Paulian 1975, *Nebulasilvius* Deschodt and Scholtz 2008, *Panelus* Lewis 1895 and *Tanzanolus* Scholtz and Howden 1987.

Species in the genus *Aphodius* were used as out-group representatives since the genus is the proposed sister taxon of the Scarabaeinae (Browne and Scholtz, 1998; Monaghan et al., 2007).

For details of the individuals and species used see Table 1.

2.2. DNA extraction and cycling conditions

Where possible, at least three individuals of a species representing each genus were included for genetic characterisation. DNA was extracted from a leg of each individual using the Roche High Pure PCR Template Preparation Kit (Roche, Penzberg, Germany).

Molecular character information was generated from five different gene regions, two mitochondrial genes – cytochrome oxidase I (COI) and 16S ribosomal RNA (16S rRNA) – a portion of the nuclear rRNA large subunit – 28S (28S rRNA) domain 2 and domain 3 – and the CPSase region of CAD. Both COI and 16S were chosen as based on previous studies of the dung beetle tribes Scarabaeini, Canthonini and Dichotomiini they appeared to be phylogenetically informative (Forgie et al., 2005; Orsini et al., 2007; Monaghan et al., 2007). Domain 2 and 3 of the 28S rRNA molecule were chosen as within insects they have a series of conserved core elements and 13 highly variable expansion segments. The expansion segments have been shown to vary greatly between insect orders allowing for the availability of a large suite of phylogenetically informative characters at higher taxonomic levels especially among taxa having diverged over a large evolutionary time scale. (For details of the 28S rRNA and its structure see Gillespie et al. (2005).

Carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase (CAD), a relatively recent addition to insect systematic studies (Moulton and Wiegman, 2004; Winterton and de Freitas, 2006; Winterton et al., 2007; Wahlberg and Wheat, 2008), is a large gene complex with CPS being the largest of CAD's three domain's; 4 kb of an approximately 6.7 kb coding stretch of DNA (Moulton and Wiegman, 2004). Recent studies on eremoneuran Diptera (Moulton and Wiegman, 2004), small-headed Diptera (Acroceridae) (Winterton et al., 2007), the Lepidopteran family Nymphalidae (Wahlberg and Wheat, 2008) and the Neuroptera (Winterton et al., 2010) have shown CAD to be phylogenetically informative, giving results comparable to morphological studies and other gene phylogenies.

For COI, 16S and 28S domain 2 and 3 polymerase chain reaction (PCR) was performed in a final volume of 50 μl containing approximately 50–100 ng genomic DNA template, 2.5 mM $MgCl_2$, 20 pmol of each primer, 10 mm dNTP's (0.25 mM of each of the four nucleotides (Promega)) and 1× buffer in the presence of 1 U of *Taq* DNA polymerase. Primers used for amplification can be seen in Table 2. Thermal cycling parameters differed for the various gene regions. Thermal cycling parameters for COI, 16S, 28S domain 3 were: initial denaturation for 90 s at 94 °C followed by 35 cycles of 94 °C for 22 s, annealing 48–50 °C for 30 s and 72 °C for 90 s with a final