Life history stage-specific diet shifts by dung beetles revealed using DNA metabarcoding

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Abstract

Life history changes typically lead to changes in resource use. Such shifts are not well understood in the dung beetles, despite recognized differences in larval and adult feeding ability. We use the flightless dung beetle *Circellium bacchus* to explore such shifts, identifying dung sources of adults using DNA metabarcoding and compare this with published accounts of larval dung sources. *C. bacchus* is traditionally considered to specialise on the dung of large herbivores for both larval and adult feeding. We successfully extracted mammal DNA from 151 adult *C. bacchus* samples (out of 172 collected), this representing 16 mammal species (ranging from elephants to small rodents), many of which are hitherto undescribed in the diet. Adult *C. bacchus* showed clear dung source preferences, especially for large herbivores inhabiting dense-cover vegetation. Our approach also confirmed the presence of cryptic taxa in the study area, and we propose this may be used for biodiversity survey and monitoring purposes. Murid rodent feces were the most commonly fed-upon dung source (77.5%) for adult *C. bacchus,* differing markedly from the large and megaherbivore dung sources used for larval rearing. These findings support the hypothesis of life history specific shifts in resource use in dung beetles, and reveal a hitherto unsuspected, but ecologically important, role of these dung beetles in consuming rodent feces. The differences in feeding abilities of the larval and adult life history stages have profound consequences for their resource use and foraging strategies, and hence the ecological role of dung beetles. This principle and its ecological consequences should be explored in other scarabaeid dung beetle species.

Keywords: biodiversity survey, *Circellium bacchus;* coprophagy, environmental DNA, next generation sequencing, megaherbivores, mitochondrial DNA, Scarabaeinae, rodent feces.

Introduction

Ontogenetic shifts give rise to ecological shifts, particularly in species with complex life histories (Werner 1988), and understanding such shifts is important to understanding the ecological role of these species. This is particularly relevant given that more than 80% of animal species show such ontogenetic shifts (Werner 1988). The massively diverse (nearly 6 000 species) and ecologically important scarabaeid dung beetle family exhibits complex life histories, with changes in the feeding ability of the different life history stages. Thus, while the larvae are typically able to ingest and digest relatively coarse plant fragments from the dung of herbivores, the adults' mouth parts constrain them to ingesting liquid and fine (< 130 μm) particulate matter (Holter 2016). However, the use of dung by dung beetles, and by extension their contribution to the detrital food chain and nutrient cycling, has focused almost exclusively on those sources used for larval rearing, with limited attention to the dung sources of adults. Here, we use Africa's largest (up to 50 mm in length) telecoprid species, the flightless *Circellium bacchus*, and DNA metabarcoding, to identify the diet sources of adults and compare this with published accounts of larval dung resources (Kryger et al. 2006).

The dung sources of *C. bacchus*, for adult consumption and brood ball construction, has been described anecdotally or estimated using opportunistic observations of beetles feeding on or preparing dung balls from various sources. The anecdotal information is that flightless dung beetles rely on elephant *Loxodonta africana*, Cape buffalo *Syncerus caffer* and black rhinoceros *Diceros bicornis* dung, and based on this, Chown *et al.* (1995) concluded that this species depends on black rhinoceros dung for its persistence. Kryger *et al.* (2006) observed flightless dung beetles consuming dung of elephant, buffalo, rhino, "various antelope", monkey *Chlorocebus pygerythrus*, human, hare *Lepus* sp. and ostrich *Struthio camelus*. This sampling was not systematic in terms of availability of different dung sources. Using a limited cafeteria-style experiment, Kryger et al. (2006) also estimated dung source preferences by *C. bacchus* for adult feeding and brood ball construction, showing that dung preferences varied among elephant, black rhinoceros, buffalo and cattle *Bos taurus*. Kryger *et al.* (2006; p. 201) concluded there is "distinct preference for feeding on elephant dung early in the morning" and that "cattle/buffalo dung was preferred later in the day".

For brood ball material, bovids (buffalo and cattle) were apparently preferred over megaherbivores (elephant and rhino; Kryger et al. 2006). Based on the above, there are two contrasting views with regard to the diet resource use of this species: it is either a large mammalian herbivore specialist or is a mammalian generalist.

DNA metabarcoding is increasingly used to identify taxa in sampled material, such as plant species in the dung of herbivores, prey species in the gut contents of carnivores or in soil samples (review in Pompanon et al. 2012; Shehzad et al. 2012; Taberlet et al. 2012a; Yoccoz et al. 2012;). Animal dung includes not only the DNA of ingested food items but also DNA from the animal providing the dung (Shehzad et al. 2012; De Barba et al. 2014). Thus, we expected adult *C. bacchus* to ingest DNA from the animal species on whose dung they had fed, and that this would be present in the feces and could be used to identify the source of the dung. We used this approach to test the hypotheses that the flightless dung beetle is a large herbivore dung specialist or alternatively is a generalist, and that adult beetle diets differ from that reported for the larvae (i.e. the sources used for brood ball construction; from Kryger et al. 2006). We demonstrate substantial shifts in resource use and preference by the adults compared to the larvae, this despite the fact that adults are responsible for the acquisition of the material for both adults and larvae. Our findings confirm the detection of dung source DNA in dung beetle feces, represent the first systematic survey of this dung beetle's adult diet and provide novel insights into dung beetle diet preferences. Importantly, we provide evidence for life history level shifts in diet and reveal a cryptic functional role for this species.

Study design

Sampling

Flightless dung beetles were sampled in the Main Camp and Colchester sections (collectively 26 500 ha in area) of the Addo Elephant National Park, South Africa, between January and February 2014, a period of high dung beetle activity (Kryger et al. 2006). *C. bacchus* is within a monotypic genus of uncertain taxonomic position and is unusual by virtue of its flightlessness and strict ectothermy (Chown et al. 1995; Davis et al. 2008a). The fragmented status of the population,

apparent contraction in distribution range and slow reproduction (Chown et al. 1995) has led to suggestions that this species should be considered threatened (Kryger et al. 2006). Although protected in some conservation areas, most notably the AENP, tourist activities represent an additional threat through roadkills (Hayward et al. 2010). These attributes have led to this beetle attracting scientific interest and conservation concern, as well as achieving charismatic fauna status for wildlife viewing among tourists (Kerley et al. 2003) and legal protection; these latter two achievements being uncommon among terrestrial invertebrates.

The AENP is about 60 km north east of Port Elizabeth on the south east coast, annual rainfall is 450 mm pa, with temperatures varying between summer maxima of *ca*. 32°C and winter minima of *ca*. 5°C (Weather SA). The AENP is recognised as supporting the largest population of flightless dung beetles (Kryger et al. 2006). In addition, the Main Camp and Colchester sections (which form a discrete, fenced unit) support a wide diversity of mammals (52 species, excluding volant and fossorial species; Swanepoel 1975; Boshoff et al. 2002; Hayward et al. 2007), most prominent among the herbivores being elephant, black rhino and buffalo, while the apex predators are represented by lions *Panthera leo*, leopards *P. pardus*, and spotted hyaena *Crocuta crocuta*. There is also a diverse avian and reptile fauna.

Dung beetle sampling comprised locating individuals active on roads or trails across the study area (irrespective of habitat type), i.e. sampled beetles were not associated with dung balls or dung. When picked up, dung beetles either defecate within about 5 seconds or take much longer (pers obs). Fecal samples from beetles that defecated on being picked up were wrapped in Kimwipes paper (Kimberly-Clark) and immediately placed into labelled plastic vials containing silica gel. Dung beetles that did not defecate were released within 30 seconds of being picked up and not sampled. The fecal samples were preserved dry in silica gel until DNA extraction. It was difficult to prevent possible human contamination during the sampling, i.e. we did not wear gloves and facial masks. Instead we elected to remove such potential contamination at the data analysis stage.

142

143 DNA extraction, amplification and sequencing

144 DNA extractions were carried out using a phosphate buffer protocol, modified from Taberlet *et al.*
145 (2012b). Each fecal sample was put in an Eppendorf tube containing 500 µL of saturated
146 phosphate buffer (Na_2HPO_4; 0.12 M; pH 8), and shaken gently for 15 min (45 rpm). The resulting
147 mixture was centrifuged at 11 000 g for 10 min. The next steps were performed using the
148 NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions,
149 and skipping the lysis steps. Four hundred µL of the supernatant was added to 250 µL of SB
150 buffer, loaded onto the extraction column, and washed once with SB and SW1 buffers, and twice
151 with SW2 buffers. The elution was done with 100 µL of SE buffer. A negative extraction control
152 was included into each batch of 23 dung beetle fecal samples, using the phosphate buffer as
153 starting material.

154 For DNA amplifications, we used a primer pair targeting a short but informative fragment of
155 the 16S mitochondrial gene of mammals (Giguet-Covex et al. 2014). The forward and reverse
156 primer sequences are 5'-CGAGAAGACCCTATGGAGCT-3' and 5'-CCGAGGTCRCCCCAACC-3',
157 respectively. To discriminate samples and PCR replicates after sequencing, both forward and
158 reverse primers were tagged with 8-nucleotide labels (hereafter designated as "tag") with at least
159 three nucleotide differences among each of them. Furthermore, three additional random
160 nucleotides were added on the 5'-end of each primer, in order to allow efficient detection of the
161 different clusters during the sequencing step. For each sample and each replicate, the same tag
162 was used on both primers, i.e. on both sides of the PCR product (Schnell et al. 2015, Taberlet et
163 al. 2018).

164 Two PCRs per sample and per control were carried out, including the fecal sample extracts,
165 the extraction negative controls, the PCR negative controls, and the PCR positive controls (DNA
166 extract from *Didelphis marsupialis*). We used the AmpliTaq Gold® 360 Master Mix (Applied
167 Biosystems™, Foster City, CA, USA), in a final volume of 20 µl containing 2 µl of DNA extract
168 (including the extraction negative controls), 0.2 µM of each primer, and 0.16 µL of bovine serum
169 albumin (BSA, Roche Diagnostic, Basel, Switzerland). To reduce the amplification of human DNA,

we added a human blocking oligonucleotide (5'-CCACCGAAATTTTTAATGCAGGTTTGGTAGTT-C3-3') in each PCR, at a final concentration of 2 µM. The design of this blocking oligonucleotide was done according to Vestheim & Jarman (2008). The PCR cycling parameters were: 10 minutes at 96°C for activating the polymerase, and then 45 cycles with denaturation for 30 s at 96°C, annealing for 30 s at 50°C, elongation for 60 s at 72°C, with a final extension for 420 s. All PCR products, including samples and controls, were mixed together and purified (MinElute™ PCR purification kit, Qiagen, Hilden, Germany). The library preparation and the sequencing was outsourced (Fasteris SA, Geneva, Switzerland). The library was prepared using the MetaFast protocol (www.fasteris.com/metafast) and the sequencing carried out on the HiSeq 2500 sequencing platform (Illumina, San Diego, CA, USA) with a paired-end approach (2 x 125 bp).

Sequence data analysis

The sequence reads were analyzed using OBITools (Boyer et al. 2016). First, the direct and reverse reads corresponding to a single molecule were aligned and merged using the *illuminapairedend* program, taking into account data quality during the alignment and the consensus computation. Primers and tags were then identified using the *ngsfilter* program. Only the amplified region of the sequences with a perfect match on tags and a maximum of two errors on primers were recorded for the subsequent analysis, keeping the information about sample names. Strictly identical sequences were clustered together using the *obiuniq* program, keeping the information about their distribution among samples. Sequences shorter than 60 bp or longer than 90 bp, or with occurrence lower than 1000 in the whole dataset were excluded using the *obigrep* program. Potential PCR/sequencing errors were identified and removed using the *obiclean* program. We kept only sequences identified at least once as "head" or "singleton" in the different PCRs (i.e. "head" are sequences that are at least twice as abundant as other sequences differing by a single change, "singleton" are sequences that have no other sequences differing by a single change; see Boyer et al. 2016 for further explanations). Taxon assignation was achieved using the *ecotag* program. The reference database for the taxonomic assignment was built by extracting the relevant part of the mitochondrial 16S gene from EMBL nucleotide library (release 126) using the

198 *ecoPCR* program (Ficetola et al. 2010). All sequences with a best identity lower than 0.86 when
199 compared to any sequence in the reference database were removed, as they potentially
200 correspond to chimeras or to non-specific amplifications (Taberlet et al. 2018). Mammalian DNA
201 was considered as present in a scat sample if at least one of two PCR replicates showed more
202 than 100 sequence reads.
203 Finally, we inspected the automatic taxonomic assignments of sequences manually and
204 considered species-level identities reliable only if these matched near-perfectly (≥ 98% identity) to
205 a single species in the reference database. Close, but non-identical, matches (88-98% identity)
206 were consistently made at the genus level, and checked against the occurrence of these taxa in
207 the study area.
208
209 Herbivore dung production
210 Estimates of large herbivore dung production were derived from the SANParks mammal census
211 data for 2013 for the Main Camp and Colchester sections of the AENP, derived from systematic
212 aerial counts (SANParks Unpublished data). These dung production estimates were calculated for
213 each censused megaherbivore and ungulate species, as well as the undetected blue duiker
214 *Philantomba monticola* (for a total of 11 large herbivore species). Dung production estimates were
215 based on mass specific models of herbivore food intake (Owen-Smith 1992), based on ¾ adult
216 female body mass for each species (Hayward and Kerley 2005), adjusted for ruminant/hindgut
217 fermenter digestive efficiency (Owen-Smith 1992).
218
219 Statistical analysis
220 Dung beetle diet was described as the frequency-of-occurrence of diet sources across fecal
221 samples at genus or species level. To assess the adequacy of our sample sizes, we generated an
222 accumulation curve (with 50 random resamplings) of diet sources recorded per sample (Online
223 Resource 1). However, because this curve did not reach a clear asymptote, we estimated the total
224 number of diet sources with a non-parametric species richness estimator (Foggo et al. 2003).

Differences between observed and expected counts provided an estimate of the variation in diet information at the upper-limit of sampling effort.

Large herbivore dung source preference by flightless dung beetles was estimated using Jacob's index (Jacobs 1974) based on the proportion of estimated dung production and proportion of records of dung beetle consumption of the dung for each of the censused herbivore species. Jacob's index varies between + 1, for maximum preference (i.e. where dung consumption is greater than dung production), and – 1, for maximum avoidance (i.e. where consumption is less than production). Preferences were calculated for each taxon, and these data were used to calculate Jacob's index for the digestive morphology guilds (ruminants, hindgut fermenters), feeding guilds (grazers, browsers, mixed feeders) and habitat use guilds (open habitat, closed habitat, mixed use of open and closed – see Online Resource 2 for guild data).

Results

DNA metabarcoding results

We analyzed a total of 172 dung beetle fecal samples, together with eight extraction negative controls using phosphate buffer as starting material, four extraction negative controls using Kimwipes paper as starting material, three PCR negative controls, and four PCR positive controls. After the *illuminapairedend* and *ngsfilter* programs (assembling forward and reverse reads, and identifying primers and tags), we obtained a total of 3,967,490 sequences. The dereplication yielded 163,798 different sequences. Removing the sequences occurring only once in the whole dataset decreased the number of different sequences to 48,189. The automatic filtering and taxonomic assignment described in Study design yielded 68 molecular operational taxonomic units. The obvious contaminants were then removed. Among these, human and human-related sequences represented about 35% of the dataset at this stage. This level of human contamination was expected according to our sampling protocol (see Study design). Additionally, we observed a few cow *Bos taurus* and pig *Sus scrofa* sequences, these being known contaminants in PCR kits (Leonard et al. 2007) and not occurring in the study site. We also obtained red deer *Cervus elaphus* contamination in a single replicate of five dung beetle fecal samples. This contamination

most probably comes from the hundreds of red deer scats that were extracted the day before in the same laboratory, as there are no known cervid populations in or around AENP. After removing human, pig, cow and red deer contaminants, the number of MOTUs decreased to 37. A final manual inspection of the remaining sequences yielded 16 putative mammalian species distributed among the 151 dung beetle fecal samples that produced usable sequences (see Online Resource 3). With the exception of two genera of the Murid family (*Micaelamys* and *Otomys* - the latter following the monogeneric treatment of the Otomyini (Taylor et al. 2004), and one member of the Bovidae (*Cephalophus*) that could only be identified to genus level, the majority of diet sources were identified to species-level.

These 16 taxa accounted for 86.5% of the variation in dung beetle dietary information at the upper limit of sampling effort. This suggests that our sample size was appropriate to describe the sources of the diet.

Dung source use

Despite the marker also potentially amplifying bird, reptile and amphibian DNA, we detected only mammal DNA in *C. bacchus* feces. The identified mammal diet sources ranged in body size from the elephant (2 000 - 6 000 kg) to the 43 g striped field mouse *Rhabdomys pumilio* (Fig. 1), and spanned 6 taxonomic orders. Murid rodents provided 77.5% of the diet sources. In terms of broad feeding guilds, these dung source taxa were dominated by herbivores, with only a single record of one carnivore species' (*Canis mesomelas*) scat being consumed. The DNA sequences from dung beetle feces indicate the presence of some previously unrecorded taxa in the study area. These include a third species of *Otomys* (only two are considered confirmed for the study area; Swanepoel 1975), and a record for the duiker genus *Cephalophus*. It is noteworthy that one of the dung sources species, the common warthog *Phacochoerus africanus,* is an introduced species that is now considered invasive in the AENP and surrounds (Mgqatsa 2010). These records represent only 27% of the 52 non-volant, non-fossorial mammal species currently recorded as occurring in the study area, indicating selectivity of dung source use by *C. bacchus*. Contrary to expectations, the frequency of dung source use does not increase with body size for the 16 taxa included in the

281 analyses (R^2 = 0.02, $F_{1,14}$ = 0.27, P = 0.613; % Frequency of occurrence = 9.11 - (1.20 * log(body
282 mass (kg)).
283
284 Dung source preferences
285 The flightless dung beetle showed clear preferences or avoidance for dung sources among the
286 large herbivore species for which we have both dung production and consumption estimates (Fig.
287 2). The traditionally considered important or preferred sources of dung (elephant, black rhino,
288 buffalo) were all avoided, while the dung of three smaller bovids and the two suids (the latter
289 including the invasive warthog) were preferred, but that of three other large bovids was avoided
290 (Fig. 2; Online Resource 2).
291 Dung source preference was negatively related to body size for the 11 large herbivore
292 species for which preference data were available (R^2 = 0.52, $F_{1,9}$ = 9.75, P=0.013; D = 1.63-(0.74 *
293 log(body mass (kg)). In terms of the digestive morphology guilds, ruminants were marginally
294 preferred (D = 0.19) over hindgut fermenters (D = - 0.19), although there was a roughly equal
295 distribution of both guilds in either the preference or avoidance category (c.f. Fig. 2 and Online
296 Resource 2). The dung of the browsing guild was consistently preferred (D = 0.52), while that of
297 the mixed feeders (D = - 0.31) and the grazers (D = - 0.08) were avoided. The clearest patterns
298 were among guilds of species characteristic of different habitats (Fig. 2): dung of species that use
299 dense, closed vegetation was consistently preferred (D = 0.63), while that of open habitat species
300 was consistently avoided (D = - 0.86). The dung of species that show mixed use of open and
301 dense habitats was avoided (D = - 0.29), although this was not always the case (Fig. 2).
302
303 **Discussion**
304 The findings we present emerge from a novel approach for exploring the functional role of dung
305 beetles, and support the hypothesis of DNA material from the dung source occurring in dung beetle
306 feces. DNA metabarcoding has rarely been applied to describing the diet of invertebrates based on
307 feces (but see Ibanez et al. 2013; Gomez and Kolokotronis 2016; Kaunisto et al. 2017; Rodgers et
308 al. 2017) and can clearly be applied to identifying diet sources for coprophagous taxa, as well as

detritivores. DNA metabarcoding can also be applied to the identification of the source of fecal material, and hence to identifying the source of dung balls, even those not associated with an identifiable dung deposit. Based on this, it is clear that DNA metabarcoding-based studies have the potential to considerably expand our understanding of the functional role of species that are otherwise difficult to study. Furthermore, DNA metabarcoding also allowed the possible identification of two taxa apparently unrecorded at the study site, the third *Otomys* species and the *Cephalophus,* although we could not resolve which species these represent. The former is not surprising, as de Graaff (1974) recorded *O. angoniensis,* in addition to *O. irroratus and O. unisulcatus,* but this was later discounted (Swanepoel and Branch 1982) due a lack of further records. In contrast, the natural range limit of the closest *Cephalophus* is at least 1000 km north-east of the study site. A possible explanation for this record is that representatives of this genus may have been introduced by neighbouring landowners, with escapees subsequently invading the AENP. Both these possibilities require further testing.

The DNA metabarcoding of dung beetle feces also served to detect some taxa in this national park, whose presence is missed by conventional census strategies. Thus, *Philantomba monticola*, the blue duiker is a small, dense vegetation-dwelling antelope known to occur in the AENP, and was not recorded in the aerial census (SANParks Unpublished data), but was detected in the dung beetle diet. This detection of cryptic species may also extend to identifying species that may be absent. The Cape grysbok *Raphicerus melanotis*, although recorded in the AENP historically (Penzhorn 1971), was not detected in the aerial census or the dung beetle diet. Given that this species is characteristic of dense cover and uses latrines (Kerley et al. 2010), its dung may be expected to serve as an attractive food source (see later). Its absence from both survey approaches calls into question its persistence in the area. Based on this, we suggest that DNA metabarcoding of dung beetle feces can be used as an efficient and cost effective biodiversity survey and monitoring tool, as proposed for DNA extracted from leeches (family Haemadipsidae; Schnell et al. 2012) and carrion flies (families Calliphoridae and Sarcophagidae; Calvignac-Spencer et al. 2013).

The dung sources consumed by adult *C. bacchus* presented here differ substantially from published findings, which have emphasized the importance of megaherbivore dung. In particular, the absence of any records of *C. bacchus* feeding on the dung of the black rhinoceros conflicts with speculation by Chown *et al.* (1995) that *C. bacchus* was dependent on the black rhinoceros for a reliable dung source. These differences may reflect the lack of systematic approaches in previous studies, the emphasis on studying dung beetles at large dung sources, or misidentification of the dung sources. In addition to these sampling artifacts, the variation in observed dung use may reflect life history level variation in the use of dung by *C. bacchus.* These issues are expanded on below.

The high levels of elephant dung production in the AENP (over 50% of estimated large herbivore dung production – Online Resource 4) means that sampling *C. bacchus* dung use based on non-stratified sampling of dung sources (as apparently done by Kryger *et al.* (2006), and the various anecdotal descriptions of dung use by *C. bacchus*) would result in an overestimate of the importance of particularly this source for dung beetles. Clearly, well-designed, systematic sampling is needed to reliably assess resource use in cases where resources show such overbearing variances in availability. Furthermore, most published studies of resource use by *C. bacchus* have been focussed on observing beetles forming either brood or feeding balls from dung piles (e.g. Kryger et al. 2006). A consequence of this approach (in addition to the resource availability bias raised above) is that the use of smaller fecal deposits (e.g. rodent droppings) would be completely overlooked, as such deposits would rarely be substantial enough to form feeding or brood balls. A corollary of this is that the use of rodent droppings by *C. bacchus* may have been entirely overlooked in the past as observers were not sensitive to the need to monitor *C. bacchus* feeding behaviour when not engaged in dung ball formation. Finally, a further sampling artefact may arise due to the misidentification of dung being used by *C. bacchus*, although the extent of this is typically not known. The approach used in the present study, however, avoided this risk.

A more interesting functional explanation of these differences may lie in the understanding of the differences in feeding strategies of the dung beetle adult and larval life history stages. Larvae are able to ingest and digest cellulose-rich material from the coarse particulate matter in dung

balls, which are provided by the adults (Davis et al. 2008b). In contrast, adults are constrained in their ability to ingest coarse material, and rely instead on ingesting fluid and extremely fine particulate matter for their nutrition (Holter 2000). Thus, the brood ball preparation and adult nutritional requirements place differing constraints on the use of dung resources. The former has a limit in terms of the minimum amount of dung required to form a brood ball - these range from 22-85 g of dry mass (e.g. Kryger et al. 2006). In contrast, adult feeding does not have such a volume constraint, and fluid content, particle size and the C:N ratio is more important (Holter 2016). Based on this, we hypothesize that *C. bacchus* exhibits different foraging strategies, depending on whether the focus is on brood ball formation or adult feeding. Thus, for brood ball formation the dung source must be large, and foraging *C. bacchus* need to locate dung from species that either have large dung boli (e.g. elephant, buffalo) or that use latrines (e.g. black rhino). In this context, *C. bacchus* can be hypothesized to be following a quantity maximization strategy in their dung source preferences for brood ball preparation. This would explain the published focus on the use of the dung of these taxa by *C. bacchus*. In contrast, feeding adult *C. bacchus*, not having such a volume constraint, may either adopt a quality maximization strategy or a time/effort minimization strategy for their location and use of dung resources. Furthermore, differences in dung sources for brood ball formation and adult feeding have the potential to reduce competition between feeding adults and those forming brood balls, thus effectively increasing resource availability.

There is limited information to test which of these strategies is employed by feeding adult *C. bacchus*. Ruminant and rodent feces are characterized by smaller particle size than that of hindgut fermenters (Owen-Smith 1992), but the filtering out of coarse material during ingestion by adults (Holter 2016) makes this less important. Limited data suggest better C:N ratios in ruminant than megaherbivore (hindgut fermenters) feces (Holter and Scholtz 2007), although such information is not available for the smaller non-ruminants and rodents. Available preference data is limited to the larger herbivore species, and does indicate a preference by *C. bacchus* for feeding on ruminant feces. This provides partial support for the quality maximization strategy. Relevant data for the time/effort minimization strategy as an explanation for dung source use by adult *C. bacchus* is currently not available and would require quantified field observations of foraging effort.

An additional aspect of dung preferences displayed by *C. bacchus* relates to the preference displayed for dung of herbivores that typically use densely vegetated habitat (Fig. 2). This may reflect habitat-specific competitive abilities of this dung beetle. The fact that *C. bacchus* is flightless, means that it is ectothermic and limited to walking when foraging, and is at a competitive disadvantage with heterothermic, flying dung beetles for both the location and use of dung deposits (Chown et al. 1995). The flightlessness of *C. bacchus* has been interpreted as an adaptation to densely wooded habitats (Chown et al. 1995), and such dense vegetation hinders access for the flying dung beetles. Based on these hypotheses, it may be predicted that *C. bacchus* has a competitive advantage when foraging in dense vegetation, and this should be reflected in its feeding preferences for dung from herbivores characteristic of such dense, closed vegetation. This is supported by diet source and preference data provided here.

The important role of dung beetles in breaking down nutrients otherwise trapped in fecal deposits is widely recognised, especially for large vertebrate dung (Nichols et al. 2009). The energy equivalence rule (Damuth 1981) suggests that large and small mammal dung deposition may be of similar orders of magnitude. Thus, mechanisms for the breakdown of rodent dung should also be important in ecosystem functioning. Based on this, and observations of the extensive use of rodent dung by adult *C. bacchus*, we suggest that *C. bacchus* adults and larvae may collectively be serving an important role in the breakdown of mega-, meso- and microherbivore dung. Following Nichols *et al.* (2009), *C. bacchus* may be important for maintaining ecological processes and possible ecological cascades, a hitherto unrecognized functional role with regard to rodent feces.

The findings presented here not only expand our understanding of the functional role of *C. bacchus,* but also highlight the value of DNA-metabarcoding in exploring such patterns. In addition, the hypothesis of ontogenetic shifts leading to ecological shifts is supported. Considering that the larval/adult differences in feeding abilities are plesiomorphic, we expect such ecological shifts across the diversity of scarabaeid dung beetles. Clearly, the differences in feeding abilities of the larval and adult life history stages has profound consequences for their resource use and foraging

419 strategies, and hence the ecological role of dung beetles. This principle and its ecological
420 consequences needs to be explored in other scarabaeid dung beetle species.
421
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Competing interests. We declare that we have no competing interests

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Data accessibility. Data are available from the Dryad Digital Repository: http://dx.doi.org/ §§§§§§§§§§§§ (Kerley et al. §§§§).

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535

Figure headings

Fig. 1 Frequency of occurrence of diet sources recorded in flightless dung beetle *Circellium bacchus* fecal samples in the Main Camp and Colchester Sections of the Addo Elephant National Park.

Fig. 2 Relative preferences for dung of each of the censused large herbivores (in the Main Camp and Colchester Sections of the Addo Elephant National Park) by flightless dung beetles *Circellium bacchus*, estimated using Jacob's index, where D > 0 indicates preference and D < 0 avoidance. See Online Resource 2 for species details.

Supplementary material

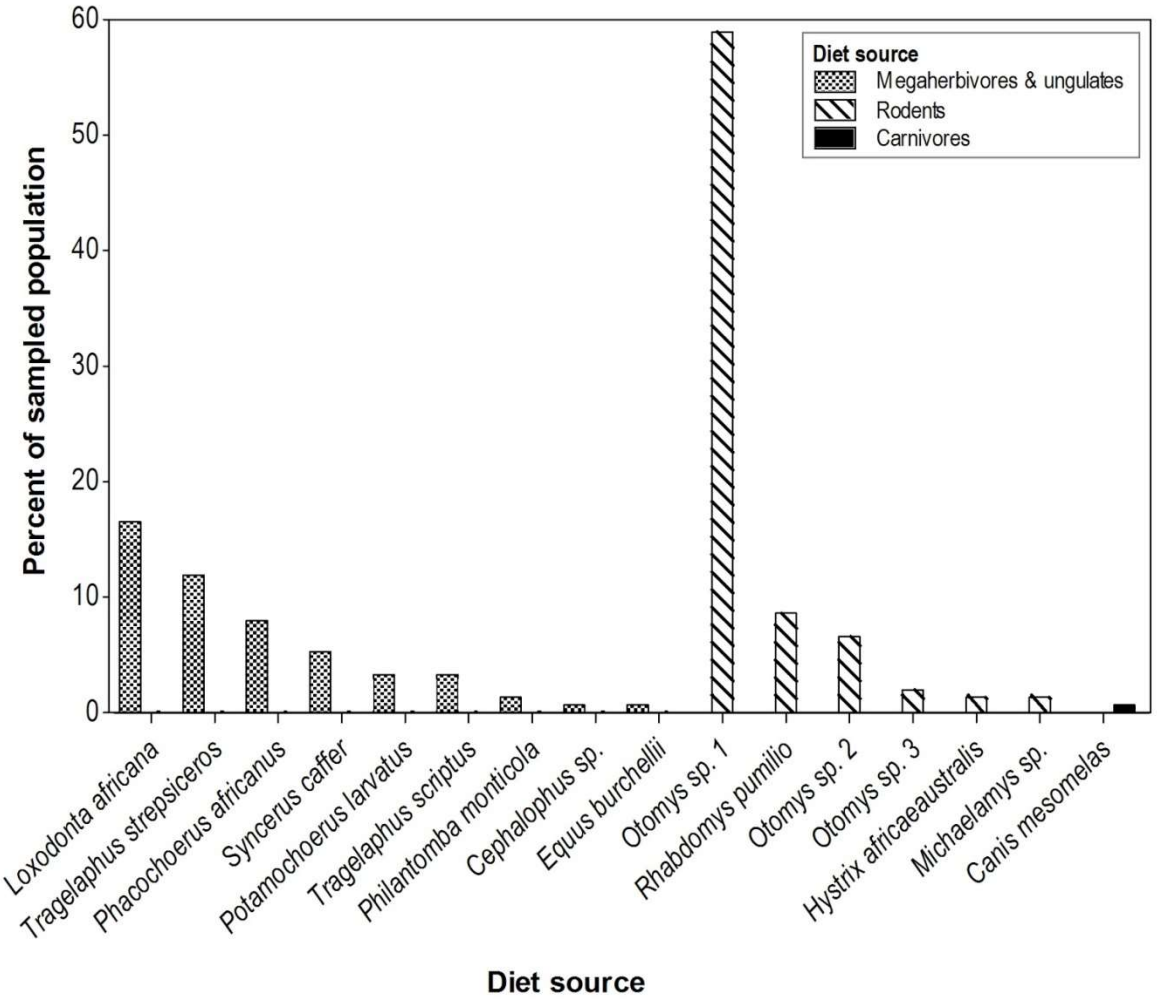
Online Resource 1 Mean accumulation curve (50 random resamplings) of diet sources (putative species) recorded per dung beetle fecal sample.

Online Resource 2 Broad guild status (feeding, digestive morphology and habitat use guilds) for the herbivorous mammal species recorded in the 2013 SANParks census of the Main Camp and Colchester Sections of the Addo Elephant National Park, and potentially providing dung sources for the flightless dung beetle *Circellium bacchus* (see Fig. 2). The blue duiker, although not included in the census, did serve as a dung source (see Fig. 1) and is included here. Feeding guild comprises browser, grazer or mixed feeder (Online Resource 5a), the digestive morphology guild comprises ruminant or hind gut fermenter (Online Resource 5a), and habitat use comprises open habitat use, closed habitat use or mixed open and closed habitat use (Online Resource 5a-c).

Online Resource 3 Mammalian sequences (fragment of mitochondrial 16S gene) obtained from dung beetle *Circellium bacchus* fecal samples collected in Addo Elephant National Park. The scientific name attributed to each sequence is deduced from the best identity found in the release 126 of the EMBL database, and taking into account occurrence of the mammalian species occurring in the study area. For *Otomys* sp. 3 and *Rhabdomys pumilio*, we had four and three different sequence variants, respectively. We assumed that these closely related variants belong to the same species, but in the absence of extra data we cannot exclude the presence of additional species within *Otomys* or *Rhabdomys*.

Online Resource 4 Estimated relative dung production by large herbivores (elephants and ungulates) in the Main Camp and Colchester Sections of the Addo Elephant National Park, based on census data (SANParks Unpublished data), ¾ female body mass and digestive efficiency.

574 **Figures**



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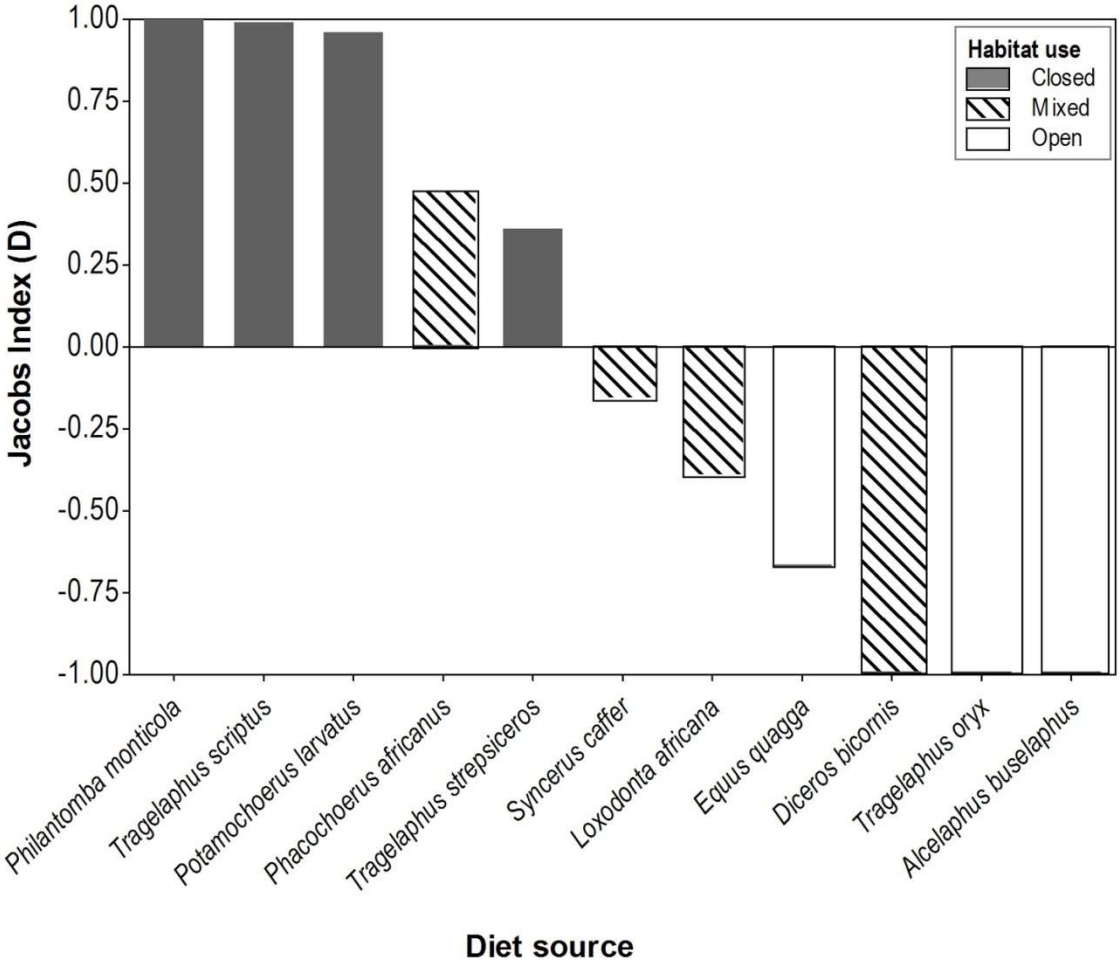
576 **Figure 1**



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578 **Figure 2**