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BEHAVIORAL ECOLOGY–ORIGINAL RESEARCH

Diet shifts by adult flightless dung beetles *Circellium bacchus,* revealed using DNA metabarcoding, reflect complex life histories

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Abstract

Life history changes may change resource use. Such shifts are not well understood in the dung beetles, despite recognised 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 comparing these 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* fecal samples, 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 that 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 scarabaeids.

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 6000 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

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sources used for larval rearing, with limited attention to the dung sources of adults, or the ecological role of adults. If adults display a shift in dung source use compared to that used by larvae, it would open up the prospects of developing a new understanding of their role in ecosystem functioning. 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, have been described anecdotally or estimated using opportunistic observations of beetles feeding on or preparing dung balls. The anecdotal information is that these 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 the species depends on black rhinoceros dung for its persistence. Kryger et al. (2006) observed flightless dung beetles consuming the 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 (only four dung types offered) cafeteria-style experiment, Kryger et al. (2006) also estimated dung source preferences by *C. bacchus* for adult feeding and brood ball construction, showing that preferences varied between elephant, black rhinoceros, buffalo, and cattle *Bos taurus* dung. Kryger et al. (2006; p. 201) concluded that 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 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 also 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). Our approach represents the first systematic survey of this dung beetle's adult diet and provides 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 (AENP), 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 northeast of Port Elizabeth on the southeast 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 (each location recorded with a handheld GPS) individuals active (i.e., not associated with dung balls or dung) on roads or trails in open (representing roughly 28% of the study area) and closed (72%) habitats across the study area. When picked up, dung beetles either defecate within about 5 s 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 s of being picked up and not sampled. We sampled a total of 172 dung beetles in this way, spread more or less evenly between open (47% of samples) and closed (53%) habitats. The fecal samples were preserved dry in silica gel until DNA extraction. Because it was difficult to prevent possible human contamination during the sampling, we did not wear gloves and facial masks. Instead, we elected to remove such potential contamination at the data analysis stage. Only 2% of the total number of sequences obtained (see below) were of human mitochondrial DNA and it is not clear whether this contamination occurred during sampling in the field and/or through the susceptibility of laboratory reagents to contaminating DNA (Leonard et al. 2007).

DNA extraction, amplification, and sequencing

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

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

Two PCRs per sample and per control were carried out, including the fecal sample extracts, the extraction negative controls, the PCR negative controls, and the PCR positive controls (DNA extract from *Didelphis marsupialis*). We used the AmpliTaq Gold® 360 Master Mix (Applied Biosystems™, Foster City, CA, USA), in a final volume of 20 µL containing 2 µL of DNA extract (including the extraction negative controls), 0.2 µM of each primer, and 0.16 µL of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland). To reduce the amplification of human DNA, we added a human blocking oligonucleotide (5′-CCACCGAAA TTTTTAATGCAGGTTTGGTAGTT-C3-3′) in each PCR, at a final concentration of 2 µM. The design of this blocking oligonucleotide was done according to Vestheim and Jarman (2008). The PCR cycling parameters were: 10 min 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 were 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 × 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 data set 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" (i.e., sequences that are at least twice as abundant as other sequences differing by a single change) or "singleton" (i.e., sequences that have no other sequences differing by a single change) in the different PCRs (Boyer et al. 2016). 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 *ecoPCR* program (Ficetola et al. 2010). All sequences with a best identity lower than 0.86 when compared to any

sequence in the reference database were removed, as they potentially correspond to chimeras or to non-specific amplifications (Taberlet et al. 2018). Mammalian DNA was considered as present in a fecal sample if at least one of two PCR replicates showed more than 100 sequence reads.

Finally, we inspected the automatic taxonomic assignments of sequences manually and considered species-level identities reliable only if these matched near-perfectly (≥ 98% identity) to a single species in the reference database. Close, but non-identical, matches (88–98% identity) were consistently made at the genus level, and checked against the occurrence of these taxa in the study area.

Herbivore dung production

Estimates of large herbivore dung production were derived from the SANParks mammal census data for 2013, which comprised systematic total aerial counts (helicopter-based, with two trained observers and a data recorder) of the entire Main Camp and Colchester sections of the AENP (SANParks Unpublished data). Dung production estimates were calculated for each censused megaherbivore and ungulate species, for a total of 11 large herbivore species. Aerial counts are inevitably undercounts, with the degree of the undercount varying between species according to their visibility (Redfern et al. 2002). While we did not attempt to correct for these variable and unknown biases (Jachmann 2002), we generally obtained large count totals across large herbivore species that reduced sampling uncertainty. Notable exceptions included counts for the less conspicuous and cover-loving black rhino, bushbuck *Tragelaphus scriptus*, bushpig *Potamochoerus larvatus,* and blue duiker *Philantomba monticola,* which we presume caused uncertainty in our estimates of dung production for these species. Dung production estimates were based on mass-specific models of herbivore food intake (Owen-Smith 1992), based on ¾ adult female body mass for each species (Hayward and Kerley 2005), adjusted for ruminant/hindgut fermenter digestive efficiency (Owen-Smith 1992).

Statistical analysis

Dung beetle diet was described as the frequency of occurrence of diet sources across fecal samples at genus or species level. To assess the adequacy of our sample sizes, we generated an accumulation curve (with 50 random resamplings) of diet sources recorded per sample (Online Resource 1). However, because this curve did not reach a clear asymptote, we estimated the total 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 Jacobs'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. Jacobs'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 Jacobs's index for the digestive morphology guilds (ruminants and hindgut fermenters), feeding guilds (grazers, browsers, and mixed feeders), and habitat use guilds (open habitat, closed habitat, and 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 data set 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 (MOTU). The obvious contaminants were then removed. Among these, human and human-related sequences represented about 35% of the data set at this stage. This level of human contamination was expected according to our sampling protocol (see "Study design"). In addition, we observed a few cow *B. 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 (2000–6000 kg) to the 43 g striped field mouse *Rhabdomys pumilio* (Fig. 1) and spanned six 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 analyses ($R^2 = 0.02$, $F_{1,14} = 0.27$, $P = 0.613$; % Frequency of occurrence = 9.11 − (1.20 * log(body mass (kg)).

Dung source preferences

The flightless dung beetle showed clear preferences or avoidance for dung sources among the large herbivore species for which we have both dung production and consumption estimates (Fig. 2). The traditionally considered important or preferred sources of dung (elephant, black rhino, and buffalo) were all avoided, while the dung of three smaller bovids and the two suids (the latter including the invasive warthog) were preferred, but that of three other large bovids was avoided (Fig. 2; Online Resource 2).

Dung source preference was negatively related to body size for the 11 large herbivore species for which preference data were available ($R^2 = 0.52$, $F_{1,9} = 9.75$, $P = 0.013$; $D = 1.63 - (0.74$ * log(body mass (kg)). In terms of the digestive morphology guilds, ruminants were marginally

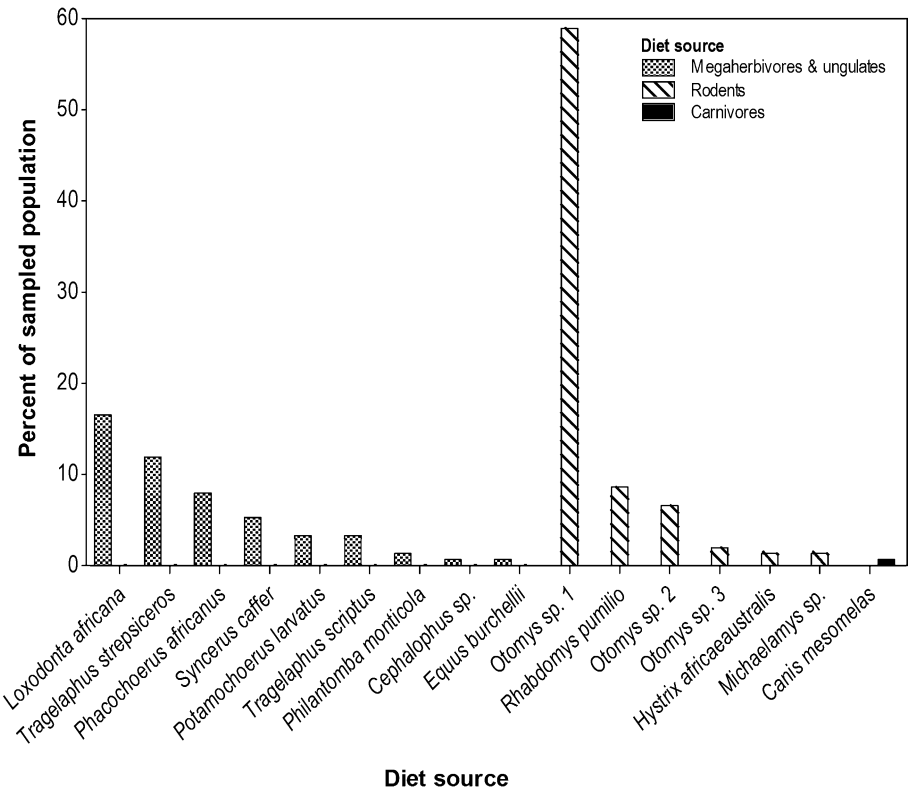

Fig. 1 Frequency of occurrence of diet sources recorded in flightless dung beetle *Circellium bacchus* fecal samples in the Main Camp and Colchester Sections of the Addo Elephant National Park

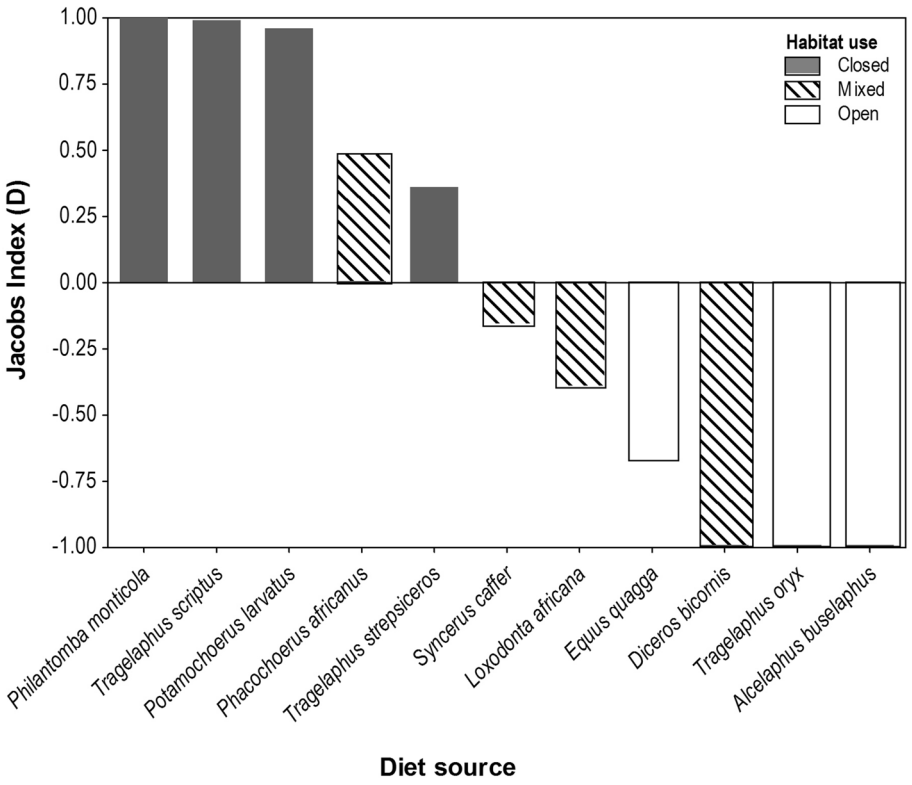

Fig. 2 Relative preferences for dung of each of the censused large herbivores (in the Main Camp and Colchester Sections of the Addo Elephant National Park) by flightless dung beetles *Circellium bacchus*, estimated using Jacobs's index, where $D > 0$ indicates preference and $D < 0$ avoidance. See Online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

preferred ($D = 0.19$) over hindgut fermenters ($D = -0.19$), although there was a roughly equal distribution of both guilds in either the preference or avoidance category (cf. Figure 2 and Online Resource 2). The dung of the browsing guild was consistently preferred ($D = 0.52$), while that of the mixed feeders ($D = -0.31$) and the grazers ($D = -0.08$) were avoided. The clearest patterns were among guilds of species characteristic of different habitats (Fig. 2): dung of species that use dense, closed vegetation was consistently preferred ($D = 0.63$), while that of open habitat species was consistently avoided ($D = -0.86$). The dung of species that show mixed use of open and dense habitats was avoided ($D = -0.29$), although this was not always the case (Fig. 2).

Discussion

Our findings emerge from a novel approach to exploring the functional role of dung beetles, and support the hypothesis that DNA material from the dung source occurs in dung beetle feces. Thus, we were able to identify the diet sources of these beetles, and incidentally detected the possible presence of cryptic dung-providing species using this approach. The findings also support the hypothesis that adult dung beetle diets differ from that of larvae, and provide insights into dung source preferences at a taxonomic (mammal taxa providing the dung) and functional (habitat use of the dung sources) level. Finally, our findings highlight the complementary ecological role that adult dung beetles may be playing in terms of dung breakdown. These points are expanded on below.

Detecting diet sources and cryptic species

DNA metabarcoding is rarely used to describe the diet of invertebrates from feces (but see Ibanez et al. 2013; Gómez and Kolokotronis 2016; Kaunisto et al. 2017; Rodgers et al. 2017) and can clearly be applied to identifying diet sources for coprophagous taxa and detritivores. DNA metabarcoding can also be applied to identify the source of fecal material, and hence to identify the source of dung balls. 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 allowed the possible identification of taxa unrecorded at the study site (i.e., the third *Otomys* species and the *Cephalophus*) and served to detect taxa, whose presence is missed by conventional census strategies. Thus, *P. monticola*, the blue duiker, is a small, dense vegetation-dwelling antelope known to occur in the AENP, and 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, although the statistical limits on detecting such absences are currently unknown. 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. 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; Rodgers et al. 2017).

Shifts in dung beetle diet sources

The dung sources consumed by adult *C. bacchus* presented here differ substantially from published findings that emphasize 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 black rhinoceros for a reliable dung source [Chown et al. (1995) provide no data on dung use]. These differences may reflect the lack of systematic approaches in the previous studies, the earlier emphasis on studying dung beetles at large dung sources, or misidentification of the dung sources. In addition to these sampling artefacts, 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. Furthermore, most published studies of resource use by *C. bacchus* have focussed on observing beetles forming either brood or feeding balls from dung piles (e.g., Kryger et al. 2006). As a consequence, the use of smaller fecal deposits (e.g., rodent droppings) would be completely overlooked, because these 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. Possible changes in the availability of black rhino dung between these studies cannot serve as an explanation of the absence of any records of adult *C. bacchus* feeding on this resource, because we were able to record dung beetles feeding on the dung of other underrepresented (vide the census data) taxa (e.g., bushbuck, bushpig, and blue duiker). Alternatively, our ability to detect black rhino DNA in the feces might have been influenced by species-specific biases in the survival of DNA during digestion (Deagle and Tollit 2007). For dung beetles, however, the mammalian DNA ingested corresponds mainly with intestinal cell remains and it is unlikely that persistence of these cell remains differ between mammalian species. As a consequence, a difference in detection probability is unlikely. The only potential problem could be a mismatch between the primers and their target sequences, but these target sequences are highly conserved among mammalian species (Taberlet et al. 2018). Finally, sampling artefacts may arise due to the misidentification of dung used by *C. bacchus* in the published studies mentioned above. Although the extent of this is typically not known, the approach used in the present study avoided this latter risk.

A more interesting functional explanation of these differences may lie in 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 to 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 are 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 and 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* 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*.

Dung preferences

Available preference estimates are limited to the larger herbivore species, and do indicate a preference by *C. bacchus* for feeding on ruminant feces. This provides partial support for the quality maximization strategy discussed above. Relevant data for the time/effort minimization strategy for adult *C. bacchus* are 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 for the dung of herbivores that typically use densely vegetated habitat (Fig. 2), bearing in mind the possible underestimate of cover-loving herbivore numbers, and hence overestimate of their dung preferences. These preferences 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 our diet source and preference data.

Functional implications

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, 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 need to be explored in other scarabaeid dung beetle species.

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Author contribution statement GIHK, ML, PT, and EC conceived and designed the study, collected and analyzed the data, and wrote the manuscript; GFF, FB, AB, and DR extracted and amplified DNA and contributed to the writing of the paper.

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Compliance with ethical standards

Conflict of interest We declare that we have no competing interests.

Data accessibility Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.rm2n41j.

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