Pertanika J. Trop. Agric. Sci. 40 (4): 543 – 552 (2017)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Interaction between the Long-Tailed Macaque and the Dung Beetle in Langkawi

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ABSTRACT

The interaction between dung beetles and primates was studied at Telaga Tujuh, Langkawi Island, Malaysia using such as observation and sampling method. The dung beetles were caught, and their dung balls collected. The beetles were identified as *Paragymnopleurus maurus*, and their dung balls were identified as originating from *Macaca fascicularis*, using a molecular approach involving the cytochrome *b* (Cytb) marker. This is the first record of *Paragymnopleurus maurus* from the study site in the Langkawi Islands and from this part of Malaysia. *Paragymnopleurus maurus* is attracted to the omnivorous dung of *M. fascicularis*, because it is a preferred food source for the beetle. Daytime is the active period for *P. maurus* and this study shows that the foraging area of *P. maurus* is restricted to the forest, even though the beetle's food source (*M. fascicularis*'s dung) can be found outside the forest.

Keywords: Primate, new record, dung beetle, species interactions, Langkawi Island

ARTICLE INFO

Article history:
Received: 23 February 2016
Accepted: 06 June 2017

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ISSN: 1511-3701

INTRODUCTION

Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) perform many crucial roles in the ecosystem, including soil enrichment, nutrient cycling, seed dispersal (Willson et al., 1990; Jordano, 1992), fly control (Haufe, 1989; Guglielmone et al., 1999; Nichols et al., 2008). The most important characteristic of dung beetles is their preference for animal faces as a food source and breeding medium. In addition to faces, dung beetles also use decaying matter as food during their adult and larval stages (Halffter & Matthews, 1966; Halffter & Edmonds, 1982).

The survival of dung beetles are highly dependent on other animals, especially mammals Some species of dung beetles are specific with regard to habitat selection (Hanski & Camberfort, 1991) and may not be able to survive in open vegetation where the number of small- or medium-sized mammals producing faces is lower. A study conducted by Davis (2000) in Borneo reported that the dung beetle diversity was lower in logged forests than in undisturbed forest areas.

The relationship between dung beetles and mammals are likely to be more specific than previously expected; for example, as documented by Hanski and Camberfort (1991), herbivorous, omnivorous, and carnivorous mammals will attract different species of dung beetles.

Three primate families are found on Langkawi Island: Hylobatidae, Lorisidae, and Cercopithecoidea. Likewise, the superfamily Cercopithecoidea can be divided into two subfamilies: Cercopithecinae and Colobinae. The genus *Macaca*, a representative of the omnivorous Cercopithecinae subfamily, has three species that are found in Malaysia: *M. fascicularis* (long-tailed macaques), *M. nemestrina* (pig-tailed macaques), and *M. arctoides* (stump-tailed macaques). Of the Cercopithecinae, only *M. fascicularis* and *M. nemestrina* are found on Langkawi. The genera *Trachypithecus, Presbytis,* and *Nasalis* represent colobines, or the leaf-eating group (Bennett, 1991); *Presbytis* and *Trachypithecus* are specific to the Langkawi Islands. Different primate species tend to have different lifestyles (i.e., arboreal or terrestrial) and different diets (Fam & Nijman, 2011). Therefore, different species of dung beetles are associated with primate species that have different diets. The objective of this study was to record the interactions between the dung beetles and the primates inhabiting this island ecosystem.

METHOD

Study Site

The sampling site selected for this study is the Telaga Tujuh Waterfalls, near the Mat Chinchang forest, a tropical mixed dipterocarp rainforest. The forest is on the island of Langkawi, located on the west coast of Peninsular Malaysia (6° 22.162′ N, 99° 40.827′ E).

Sampling of Dung Beetles and Faeces

Field observations were conducted to search for any interactions between primates and dung beetles that specialise in rolling dung. The observation of macaque troops was conducted from 21–23 August 2015, from 10am to 1pm each day, with 45-minute observation period and 15-minute interval.

The conditions were bright and sunny throughout the sampling period. Only diurnal species was observed because it coincided with the specific active period of the dung beetles under study (Davis, 1999; Niino et al., 2014). No passive trapping or bait trapping was used to collect the dung beetles. However, when any rolling dung balls were observed, both the dung beetle and the dung ball were collected. The collected dung beetles were preserved and identified in Universiti Kebangsaan Malaysia (UKM) laboratory using available taxonomic keys (Ochi et al., 1996; Ek-Amnuay, 2008); the diagnostic characters were elongated legs and two frontal lobes on the clypeus. Photographs of the dung beetles were taken using a Canon EOS 6D camera attached to a stereomicroscope (Zeiss Stemi SV11). Faeces from *M. fascicularis* and from beetles' dung balls were taken to the UKM laboratory to identify the associated macaque species using a molecular approach. To avoid cross-contamination, only a single faecal sample was collected per session and was carefully stored in a 15-ml vial with 99% ethanol, to preserve the traces of DNA in the faces.

The faecal samples were visually examined to identify the target species; usually, the genus *Macaca* has brown-coloured faces, while that of *Trachypithecus* is greenish. The colours are different because of the omnivorous and frugivorous diets of the macaques and the strictly herbivorous and folivorous diets of the langurs (called "*lutung*" locally). Fresh samples of the faces were easily found by looking for flies or by observing the dung beetles rolling the faces into dung balls.

DNA Extraction

The DNA was extracted from 0.2–0.4-g samples of the faces (2 replicates) using the innuPREP Stool DNA Kit (Analytik, Jena, Germany), following the manufacturer's protocol.

Polymerase Chain Reaction (PCR)

To confirm the identity of the macaque species, PCR was conducted using the mitochondrial DNA Cytochrome *b* (Cytb) as a primer (Table 1). This primer was designed specifically to avoid species cross-contamination. A sequence of *M. fascicularis* was retrieved from GenBank to be used as template in designing primer. The Primer-BLAST program was used to generate species-specific primer (Ye et al., 2012). The Needleman-Wunsch global alignment algorithm (Needleman & Wunsch, 1970) is used by Primer-BLAST to check the specificity of the primer pairs to the template sequences. To choose the best primer pair, the physical characteristics such as length (mer), guanine-cytosine (GC) content (%), molecular weight (g/mole), extinction coefficient at $260_{nm,}$ dimer and complementary sequences of the primer pairs were analysed using Oligo Analyzer 3.1. By using the Phusion Flash-High-Fidelity PCR Master Mix (Thermofisher scientific) which contains Phusion Flash II DNA Polymerase, 2x reaction buffer, dNTPs and $MgCl_2$ as PCR reagent, a three-step PCR process was employed, including an initial denaturation at 98°C held for 10 seconds, followed by 30 cycles of denaturation at 98°C for 1 second, 50°C of annealing for 30 seconds, 72°C for 15 seconds of extension, and a final extension at 72°C for 1 minute (see Table 2).

Table 1
The designed primer sequences for PCR

Primer name	Sequence (5'-3')	Locus	Annealing Temperature (°C)	Species
Latiff1018_F	CAATACACTACTCACCAGAC	Cyt *b*	50.0	*M. fascicularis*
Latiff1069_R	TAGGTTGTTTTCGATTAGGG			

Table 2
PCR components in DNA amplification

Chemical	Volume (x1) (µl)
Phusion Flash High Fedelity (Mastermix)	10.0
100 µM Primer Forward	1.0
100 µM Primer Reverse	1.0
Template DNA	1.0
dd	7.0
TOTAL	20.0

RESULTS

Dung Beetle Species

Field observations showed that only a single species of dung beetle had a strong preference for primate faces. Dung-rolling beetles of the species *Paragymnopleurus maurus* (Figure 1 & Figure 2) consumed primate faces as their main food source from this area.

Figure 1. Dung beetle *Paragymnopleurus maurus* making a dung ball and ready to roll it to other places

Figure 2. *Paragymnopleurus maurus*

Primate Species

Faeces rolled by *P. maurus* were collected to determine their host DNA because, aside from hair follicles, faces are the best non-invasive source of DNA from primates (Inoue et al., 2007; Marangi et al., 2015). The entire mitochondrial DNA (mtDNA) sample from the faeces were successfully extracted using the standardised method described in the manufacturer's protocol. The PCR products with the most visible bands on the agarose gel (Figure 3) were sequenced to analyse the effectiveness of the primer and the PCR optimisation.

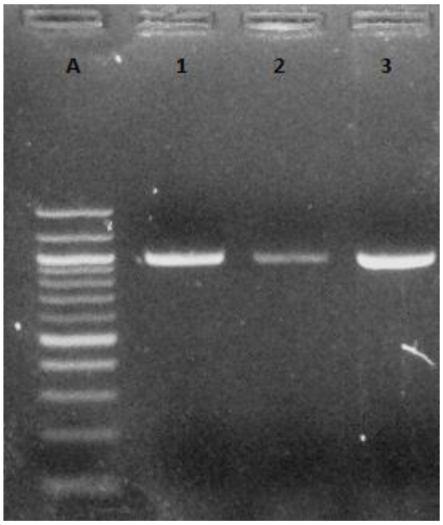

Figure 3. Result of PCR process on 1.5% agarose gel. Well A=100bp molecular marker

The sequences obtained were analysed using the GenBank BLAST to ensure that the targeted loci of the selected species were amplified. The parameters used to ensure the specificity of the DNA sequences included the percentage of queries covered by alignment to the database sequence, the best expected value (E-Value) of the alignments from the database sequence, and the highest percent identity (Max Identity) of all the query-subject alignments. The sequence obtained from the PCR product had 98% specificity to the database sequence of *M. fascicularis,* agreeing with the Max Identity and an E-Value ≤0. The specifically designed primer successfully amplified the targeted loci, and the overall molecular approach confirmed that the faces rolled by *P. maurus* on Langkawi Island belonged to a single species, *M. fascicularis* (Figure 4).

Figure 4. *Macaca fascicularis* (Long-tailed macaque) enjoying food in Langkawi Island, Kedah, Malaysia

DISCUSSION

Three genera of dung-rolling beetles have been recorded in Malaysia: *Paragymnopleurus*, *Ochicanthon*, and *Sisyphus*. Among these, *Paragymnopleurus* is the largest (2–4 cm in length) and was the only species found in this study. *P. maurus*, a large-bodied species, is associated with the long-tailed macaque, *M. fascicularis*. The existence of the interaction has been documented based on molecular data and live observations in the field. Though *P. maurus* is classified as large dung beetle, it is not the largest dung beetle that can be found in Malaysia (Muhaimin et al., 2015). Species such as *Heliocopris tyrannus* (Goh et al., 2014), *Catharsius renaudpauliani*, and *C. molossus* are much larger than *P. maurus* and have been previously recorded in Singapore (Ong et al., 2013). However, in this study, which was conducted during the daytime, we focused only on diurnal species of dung beetles. Niino et al. (2014) reported that *P. maurus* is active during the day, while a closely related species, *P. striatus*, is active at night. It is likely that if we had conducted our sampling activities at night, the presence of *P. striatus* might have been detected.

The interactions between a primate species and a dung beetle species were recorded for the first time at the Telaga Tujuh Waterfalls, a site adjacent to the Mat Chinchang forest on the main island of Langkawi. This dung beetle species is common, due to its wide distribution in Peninsular Malaysia (Doll et al., 2014; Niino et al., 2014), Borneo (Davis, 2000; Davis et al., 2001), and the Oriental region (Davis et al., 2002). Unlike the genus *Ochicanthon*, which prefers to consume carrion (Krikken & Huijbergts, 2007), *Paragymnopleurus* is similar to the genus *Sisyphus* in terms of food preference.

According to Lee et al. (2009), large-bodied dung beetle species are more sensitive to forest disturbances than smaller species. This is because large dung beetles require big forest areas inhabited by large population of mammals. As the mammals provide food and a breeding medium for the dung beetles, declining population of mammals directly affect the survival of dung beetles. This relationship was documented by Scheffler (2005) and Gardner et al. (2008), who observed the same direct correlations between the mammal and dung beetle populations in the tropical forests of Brazil. However, a different circumstance was observed for *P. maurus,* which seems to be more resistant to habitat disturbance and can thrive in smaller forest patches. According to Qie et al. (2011), *P. maurus* can also be found in the small fragmented and isolated recreational forest patches of Kenyir Lake, Terengganu. Lee et al. (2009) discovered the same dung beetle species in small forest patches in Singapore, a case similar to that of Langkawi Island, in which island effects and similar abiotic factors prevail.

Regarding the dung preference of *P. maurus*, Hanski and Camberfort (1991) claimed that *P. maurus* is closely associated with the dung of omnivorous animals. However, this species is also found on carrion (Sakai & Inoue, 1999), human dung (Davis et al., 2000), cattle dung (Muhaimin et al., 2015), and pig dung (Slade et al., 2007). *P. maurus* specimens have also been collected from elephant dung (Doll et al., 2014; Goh et al., 2014), which is their food source and breeding medium. In this study, *P. maurus* preferred the dung of the omnivorous primate *M. fascicularis*. This indicates that either *P. maurus* is a generalist or that it has expanded its food preferences due to the scarcity of its preferred food source. Further research should be conducted to show the actual diet preference of this species.

The long-tailed macaque *M. fascicularis* is a mutualist–edge species, resilient and well-adapted to interaction with humans (Abdul-Latiff et al., 2014a, 2014b). Garbage dump sites provide the macaques with ample food sources. However, the dung beetle *P. maurus* is incapable of venturing far out into human areas because its flying ability and mobility is limited (Niino et al., 2014) and specific requirements such as the forest floor and vegetation types that only the forest can provide (Doube, 1983; Andresen, 2005). Understanding the interactions and preferences of dung beetles, both tunnellers and rollers, with regard to primate dung is crucial for understanding the dynamics of the surrounding ecosystems (Nichols et al., 2007). This study has reported the interactions between the dung beetle *P. maurus* and the macaque *M. fascicularis*. Future works should include the identification of the components that attract this dung beetle to the faces of the long-tailed macaque, as well as the mode of seed dispersal involved in this interaction. Further and more specific studies on the rich biodiversity of the Langkawi Islands should be conducted for developing community-level conservation strategies for this invaluable UNESCO Geopark site.

CONCLUSION

This study has shown *Paragymnopleurus maurus* has direct interactions with the long-tailed macaque (*M. fascicularis*); this dung beetle evidently prefers omnivorous faces, a main diet of this primate. This study can contribute to knowledge by showing interactions among species, utilising practical methodologies and field samples such as the dung or faces of primates and other vertebrate hosts.

ACKNOWLEDGEMENT

The authors thank Mr. Danny Haelewaters and Mr. Tristan Wang from Harvard University in Cambridge, Massachusetts, USA for the original photographs taken during the mini-expedition to Langkawi Island. Thanks also to Prof. Dr. Maimon Abdullah for her critical comments and in editing the manuscript. We are grateful to the Langkawi Research Centre (PPL), UKM for their kind hospitality during our field study. This research was funded by the following grants: FRGS/1/2014/SG03/UKM/02/1 and GUP-2016-022.

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