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# Elucidating the diet and foraging ecology of the island flying fox (*Pteropus hypomelanus*) in Peninsular Malaysia through Illumina Next-Generation Sequencing

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There is an urgent need to identify and understand the ecosystem services provided by threatened animal species such as flying foxes. The first step towards this is to obtain comprehensive data on their diet. However, the volant and nocturnal nature of flying foxes presents a challenging situation, and conventional microhistological approaches to studying their diet can be laborious and time-consuming, and provide incomplete information. We used Illumina Next-Generation Sequencing (NGS) as a novel, non-invasive method for analysing the diet of the island flying fox (*Pteropus hypomelanus*) on Tioman Island, Peninsular Malaysia. Through NGS analysis of flying fox droppings over eight months, we identified at least 29 Operationally Taxonomic Units comprising the diet of this giant pteropodid, spanning 19 genera and 18 different plant families, including one new family not previously recorded for pteropodid diet. NGS was just as successful as conventional microhistological analysis in detecting plant taxa from droppings, but also uncovered six additional plant taxa. The island flying fox's diet appeared to be dominated by figs (*Ficus* sp.), which was the most abundant plant taxon detected in the droppings every single month. Our study has shown that NGS can add value to the conventional microhistological approach in identifying food plant species from flying fox droppings. However, accurate and detailed identification requires a comprehensive database of the relevant plant DNA, which may require collection of botanical specimens from the study site. Although this method cannot be used to quantify true abundance or proportion of plant species, nor plant parts consumed, it ultimately provides a very important first step

towards identifying plant taxa in pteropodid diet.

1 **Elucidating the diet and foraging ecology of the island flying fox**  
2 **(*Pteropus hypomelanus*) in Peninsular Malaysia through Illumina**  
3 **Next-Generation Sequencing**

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22

23 **ABSTRACT**

24 There is an urgent need to identify and understand the ecosystem services provided by threatened  
25 animal species such as flying foxes. The first step towards this is to obtain comprehensive data  
26 on their diet. However, the volant and nocturnal nature of flying foxes presents a challenging  
27 situation, and conventional microhistological approaches to studying their diet can be laborious  
28 and time-consuming, and provide incomplete information. We used Illumina Next-Generation  
29 Sequencing (NGS) as a novel, non-invasive method for analysing the diet of the island flying fox  
30 (*Pteropus hypomelanus*) on Tioman Island, Peninsular Malaysia. Through NGS analysis of  
31 flying fox droppings over eight months, we identified at least 29 Operationally Taxonomic Units  
32 comprising the diet of this giant pteropodid, spanning 19 genera and 18 different plant families,  
33 including one new family not previously recorded for pteropodid diet. NGS was just as  
34 successful as conventional microhistological analysis in detecting plant taxa from droppings, but  
35 also uncovered six additional plant taxa. The island flying fox's diet appeared to be dominated  
36 by figs (*Ficus* sp.), which was the most abundant plant taxon detected in the droppings every  
37 single month. Our study has shown that NGS can add value to the conventional  
38 microhistological approach in identifying food plant species from flying fox droppings.  
39 However, accurate and detailed identification requires a comprehensive database of the relevant  
40 plant DNA, which may require collection of botanical specimens from the study site. Although  
41 this method cannot be used to quantify true abundance or proportion of plant species, nor plant  
42 parts consumed, it ultimately provides a very important first step towards identifying plant taxa  
43 in pteropodid diet.

44

45

## 46 INTRODUCTION

47 Understanding the contribution of animals to the functioning of rainforests has become an  
48 important issue in conservation biology. Conservation studies are now recognizing the need to  
49 collect qualitative and quantitative information on trophic relationships between animals and  
50 plants, not only to identify potential ecosystem service providers (Pompanon et al. 2012; Hibert  
51 et al. 2013), but also to inform management interventions for threatened species (Valentini et al.  
52 2009a; Ando et al. 2013).

53 Bats (Order: Chiroptera) provide important ecosystem services such as insect pest  
54 suppression, pollination, and seed dispersal (Fujita and Tuttle 1991; Kunz et al. 2011).  
55 Characterising their diet is a fundamental step towards understanding their ecological roles. Due  
56 to their nocturnal and volant nature, invasive analysis (by capturing individuals) or indirect  
57 methods (by collecting droppings) have traditionally been used to study bat diets. Indeed, insect  
58 fragments found in faecal and stomach contents of insectivorous bats have facilitated the  
59 investigation of their trophic interactions (Clare 2014) and role in agricultural pest regulation  
60 (Kunz et al. 2011). Similarly, microscope analyses of pteropodid faeces have provided insights  
61 into their interactions with various plants (Bumrungsri et al. 2007), and their roles in pollination  
62 (Bumrungsri et al. 2013) and seed dispersal (Sritongchuay et al. 2014). However, studies on  
63 phytophagous bat diets to date have relied on physical identification of food plant species –  
64 either through direct observations of foraging bats, or microhistological identification of seeds,  
65 pollen, fruit fibres and leaf fragments in faeces and ejecta. The successful use of such methods  
66 relies on several important factors such as accessibility and visibility of foraging bats, as well as  
67 the availability of expert knowledge or resources such as reference collections. Another  
68 limitation of these conventional approaches is that they require physically identifiable remains to

69 be expelled by the bats; any plant parts that were consumed or expelled solely in liquid form will  
70 be missed out in the analysis (Pompanon et al 2012). Foraging studies of wide-ranging species  
71 such as flying foxes also require the use of expensive, hi-tech equipment such as GPS collars,  
72 which is often not feasible for all projects.

73 In the Old World, fruit bats such as flying foxes (Pteropodidae: *Pteropus* spp., *Acerodon*  
74 spp.; Kingston 2010) have become increasingly threatened by hunting for bushmeat and  
75 medicine (Mildenstein et al. 2016). Identifying their diet and roles as ecosystem service  
76 providers can help strengthen arguments for their protection. It will also help us understand the  
77 wider implications of large-scale flying fox extinctions, as these giant bats are known to interact  
78 with plants on a large landscape scale, performing ecological roles over vast transboundary areas  
79 (Epstein et al. 2009). Flying foxes are likely to be particularly important players in island  
80 ecosystems where they often serve as principal pollinators and seed dispersers (Cox et al. 1991),  
81 and where maintaining their numbers at high densities is necessary for the survival of plant  
82 communities (McConkey and Drake 2006, 2007; McConkey and Drake 2015). Such data are  
83 also important to understand the drivers and potential mitigation strategies for conflicts between  
84 fruit bats and humans (Aziz et al. 2015).

85 Whilst in-depth, comprehensive dietary/foraging studies have been conducted for certain  
86 flying fox species, particularly in Australia (e.g. Boulter et al. 2005; Williams et al. 2006),  
87 Oceania (e.g. McConkey and Drake 2006; Luskin et al. 2010), Japan (e.g. Nakamoto et al. 2007,  
88 2009; Lee et al. 2009), South Asia (e.g. Mahmood-Ul-Hassan et al. 2010; Sudhakaran and Doss  
89 2012), and Indian Ocean islands (e.g. Nyhagen et al. 2005; Oleksy et al. 2015), the diets of  
90 Southeast Asian species, which are some of the most threatened due to the additional threat of  
91 commercial hunting (Mildenstein et al. 2016), remains largely unknown. Indeed, apart from a

92 few studies in the Philippines (Reiter and Curio 2001; Mildenstein et al. 2005; Stier and  
93 Mildenstein 2005), Thailand (Weber et al. 2015), and Myanmar (Win and Mya 2015), all other  
94 dietary and foraging studies on Southeast Asian Pteropodidae have focused on the smaller  
95 pteropodids (e.g. Hodgkison et al. 2004; Fletcher et al. 2012; Bumrungsri et al. 2013; Stewart et  
96 al. 2014). This is of particular concern given that out of the 67 flying fox species listed on the  
97 IUCN Red List, almost half (30 species i.e. 45%) are actually found in Southeast Asia (IUCN  
98 2016).

99         Although molecular analysis of pteropodid diets can potentially be used to overcome the  
100 obstacles outlined above, this approach has yet to be applied. Non-invasive DNA analyses of  
101 faeces have already been conducted to determine the herbivorous diets of animals such as  
102 primates (Bradley et al. 2007), marmots, bears, capercaillies, grasshoppers, molluscs, slugs  
103 (Valentini et al. 2009a), pigeons (Ando et al. 2013) and tapirs (Hibert et al. 2013), but this has  
104 never before been attempted for pteropodids or plant-based mammal diets in the Palaetropics.  
105 To date however, molecular analyses of bat diets have only been used for insectivorous species  
106 (e.g. Clare et al. 2009; Razgour et al. 2011; Zeale et al. 2011). In fact, to our knowledge, the only  
107 successful attempt to identify the diet of plant-visiting bats through molecular analysis has been  
108 done by one study in the Neotropics (Hayward 2013).

109         On Tioman Island in Peninsular Malaysia, we evaluated the utility of Next-  
110 Generation Sequencing (NGS) to identify plant species present in the droppings of the island  
111 flying fox (*Pteropus hypomelanus*), whose diet hitherto remains unknown throughout its entire  
112 range. Specifically, our study aimed to: 1) determine the feasibility of extracting amplifiable  
113 plant DNA from flying fox droppings; 2) infer spatio-temporal dietary patterns based on high  
114 throughput amplicon sequencing of the partial *rbcL* gene; and 3) evaluate the potential of NGS



115 analysis in complementing or even replacing conventional microhistological analysis to elucidate  
116 flying fox diet.

117

## 118 **MATERIALS AND METHODS**

### 119 *Study species*

120 The island flying fox (*Pteropus hypomelanus*), also known as the variable flying fox and the  
121 small flying fox, roosts gregariously, forming colonies of up to 5000 individuals. It is a  
122 widespread insular species, considered to be abundant throughout a distribution range that  
123 extends from the Maldives and Indian islands in the west to Melanesia in the east. Because of  
124 this, it is considered to be Least Concern on a global scale by the IUCN Red List; however its  
125 population trend is noted to be decreasing (Francis et al. 2008; Olival 2008).

126 In Malaysia this species is confined to small offshore islands. A study on *Pteropus*  
127 population genetics and phylogeography (Olival 2008) has shown the east coast populations off  
128 the Malay Peninsula to be a subspecies – *P. hypomelanus lepidus* – that is genetically distinct  
129 from the west coast populations of *P. hypomelanus robinsoni*. The species is listed as  
130 Endangered on the Malaysian Red List (DWNP 2010).

131 On Tioman, the island flying fox can be found roosting permanently in two villages:  
132 Tekek, on the west coast, and Juara, on the east coast (Figure 1A), and forages throughout the  
133 island (Medway 1966; Ong 2000). Monthly roost counts conducted during March-October 2015  
134 yielded estimated ranges of 675-1033 individuals in Juara, and 2178-5385 individuals for the  
135 entire island.

136

### 137 *Study site*

138 We conducted this study on Tioman Island (2°48'38" N, 104°10'38" E; 136 km<sup>2</sup>; Figure 1A),  
139 located 32 km off the east coast of Peninsular Malaysia in the State of Pahang. This research was  
140 approved by the Economic Planning Unit of Malaysia (Permit number: 3242). Much of the  
141 island inland is still covered by primary tropical rainforest, which has been designated as Pulau  
142 Tioman Wildlife Reserve (82.96 km<sup>2</sup>). It has a hilly topography, with flat areas only along the  
143 coast (Abdul 1999). The area designated as a wildlife reserve is composed of lowland mixed  
144 dipterocarp forest and hill dipterocarp forest. Most forested areas are still inaccessible due to the  
145 rugged topography, with many steep slopes and rocky outcrops (Latiff et al. 1999). The climate  
146 is tropical, uniformly warm and humid throughout the year (Hasan Basyri et al. 2001), but the  
147 island experiences the northeast monsoon from November to March (Bullock and Medway  
148 1966).

149           There are currently seven villages on the island, situated along the coastline (Fig.  
150 1A). The majority of the local people are Muslim, and therefore due to religious dietary  
151 restrictions do not hunt the bats for food or medicine (Aziz et al. submitted). As the island's  
152 marine area is also a designated Marine Park and a popular tourist destination, many of the local  
153 people are heavily involved in the tourism industry (Abdul 1999).

154           Currently, the island flying fox can only be found roosting in two villages: Tekek and Juara.  
155 Local people have reported that the flying foxes do forage in other villages on the island. Besides  
156 flying foxes, only four other pteropodid species have been recorded on the island (Lim et al.  
157 1999).

158

159 ***Study design***

160 First, we assessed the feasibility of extracting plant DNA from *Pteropus* droppings, and  
161 evaluated whether DNA sequences obtained from NGS could be matched with those from: 1)  
162 online DNA reference databases; and 2) an *in situ* reference collection created by sampling DNA  
163 from possible food plants in and around both villages. Next, we compared the performance of  
164 NGS with a conventional microscope approach to identify food plant species from flying fox  
165 droppings.

166

### 167 ***Sampling of flying fox droppings***

168 Collection of droppings took place once a month during March-October 2016 (i.e. eight months).  
169 Samples of flying fox droppings consisting of faeces and ejecta were collected for three  
170 mornings in the last week of each month from three separate day roosts in Juara (east coast) and  
171 two separate day roosts in Tekek (west coast). The number of roosts and sampling days were  
172 determined based on species accumulation curves of pollen morphospecies that were detected  
173 through preliminary microhistological analysis in June 2014. Program EstimateS (version 9.1.0;  
174 <http://viceroy.eeb.uconn.edu/estimates/>) indicated that sampling completeness (i.e.  
175 observed/estimated number of species; Soberon et al. 2000) was around 97% using this sampling  
176 regime.

177 In Juara, three suitable roost trees (Fig. 1B, right) for sampling were selected based on  
178 accessibility and also on the highest/largest amount of faecal/ejecta splatter produced under the  
179 roost, in order to maximise sample yield. As flying foxes often shifted roosts or temporarily  
180 abandoned degraded roosts, this meant that sometimes different roosts were sampled in each  
181 location every month or even every morning, although most roosts were consistently sampled  
182 each month due to their constant high occupancy and best accessibility yielding the most amount

183 of droppings every month. In Tekek, two suitable roosts, one mango and one jackfruit roost,  
184 were selected based on least human activity/disturbance, although this was consistently high for  
185 all accessible roosts at that site. However, after the first six months, the jackfruit roost was  
186 chopped down by the owner. Consequently the angšana roost, with higher human disturbance,  
187 was sampled as a replacement for the remaining two months at that site (Fig. 1B, left).

188 Plastic sheets measuring 0.8 x 1.0 m were placed under each roost after dark, once the  
189 bats had exited the roost to forage. The roosts were then visited the next morning for collection  
190 starting at 0700h and ending at 1200h (bats typically returned to the roosts around 0500-0600h);  
191 the plastic sheets were pulled out first from under the roost, and carefully moved away to a clear  
192 area for processing (Fig. 1C). As it was often difficult to differentiate faeces from ejecta  
193 (chewed-up plant parts spat out by bats during feeding), both were collected and analysed  
194 equally as 'droppings' (Fig. 1D). Droppings collected for processing were selected based on  
195 unique colour and texture, as this was assumed to be representative of plant diversity in the bats'  
196 diet. Following the approach used by Stier and Mildenstein (2005) based on short gut-passage  
197 time for flying foxes (12-34 min; Tedman and Hall 1985), we assumed that each bat voided its  
198 last meal once, and therefore each dropping represented a different individual's food choice.  
199 Droppings were collected by swabbing them with a cotton bud, then placing each individual  
200 dropping into a 5 ml Eppendorf tube containing ~1000 µl of 95% ethanol. These tubes were then  
201 kept cool in the field, either by storing in a conventional freezer or by using a portable cooler box  
202 with ice packs, for 1-3 days before being transported off the island and then stored in a -80°C  
203 freezer.

204 In order to simultaneously test the utility of NGS and compare it with conventional  
205 approaches, we collected two duplicate sets of 10 individual droppings from one single roost in

206 Juara village during a single morning on 6 May 2015. One sample set was then kept in a  
207 conventional fridge for microscope analysis, whilst the other set was stored in the -80°C freezer  
208 for molecular analysis.

209

### 210 ***Reference plant sample collection and generation of in situ rbcL sequence database***

211 In order to form an *in situ* DNA reference collection, we first checked a published list of genera  
212 of known food plants for *Pteropus* across its range (Marshall 1985), cross-checked this against a  
213 preliminary checklist of seed plants for Tioman (Latiff et al. 1999), and also obtained  
214 information on possible flying fox food plants through talking to local people in Juara. We then  
215 searched for genera of similar plants in and around the two villages with the aid of a local plant  
216 expert. The botanical identification of plants (at least to genus) were subsequently verified by a  
217 trained botanist familiar with plants from the region. When the individual of a plant matching the  
218 genera was opportunistically found, we recorded its GPS location and collected 3-5 mature  
219 leaves for DNA extraction. The leaves were stored in Ziploc bags with silica gel under cool  
220 conditions to retard decomposition rates. Leaf samples from 19 different plant species were  
221 obtained for this purpose, constituting a preliminary library (Table 1).

222 Genomic DNA was extracted from approximately 25 mg of one leaf from each plant  
223 species using DNAeasy Plant Mini Kit (Qiagen, Halden, Germany) according to the  
224 manufacturer's protocols. DNA amplifications were performed in a mastermix containing 1µL of  
225 DNA, 25µL of OneTaq Quick-Load 2X Master Mix with Standard Buffer, (New England  
226 Biolab, Ipswich, MA), 1µL of 10mM forward primer rbcLaf-M13, 1µL of 10mM reverse primer  
227 rbcLa-revM13 (Table 2), and 22µL of nuclease-free water. The PCR protocol was started with  
228 an initial denaturation step for 30 sec at 94°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at

229 48°C, 40sec at 68°C, and final elongation for 2 minutes at 68°C. The PCR products were purified  
230 using 0.8X volume ratio of Agencourt Ampure XP beads (Beckman Coulter, Inc). The purified  
231 samples were sent to 1<sup>st</sup> BASE SB for Sanger sequencing. The sequencing results were quality  
232 trimmed using CodonCode TraceViewer (<http://www.codoncode.com/TraceViewer/>) and aligned  
233 using MAFFT version 7.0 (Kato and Standley 2013).

234

### 235 *Next-generation sequencing*

236 Individual droppings were pooled according to roost (n=5, 2 in Tekek and 3 in Juara) and month  
237 (n=8), creating 40 separate mixtures for analysis. The tubes containing the daily samples were  
238 first vortexed for 2 min to homogenise the content and subsequently, 1000 µL of the sample was  
239 pipetted into another tube to form the mixture. Next, 100 µL of the mixture underwent gDNA  
240 extraction with DNAeasy Plant Mini Kit (Qiagen, Halden, Germany) according to the  
241 manufacturer's protocols. Based on the alignment, primers targeting 220bp of *rbcL* gene were  
242 designed using Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/>) on default settings (Fig. 2). Partial  
243 Illumina adapter sequences were added to the 5' end of the designed primers, *rbcL*-357F and  
244 *rbcL*-556R, to allow barcoding and sequencing on the Illumina platform. The current *rbcL* was  
245 not used for Illumina as the digested plant might be degraded; hence a shorter target region  
246 would be more optimal to investigate the diet of flying foxes (Pompanon et al. 2012).

247 PCR reaction was performed using IlluM\_*rbcL*F and IlluM\_*rbcL*R. The 20µL PCR  
248 cocktail consists of 10µL *\_Q5* Hot Start High-Fidelity 2X *Master Mix* (New England Biolab,  
249 Ipswich, MA), 1µL each of 10µM forward and reverse primer, 1µL gDNA and 7µL MilliQ  
250 water. All reactions were performed in a Veriti® 96-Well Fast Thermal Cycler with the  
251 following protocol: initial denaturation for 30 sec at 98°C, 25 cycles of 10 sec at 98°C, 30 sec at

252 55°C and 10 sec at 65°C, with a final 1 min extension at 65°C. The PCR product was purified  
253 using 0.8x vol. ratio Agencourt Ampure XP beads (Beckman Coulter, Inc). Then, 1uL of Index 1  
254 and Index 2 primers from Nextera XT kit were added to 3uL of purified PCR product and  
255 combined with 5uL of *Q5 Hot Start High-Fidelity 2X Master Mix* (New England Biolabs,  
256 Ipswich, MA). The PCR protocol was as followed: initial denaturation for 30 sec at 98°C, 25  
257 cycles of 10 sec at 98°C and 1 min at 65°C, with a final 1 min extension at 65°C.

258 The purified amplicons containing the full length Illumina adapter and appropriate unique  
259 barcode were then quantified using KAPA Library Quantification kit (Kapa Biosystems,  
260 CapeTown, South Africa) on the EcoRealTime PCR system (Illumina, San Diego, CA). Based  
261 on the qPCR data, the amplicons were normalised, pooled and subsequently sequenced on the  
262 MiSeq (2 x 250 bp paired-end run) located at the Monash University Malaysia Genomics  
263 Facility.

264

#### 265 ***Adapter trimming, OTU clustering and abundance estimation***

266 Illumina nextera adapters and the primer sequences of the reads were trimmed off using  
267 Trimmomatic v 0.33 and FastX trimmer, respectively (Bolger et al. 2014;  
268 [http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/)). The trimmed paired-end reads were then merged using  
269 PEAR (Zhang et al. 2014) using default settings. Dereplication, singleton removal and  
270 Operationally Taxonomic Unit (OTU) clustering were performed using the pipeline implemented  
271 in UPARSE (Edgar 2013). The filtered OTUs were manually inspected and those containing stop  
272 codon(s) in the open reading frame were removed from the final dataset. Subsequently, to  
273 generate relative abundance distribution for each sample, reads were mapped to the final OTUs  
274 via USEARCH and normalised to 10,000 reads (Caporaso et al. 2010).

275           The number of reads for each OTU was converted into percentage and OTU with relative  
276 abundance lower than 0.5% was eliminated. The remaining OTUs were searched against BOLD  
277 database and reference sequences obtained from this study to obtain the identity of the OTUs.  
278 The top non-redundant 10 BLAST hits for each OTU along with the generated *rbcL* sequences  
279 from this study were used for phylogenetic construction. Briefly, the sequences were combined  
280 and aligned using default settings in MAFFT version 7.0 (Kato and Standley 2013). The aligned  
281 sequences were subsequently used to construct a maximum likelihood phylogenetic tree using  
282 FastTree (-nt -gtr) (Price et al. 2009). Subsequent tree visualisation and editing were done using  
283 FigTree (<http://tree.bio.ed.ac.uk/software/figtree>).

284

### 285 *Microhistological analysis*

286 For the 10 dropping samples collected in May, we sent one set for NGS analysis (following the  
287 protocol above) and used another set for microscope analysis. First, we manually broke up the  
288 dropping contents in the tube to produce a relatively more representative liquid sample. 1-3  
289 drops of this liquid was then dropped onto a microscope slide using a pipette. Fuchsin jelly was  
290 added to this in order to stain pollen grains within the dropping, a slip cover was placed on top,  
291 and the jelly was then melted over an open flame, sealing the slip cover to the slide. The slide  
292 was then cooled down in a conventional fridge in order to allow the jelly to solidify again before  
293 examination.

294           Once the slide had cooled sufficiently, it was placed under a conventional light  
295 microscope (Leica DM E) and first examined using 10/0.25 magnification in order to detect  
296 pollen grains and other plant parts. Once pollen or other plant parts were detected these were  
297 compared with a preliminary reference collection of pollen and fig parts taken from plants at the



298 sampling site (photographed using a microscope eye-piece camera (Dino-Eye AM4023X)), as  
299 well as photos from Start (1974), S. Bumrungsri (unpublished) and Mohamed (2014). If  
300 necessary, smaller pollen grains were viewed in greater detail using 40/0.65 magnification.

301 Since any attempt to quantify abundance of pollen grains can bias the analysis towards  
302 plant species that naturally produce greater amounts of pollen than others, pollen species were  
303 assessed based on 'presence/absence' only; following the advice and approach reported by  
304 Thomas (2009), a plant species was considered present in the diet if three or more of its pollen  
305 grains were found on one single slide.

306

## 307 **RESULTS**

### 308 *NGS as a viable tool to study pteropodid diet*

309 We were able to successfully extract, amplify, and subsequently identify plant DNA from all of  
310 the collected flying fox droppings using the *rbcL* primer. This indicates that the integrity of plant  
311 DNA was not severely affected during food digestion in the flying fox gut. After the filtration, 29  
312 OTUs were recovered from the sequencing reads, nominally representing at least 19 different  
313 plant genera from 18 families detected in the droppings (Fig. 3, Table 3). In addition, the family  
314 Polygalaceae represents a new record for pteropodid diet. Fig. 4 shows the maximum likelihood  
315 phylogenetic tree that was constructed from these results. Based on sampling completeness  
316 (calculated using EstimateS) for OTU relative abundance data from five roosts (data pooled over  
317 three days) per month using Chao 1 species richness estimator (good for datasets skewed towards  
318 low abundance classes; Chao 1984), sampling completeness was relatively high for the months  
319 March, April, August, September and October (88-100%). However, sampling completeness was  
320 relatively low for May, June and July (55-79%).

321

322 ***Spatio-temporal dietary patterns***

323 The results from our NGS analysis of island flying fox droppings over eight months suggest that  
324 the diet at both Juara and Tekek during this time was dominated by four different plant taxa that  
325 each yielded more than 100 sequencing reads: *Ficus* sp. (OTU 1), *Mangifera indica* (OTU 3),  
326 *Pavetta* sp. (OTU 4); and *Uncaria* sp. (OTU 5). Spatio-temporal trends in the relative abundance  
327 of these four taxa in the diet were apparent (Fig. 5). For example, OTU 5 appeared to be  
328 consumed in similar proportions at both Juara and Tekek across all months whereas OTU 4 was  
329 consistently consumed in low proportions in Tekek yet consumed irregularly in Juara over the  
330 same period. Even between different roosts in the same site, spatio-temporal differences were  
331 observed, such as for OTU 7 (*Antiaris* sp.; Fig. 6), although this taxon was far less abundant in  
332 the diet (50 sequencing reads).

333

334 ***Microhistological vs. NGS approach***

335 Microscope analysis identified two plant taxa in flying fox droppings (Table 4). Out of 10  
336 individual droppings, three contained durian (*Durio* sp.) pollen. Two of these also contained fig  
337 parts (*Ficus* spp.). All the other droppings contained fig parts exclusively; no other plant parts  
338 were detected. Durian pollen occurred at extremely low abundance; in all cases, only 3-4 grains  
339 were detected per slide. No other pollen or plant parts were detected. On the other hand, NGS  
340 identified the same two plant taxa detected by microhistological analysis, and further identified  
341 an additional six plant taxa. *Durio* was not detected in the same samples as in those identified via  
342 microscope.

343 Using NGS for the same 10 individual samples, reads mapping to OTU 1 belonging to the  
344 genus *Ficus* were highly abundant across a majority of the samples. Only three samples

345 contained a small number of reads (<1%) mapping to OTU 17 belonging to the genus *Durio*,  
346 which to some extent correlates with the observation from microhistological analysis. The ability  
347 of NGS to identify at least six additional plant taxa with substantial relative abundance, that were  
348 completely missed by the conventional approach, underscores its potential in uncovering plant  
349 taxa previously not known to be part of flying fox diet. It is also worth noting that the relative  
350 abundance of mapped reads varied considerably among individual samples which may be an  
351 indication of inter- and/or intra-sample diet inconsistency.

352

### 353 **DISCUSSION**

354 Our study is the first to describe the diet of the island flying fox, which was previously unknown.  
355 To our knowledge, this is also the first known use of NGS to identify plant taxa in the diet of a  
356 pteropodid, which has been difficult to characterise due to this animal's volant nature, large  
357 home ranges and nocturnal foraging behaviour. Furthermore, NGS provided comparatively  
358 greater insights into its diet than conventional microhistological approaches by detecting a wider  
359 range of plants, thus highlighting the comprehensiveness and discriminatory potential of the  
360 newly designed *rbcL* primers. Through NGS, we also discovered a new food plant family  
361 previously unrecorded by other studies of pteropodid diet. In our study, attempts to use  
362 microscope analysis to identify plant parts in droppings proved to be challenging, as no pre-  
363 existing reference collection was available. Attempting to build our own comprehensive  
364 botanical reference collection for Tioman was time-consuming and labour-intensive – and the  
365 resulting collection often did not match up with the plant parts found in the flying fox droppings.  
366 Obtaining DNA from botanical specimens, however, is still a necessary step to narrow down the  
367 identity of OTUs obtained from NGS to species level. More importantly, the use of NGS allowed

368 us to identify plant species even when no physical plant parts were found in the flying fox  
369 droppings. Fourteen of the probable plant genera detected have also been recorded by botanists  
370 as being present on Tioman, including the top four genera detected most abundantly in the  
371 droppings (Latiff et al. 1999; Mohd. Norfaizal et al. 2014).

372 In order to be conservative, we have avoided assigning most OTUs in our study to species  
373 level. The only exception is OTU 3, which we identified as *Mangifera indica* based on 100%  
374 matches between the OTU, BOLD database sequence and botanical specimen sequence.  
375 Although plant identification based on DNA sequence to the species level may not be  
376 straightforward, the utilisation of partial *rbcL* gene fragments coupled with alternative taxonomic  
377 assignment based on phylogenetic tree clustering has already delivered numerous new insights  
378 into flying fox diet, and overcome severe limitations associated with traditional methods. It is  
379 also worth noting that identification to family level is highly accurate based on the partial  
380 sequence of *rbcL*, a protein coding gene associated with the chloroplast genome of all living  
381 plants. We have also demonstrated that most OTUs in our study could also be successfully  
382 assigned to genus level using this approach.

383 Other studies have successfully used different genetic approaches to identify plant species in  
384 animal diets. Valentini et al. (2009a) found *trnL* to be effective for Asian mammals, birds, and  
385 invertebrates, identifying 50% of the plant taxa found in the diets of these animals to species  
386 level. The same approach has been used for European bison (Kowalczyk et al. 2011), alpine  
387 chamois (Raye' et al. 2011), and red-headed wood pigeons (Ando et al. 2013). However, it has  
388 not been recommended to use a single DNA region for barcoding plants (Clare 2014). Indeed, a  
389 combination of target regions has been used to study the diets of large herbivores (e.g. *rbcL* with

390 *ITS-2* for African primates; Bradley et al. 2007, and *trnL* with ITS1 for lowland tapirs; Hibert et  
391 al. 2013).

392 In our study, only six OTUs had 100% matches to the sequences of botanical specimens  
393 collected from the study site, suggesting insufficient plant sampling. It is worth noting that  
394 subsequent similarity searches against the BOLD database did not recover reference sequences  
395 with 100% identity matches for all of the OTUs. This may be attributed to gaps in the database  
396 i.e. certain plant species consumed by the flying foxes may not yet have their corresponding  
397 sequences deposited in the database. This highlights the importance of building a comprehensive  
398 local sequence library beforehand, preferably specific to one's particular study site. In addition,  
399 there is also an urgent need for the BOLD database to have more representation of plant  
400 sequences from Southeast Asia and, more specifically, from Peninsular Malaysia.

401 We acknowledge that NGS approaches to diet identification are semi-quantitative  
402 because chloroplast abundance is known to be variable in different plant species and different  
403 parts of the leaf. Ultimately, the ability of NGS to accurately identify food plants will always  
404 depend on sequence specificity of the primers. While the NGS approach has proven to be useful  
405 in elucidating the island flying fox's varied diet on Tioman Island, for animals with such a highly  
406 diversified phytophagous diet, primer specificity will always be a limiting factor and there is a  
407 chance that unknown plant species will not be detected due to primer mispriming. Also, identical  
408 chloroplast DNA sequences can be present in different but related species, making it impossible  
409 to distinguish closely related plant species from each other in the diet. This could be one possible  
410 factor as to why OTU 12 had 100% identity hits with several members of family Arecaceae,  
411 making it impossible to identify this OTU to genus level, and suggesting that this particular  
412 family requires further phylogenetic investigation.

413 Another limitation of the NGS approach for generalist diets is that it does not identify  
414 which part of the plant was consumed. For animals that are specialised frugivores and  
415 nectarivores, or large terrestrial herbivores that consume entire plants whole, this may not be an  
416 issue. Flying foxes, however, are generalists which consume fruits, flowers, nectar, and even  
417 leaves (Marshall 1985). It is this dietary plasticity which allows them to perform more than one  
418 ecological role in tropical landscapes. Therefore, identifying which plant parts are actually  
419 consumed is a crucial step towards identifying the ecosystem services that these bats provide.  
420 Because of this, NGS can only provide a first step towards identifying flying fox diet, and should  
421 not be viewed as a replacement for microhistological analysis. Nevertheless, this approach has  
422 shed new light on flying fox diet by discovering new plant species that were entirely missed out  
423 by the conventional approach. Ideally, studies using NGS should be combined with micro-  
424 histological analysis in order to fill in the gaps and broaden our understanding of pteropodid diet  
425 and foraging ecology. NGS can also be used in combination with comprehensive and long-term  
426 data on plant phenology, to observe which food resources are available at which time. Following  
427 on from this preliminary study, the identification of specific food plants via NGS can now help  
428 guide more in-depth plant sample collection and phenological observations.

429 NGS did not detect *Durio* equally in the same individual droppings as those identified via  
430 microscope. This is likely due to the low abundance of this plant taxon in the droppings affecting  
431 detection probability, especially since the NGS analysis used a more general primer that was not  
432 specific to *Durio*. This pollen detection probability is another caveat to be aware of; Scanlon and  
433 Petit (2013) have cautioned that faecal subsampling methods can potentially lead to inaccurate  
434 detection of pollen in dietary studies, regardless of which method is used. The sample collection  
435 method in the field, selecting only for droppings with unique colour and texture, may have also

436 introduced a bias that could result in underestimating the proportion of a plant taxa in the diet. In  
437 particular, sampling completeness for the months May, June and July were relatively low,  
438 showing that more roosts and/or days needed to be sampled in order to obtain a complete  
439 representation of diet for these months. Interestingly, this also suggests that diet diversity, and  
440 potentially food resource diversity, were relatively higher during these three months compared to  
441 the rest of the year. Clearly, our method of collecting only droppings of unique appearance was  
442 not sufficient to reflect the full diversity of the diet; future studies should aim to collect all  
443 droppings found underneath a roost.

444         It is important to note that even with the potential underestimation, figs consistently  
445 formed the highest amount of plant taxa detected in the droppings each month, at both sampling  
446 sites. This strongly suggests that figs compose the core diet of flying foxes on the island.  
447 Although our study specifies only one (unidentified) species of fig, this is a conservative  
448 estimate. Sequencing a longer fragment of the *rbcL* gene would give better resolution, indicating  
449 whether more than one fig species was consumed. It is thus highly likely that the island flying  
450 fox plays a key role in dispersing fig seeds throughout Tioman, making these bats important  
451 keystone species for the island (Cox et al. 1991; McConkey and Drake 2015); future studies on  
452 seed dispersal and germination are required to confirm this.

453         Given the potentially short gut passage times involved (Tedman and Hall 1985),  
454 droppings collected from day roosts in the morning may bias the analysis results towards food  
455 items that were consumed only at the end of the foraging period (Schmelitschek et al. 2009).  
456 Although Banack and Grant (2003) have observed flying foxes returning to food resources that  
457 were foraged upon earlier, before then returning to day roosts, this is still a potential caveat to  
458 bear in mind; food plants that were only consumed during the start or middle of the evening may

459 not have been detected by our methods. For example, primates are known to exhibit temporal  
460 patterning in diet choice, structuring their diet throughout their foraging period with different  
461 food items; it is believed that this is due to how different foods are processed, and give energy, at  
462 different rates, and therefore helps to ensure that the animals maintain high energy levels  
463 (Robinson 1984; Ganzhorn and Wright 1994; Chapman and Chapman 1991). Given the sheer  
464 size of Tioman, and the logistical challenges of observing flying foxes foraging throughout the  
465 entire evening, the only way to overcome this possible information gap is to conduct GPS  
466 tracking studies.

467

## 468 **CONCLUSION**

469 Our study is the first to use NGS to identify potential plant species in flying fox diet, paving the  
470 way for a new approach to studying pteropodid diets. Since our NGS analysis of flying fox diet  
471 was semi-quantitative, it is not yet possible to make any definite conclusions regarding food  
472 preference vs. food availability; ultimately it is unclear to what extent sampling bias and  
473 detection probability may have influenced the type and abundance of plant taxa detected in our  
474 study. Yet some of the interesting patterns we observed are worth investigating in greater detail,  
475 particularly in combination with microhistological analysis. The results will also help to guide us  
476 in conducting more accurate and expanded phenology monitoring, and further collection of  
477 botanical samples. Further and more rigorous sampling, especially at the level of the individual  
478 animal, is required to understand the dietary patterns of this particular flying fox population,  
479 expand on the information provided here and build on our understanding of how the island flying  
480 fox may act as a strong interactor within the ecosystem of Tioman Island.

481



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490

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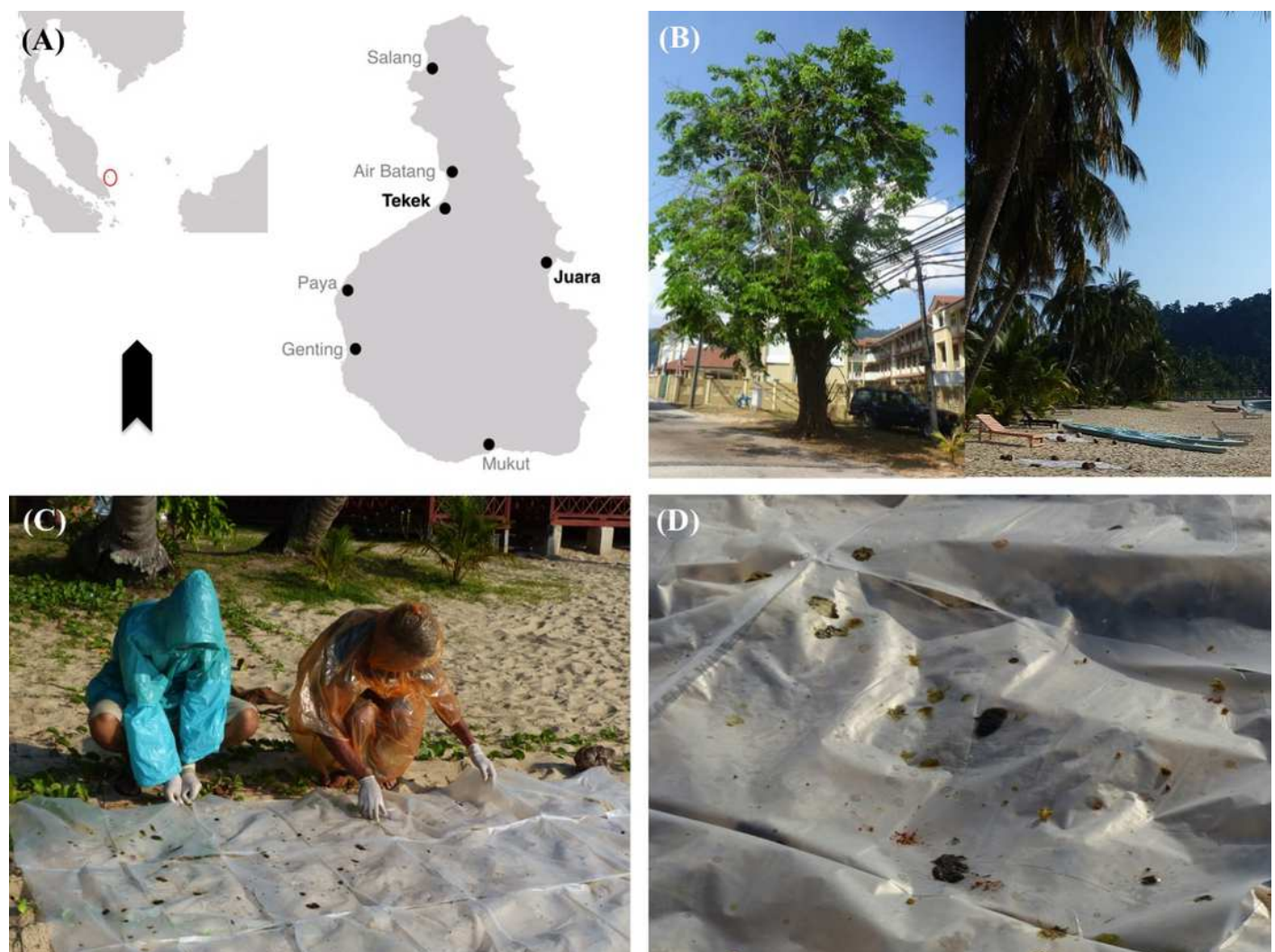
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# Figure 1

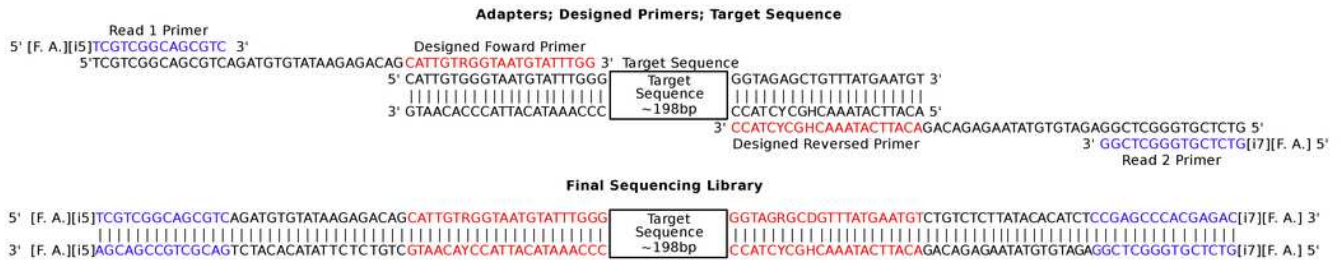
Map of study area and images of sampling site and method.

A) Map of Tioman showing sampling sites Tekek & Juara. B) Examples of flying fox roosts sampled in Tekek (left) & Juara (right). C) Collecting droppings from roosts. D) Close-up of droppings.



## Figure 2

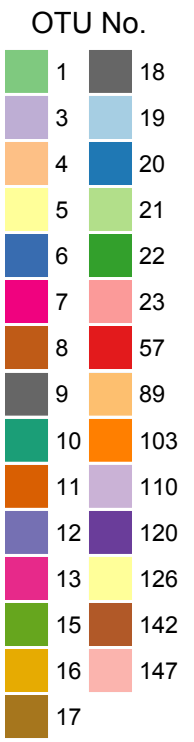
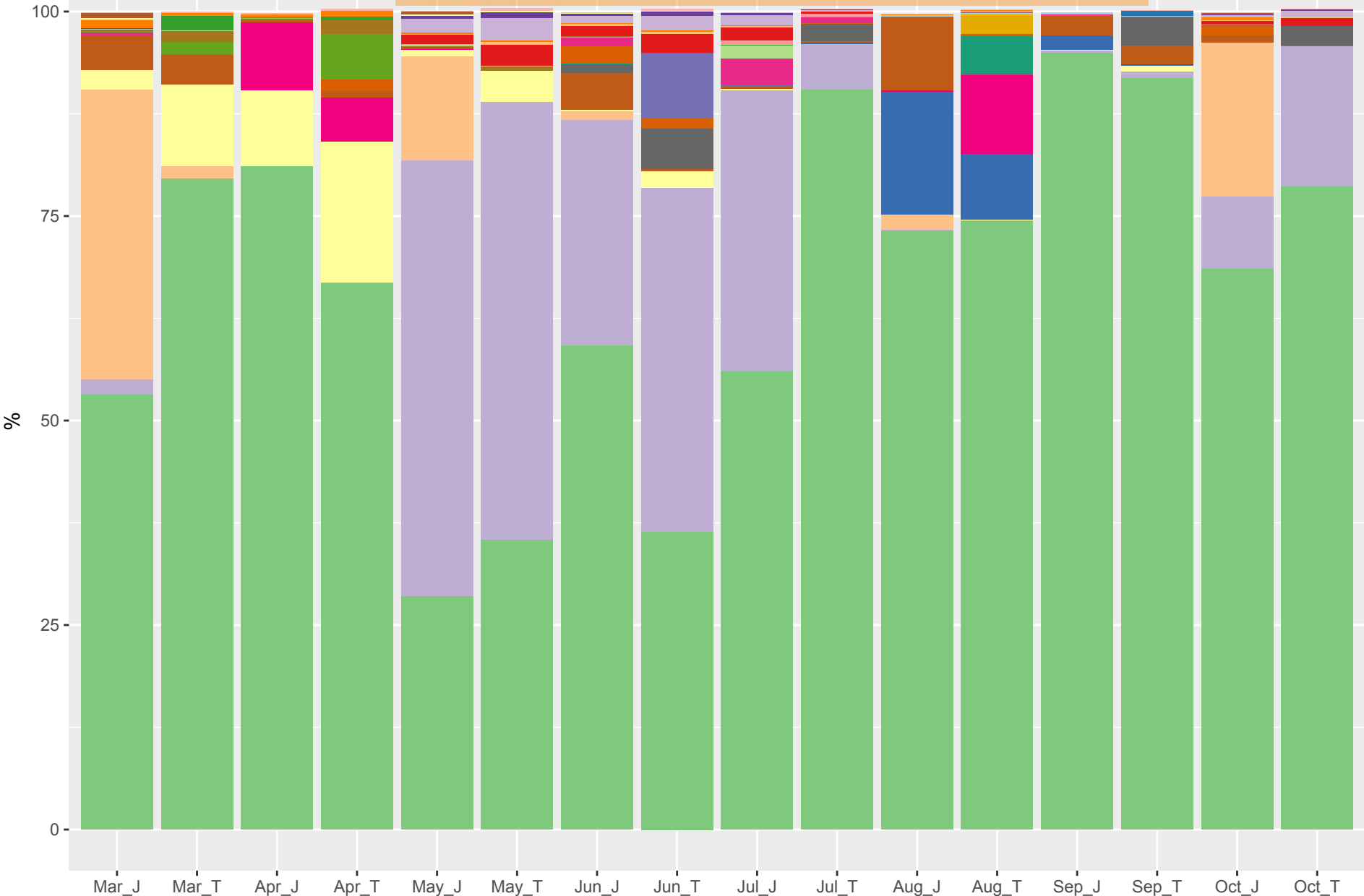
Overview of the newly designed primers and expected construct consisting of the complete Illumina adapter, dual index barcode and partial *rbcL* gene.



[F. A.] : Flowcell Annealing: AATGATACGGCGACCACCGAGATCTACAC; TAGAGCATAACGGCAGAAGACGAAC  
[15]: Index attached to Read 1 Primer  
[17]: Index attached to Read 2 Primer

**Figure 3**(on next page)

Proportion of OTU reads detected in flying fox droppings across 8 months (Mar-Oct 2015) at 2 different roosting sites on Tioman, Juara (J) and Tekek (T). Refer to Table 3 for OTU identification and corresponding number of reads.





**Figure 4**(on next page)

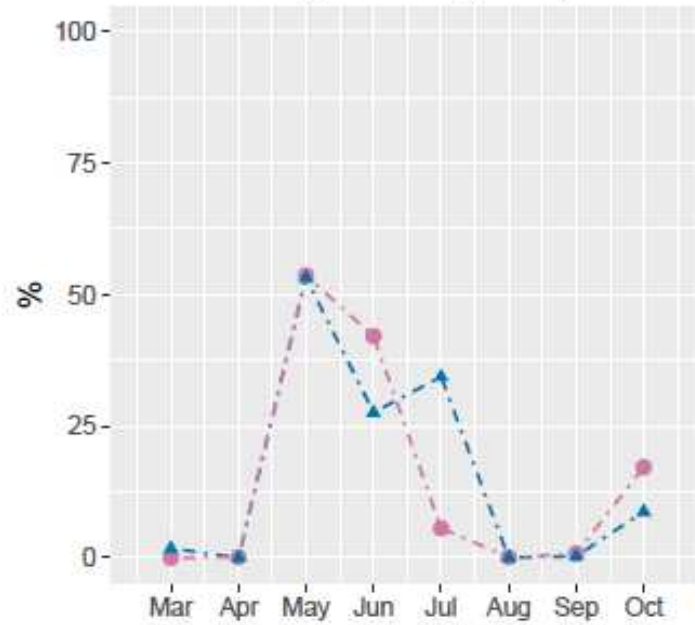
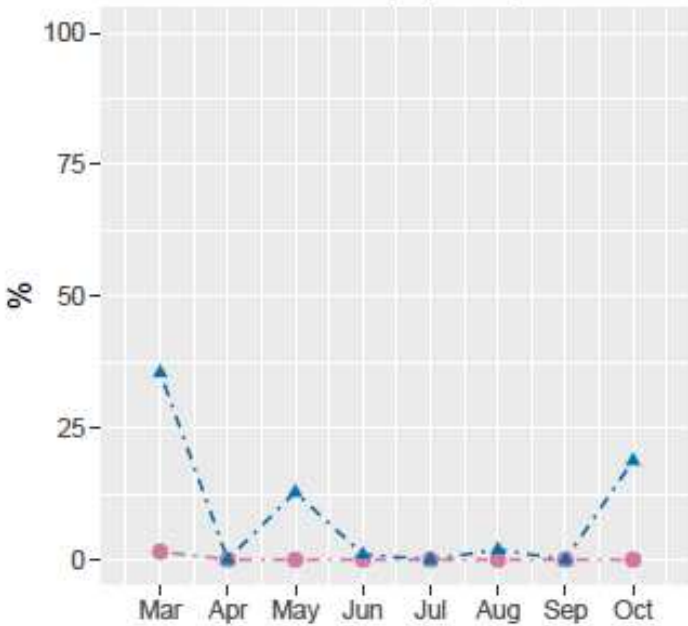
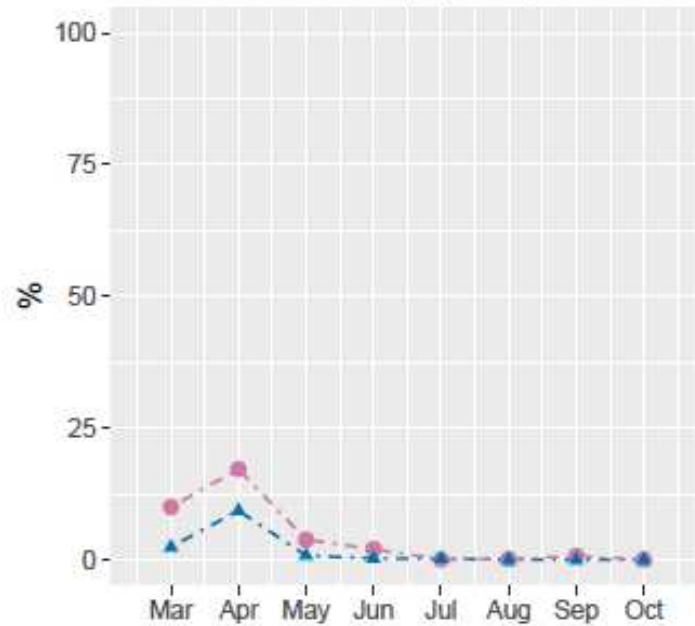
Maximum likelihood phylogenetic tree depicting the evolutionary relationship among identified OTUs from flying fox droppings, *rbcL* sequences obtained from individually collected leaf samples, and from public databases.

Values in nodes indicate ultrafast bootstrap support values and nodes with less than 50% support were collapsed. Scale bar indicates number of substitution per site.



**Figure 5** (on next page)

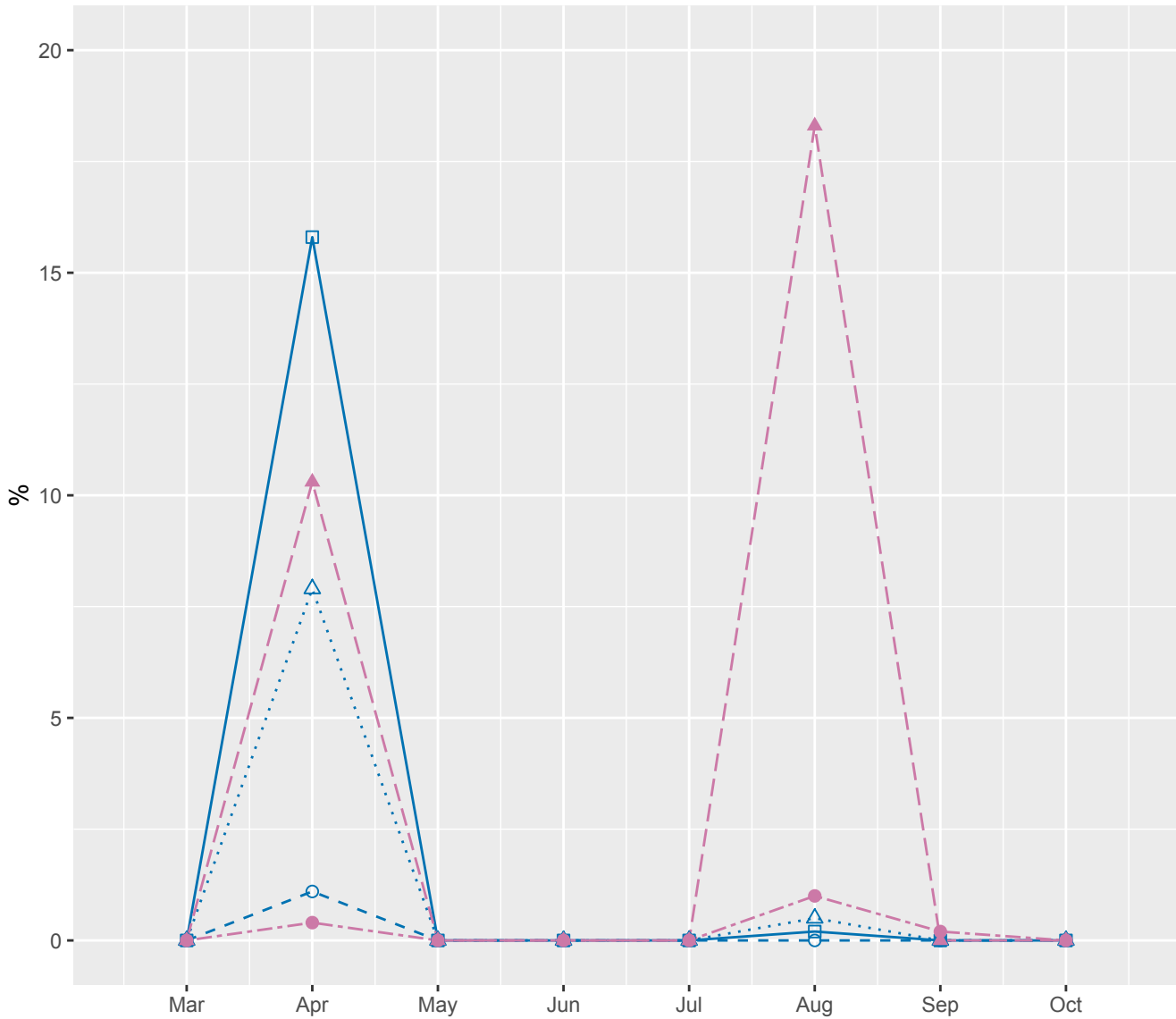
Spatio-temporal trends in consumption of the top four most dominant plant taxa detected in *Pteropus hypomelanus* droppings during March-October 2015 through NGS analysis.

*Ficus sp.* (OTU 1)*Pavetta sp.* (OTU 4)*Uncaria sp.* (OTU 5)

**Figure 6**(on next page)

Spatio-temporal trends in consumption of *Antiaris* sp. (OTU 7) showing differences between roosts during March-October 2016, suggesting possible inter-roost variation in diet.

Juara1 Juara2 Juara3 Tekek1 Tekek2



**Table 1** (on next page)

Summary information of 19 botanical specimens obtained from Tioman Island, Peninsular Malaysia.

No.	Botanical Specimen ID	GPS coordinates	Specimen code	GenBank Accession code	Closest taxon match from BOLD database
1	<i>Anacardium occidentale</i>	N2° 47.756' E104° 12.220'	PTMN12	KX618219	Anacardiaceae
2	<i>Arenga pinnata</i>	N2° 48.048' E104° 11.823'	PTMN18	KX618224	<i>Arenga</i> sp.
3	<i>Cocus nucifera</i>	N2° 47.652' E104° 12.176'	PTMN07	KX618214	Arecaceae
4	<i>Durio zibethinus</i>	N2° 47.462' E104° 12.047'	PTMN16	KX618222	<i>Durio</i> sp.
5	<i>Euphoria malaiense</i>	N2° 47.300' E104° 12.139'	PTMN13	KX618220	Sapindaceae
6	<i>Ficus</i> sp. 1	N2° 48.197' E104° 11.566'	PTMN11	KX618218	<i>Ficus</i> sp.
7	<i>Ficus</i> sp. 2	N2° 49.354' E104° 10.145'	PTMN22	KX618228	<i>Ficus</i> sp.
8	<i>Lansium parasiticum</i>	N2° 48.012' E104° 11.906'	PTMN14	KX618221	<i>Lansium</i> sp.
9	<i>Mangifera indica</i>	N2° 47.645' E104° 12.176'	PTMN20	KX618226	<i>Mangifera</i> sp.
10	<i>Mangifera odorata</i>	N2° 48.134' E104° 11.745'	PTMN01	KX148479	Anacardiaceae
11	<i>Nephelium lappaceum</i>	N2° 49.353' E104° 09.916'	PTMN23	KX618229	Sapindaceae
12	<i>Parkia speciosa</i>	N2° 48.595' E104° 10.758'	PTMN10	KX618217	<i>Parkia</i> sp.
13	<i>Streblus asper</i>	N2° 26.214' E103° 50.857'	PTMN02	KX618211	<i>Streblus</i> sp.
14	<i>Strombosia</i> sp.	N2° 48.737' E104° 10.537'	PTMN06	KX618213	<i>Strombosia</i> sp.



15	<i>Syzygium malaccense</i>	N2° 47.406' E104° 12.096'	PTMN08	KX618215	<i>Syzygium</i> sp.
16	<i>Syzygium</i> sp. 1	N2° 47.740' E104° 12.218'	PTMN09	KX618216	<i>Syzygium</i> sp.
17	<i>Syzygium</i> sp. 2	N2° 47.965' E104° 11.983'	PTMN17	KX618223	<i>Syzygium</i> sp.
18	<i>Terminalia catappa</i>	N2° 47.615' E104° 12.190'	PTMN19	KX618225	<i>Terminalia</i> sp.
19	<i>Vitex pinnata</i>	N2° 47.745' E104° 12.225'	PTMN05	KX618212	<i>Vitex</i> sp.

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**Table 2** (on next page)

Primers used in this study for the amplification of rbcL from flying fox droppings. Bold, target sequence; underlined, Illumina partial adapter

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<b>Primer Name</b>	<b>Sequence</b>
rbcLaf-M13	<u>TGTAAAACGACGGCCAGTATGTCACCACAAACAGAGACTAAAGC</u>
rbcLa-revM13	<u>CAGGAAACAGCTATGACGTAAAATCAAGTCCACCRCG</u>
rbcL-357F	<b>CATTGTRGGTAATGTATTTGG</b>
rbcL-556R	<b>ACATTCATAAACHGCYCTACC</b>
IlluM-rbcLF	<u>TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCATTGTRGGTAATGTATTTGG</u>
IlluM-rbcLR	<u>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACATTCATAAACHGCYCTACC</u>

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**Table 3** (on next page)

Identities of 29 OTUs detected in flying fox droppings based on matches with reference database and botanical specimen DNA sequences across 8 months (Mar-Oct 2015) in Tioman Island, Malaysia. Number of sequencing reads, percentage identity hits and specim

OTU no.		Closest taxon match from BOLD	Closest taxon match from NCBI		Closest botanical
(no. of reads)	Plant Family	(% identity hit)	(% identity hit)	Probable genus*	specimen match (code)
OTU 1 (2652)	Moraceae	<i>Ficus variegata</i> (100%)	<i>Ficus elastica</i> (100%)	<i>Ficus</i>	<i>Ficus</i> sp.2 (PTMN22)
OTU 3 (617)	Anacardiaceae	<i>Mangifera indica</i> (100%)	<i>Mangifera indica</i> (100%)	<i>Mangifera</i>	<i>Mangifera indica</i> (PTMN20)
OTU 4 (213)	Rubiaceae	<i>Coptosperma</i> sp. (100%)	<i>Pavetta indica</i> (100%)	<i>Pavetta</i>	
OTU 5 (106)	Rubiaceae	<i>Uncaria macrophylla</i> (100%)	<i>Uncaria attenuata</i> (100%)	<i>Uncaria</i>	
OTU 6 (67)	Ebenaceae	<i>Diospyros fasciculosa</i> (100%)	<i>Diospyros pilosanthera</i> (100%)	<i>Diospyros</i>	
OTU 7 (56)	Moraceae	<i>Antiaris toxicaria</i> (100%)	<i>Antiaris toxicaria</i> (100%)	<i>Antiaris</i>	
OTU 8 (80)	Bignoniaceae	<i>Anemopaegma album</i> (98.88%)	<i>Stereospermum annamense</i> (100%)	<i>Stereospermum</i>	
OTU 9 (24)	Muntingiaceae	<i>Muntingia calabura</i> (100%)	<i>Muntingia calabura</i> (100%)	<i>Muntingia</i>	
OTU 10 (9)	Moraceae	<i>Artocarpus heterophyllus</i> (100%)	<i>Artocarpus lakoocha</i> (100%)	<i>Artocarpus</i>	
OTU 11 (17)	Myrtaceae	<i>Syzygium malaccense</i> (100%)	<i>Backhousia</i> sp. (100%)	<i>Syzygium</i>	
OTU 12 (17)	Arecaceae	<i>Howea belmoreana</i> (100%)	<i>Roystonea oleracea</i> (100%)	-	<i>Cocos nucifera</i> (PTMN07)
OTU 13 (16)	Combretaceae	<i>Terminalia microcarpa</i> (100%)	<i>Terminalia catappa</i> (100%)	<i>Terminalia</i>	
OTU 15 (15)	Rhizophoraceae	<i>Carallia brachiata</i> (100%)	<i>Carallia brachiata</i> (100%)	<i>Carallia</i>	
OTU 16 (5)	Rutaceae	<i>Melicope elleryana</i> (100%)	<i>Pitaviaster haplophyllus</i> (100%)	-	
OTU 17 (9)	Malvaceae	<i>Durio zibethinus</i> (100%)	<i>Durio zibethinus</i> (100%)	<i>Durio</i>	<i>Durio zibethinus</i> (PTMN16)
OTU 18 (5)	Polygalaceae	<i>Xanthophyllum papuanum</i> (98.88%)	<i>Xanthophyllum yunnanense</i> (99%)	<i>Xanthophyllum</i>	
OTU 19 (3)	Olacaceae	<i>Maburea trinervis</i> (99.44%)	<i>Strombosia javanica</i> (100%)	<i>Strombosia</i>	<i>Strombosia</i> sp. (PTMN06)
OTU 20 (2)	Lamiaceae	<i>Vitex cofassus</i> (100%)	<i>Vitex peduncularis</i> (100%)	<i>Vitex</i>	<i>Vitex pinnata</i> (PTMN05)

OTU 21 (5)	Salicaceae	<i>Flacourtia indica</i> (100%)	<i>Flacourtia indica</i> (100%)	<i>Flacourtia</i>
OTU 22 (4)	Chrysobalanaceae	<i>Maranthes glabra</i> (100%)	<i>Maranthes kerstingii</i> (100%)	<i>Maranthes</i>
OTU 23 (3)	Annonaceae	<i>Pseuduvaria froggattii</i> (100%)	<i>Pseuduvaria indochinensis</i> (100%)	<i>Pseuduvaria</i>
OTU 57 (26)	Anacardiaceae	<i>Faguetia falcata</i> (97.19%)	<i>Mangifera odorata</i> (98%)	<i>Mangifera</i>
OTU 89 (3)	Anacardiaceae	<i>Mangifera indica</i> (97.04%)	<i>Mangifera odorata</i> (98%)	<i>Mangifera</i>
OTU 103 (10)	Moraceae	<i>Ficus variegata</i> (96.63%)	<i>Ficus elastica</i> (97%)	<i>Ficus</i>
OTU 110 (23)	Moraceae	<i>Drypetes roxburghii</i> (95.51%)	<i>Drypetes roxburghii</i> (96%)	-
OTU 120 (7)	Moraceae	<i>Ficus variegata</i> (96.43%)	<i>Ficus religiosa</i> (96%)	<i>Ficus</i>
OTU 126 (2)	Anacardiaceae	<i>Schinus molle</i> (97.19%)	<i>Mangifera indica</i> (97%)	<i>Mangifera</i>
OTU 142 (4)	Rubiaceae	<i>Coptosperma nigrescens</i> (97.62%)	<i>Coptosperma rhodesiacum</i> (98%)	-
OTU 147 (2)	Rubiaceae	<i>Uncaria tomentosa</i> (96.61%)	<i>Uncaria tomentosa</i> (97%)	<i>Uncaria</i>

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NB: OTU numbers are not in running sequence because OTUs that were possible chimeras were removed from filtering process

\*Based on known geographical occurrences in Malaysia with reference to plant distribution information from Flora Malesiana (<http://floramalesiana.org/>) and Wikipedia

**Table 4**(on next page)

Comparison of microscope vs. NGS analyses in detecting food plants present in 10 individual flying fox droppings collected on 6 May 2015.

\*Not detected in the 8-month analysis, therefore no corresponding OTU number.

Identified food plant	Microscope										NGS										OTU
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	no.
<i>Durio</i> sp.	x						x		x						x			x	x		17
<i>Ficus</i> sp.		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1
<i>Mangifera</i> sp.											x	x	x	x	x	x	x	x	x	x	3
<i>Strombosia</i> sp.												x	x	x					x		19
<i>Terminalia</i> sp.											x	x			x	x	x				13
Arecaceae											x	x	x	x	x	x	x	x	x	x	12
<i>Uncaria</i> sp.											x					x					5
Sapindaceae											x		x		x	x			x	x	*

1