

1 **Title:**

2 Impact of Urbanisation and Agriculture on the Diet of Fruit Bats

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14

15 **ABSTRACT**

16 The expansion of cities and agricultural plantations have unpredictable impacts on biodiversity
17 and ecosystem services. Yet some species are capable of tolerating anthropogenic impacts and
18 continue to provide ecological services in highly disturbed landscapes. The objective of this study
19 was to use DNA barcoding to identify digested plant materials and seeds in the faeces of
20 frugivorous bats (*Cynopterus brachyotis*) and investigate whether (1) *C. brachyotis* in urban and
21 agricultural areas exploit cultivated and exotic plants as a novel food resource and as a
22 consequence, potentially facilitate the invasion of cultivated and exotic plants, or whether (2) *C.*
23 *brachyotis* exploit native plants and as a consequence, potentially promote forest regeneration. A
24 native species, *Ficus fistulosa*, was the most frequently detected plant and the seeds were found
25 in bat faeces from all sampling sites suggesting the potential of fruit bats in dispersing seeds.
26 However, we also detected several exotic plants in the faeces of *C. brachyotis* which suggests
27 that the fruit bats exploit novel food resources at all sites. We recorded a diverse diet of *C.*
28 *brachyotis* at an oil palm plantation which indicated that the fruit bats are not predominantly
29 feeding on oil palm fruits. By using DNA barcoding, we detected plants that have not been
30 reported in previous studies of the diet of *C. brachyotis*, although we could not identify which
31 part of the plant was being consumed by the fruit bats. Given the varied diet of *C. brachyotis*, the
32 potential of this bat to adapt to changing landscapes is high and they are likely dispersing seeds
33 of native pioneer plants (*Ficus*).

34 **KEYWORDS**

35 DNA barcoding, land use, oil palm plantation, seed dispersal, species' interaction, urbanisation

36 **INTRODUCTION**

37 Between 2000 and 2010, the area of urban land expanded by more than 22% in East-Southeast
38 Asia (Schneider et al. 2015). In Peninsular Malaysia, urban land is expanding 1.5% annually
39 (Schneider et al. 2015), and the land area used for oil palm plantation is expanding 7% annually
40 (Butler 2013). Such changes in land use are often associated with alterations to biogeochemical
41 cycles, climate and biodiversity (Grim et al. 2008; Fitzherbert et al. 2008), for example, the
42 introduction of exotic species in human-dominated areas (Grim et al. 2008; Fitzherbert et al. 2008)
43 which may compete with and extirpate native species (Faeth et al. 2005; McConkey et al. 2012).
44 However, despite losses of biodiversity, important ecological processes still take place in urban
45 and agricultural habitats. For example, botanical and residential gardens in urban areas provide
46 diverse food resources and nesting areas to bees (Sing et al. 2016) which pollinate garden plants,
47 while birds and bats continue to survive in urban areas and can provide critical seed dispersal
48 services for native plants, particularly for pioneer species such as *Ficus* (Tan et al. 2000; Corlett
49 2005). Understanding how ecosystem services in human modified environments are maintained,
50 albeit often involving exotic species and novel interactions (Corlett 2005), is a serious and
51 growing challenge. As a first step it is important to understand how a population uses resources
52 in natural versus human modified environments.

53 The Lesser Dog-faced Fruit Bat (a species complex often reported as *Cynopterus brachyotis*;
54 Campbell et al. 2004; Wilson et al. 2014) is the most common bat in Peninsular Malaysia and is
55 abundant in primary and secondary forests, agricultural land, and urban areas (Campbell et al.
56 2004; Jayaraj et al. 2012). Because of its ubiquitous presence, *C. brachyotis* is an excellent model
57 of ecological flexibility with a potentially important role in seed dispersal. *C. brachyotis* has been

58 reported feeding on sixteen plant species in primary forest (Hodgkison et al. 2004), 66 plant
59 species in secondary forests (Tan et al. 1998) and 38 species in urban areas (Tan et al. 2000).
60 While *C. brachyotis* in urban areas demonstrated distinct food preferences during fruiting seasons
61 (Tan et al. 2000), *C. brachyotis* in primary forest exploited both “steady state” and “big bang”
62 plants and has not shown variation in diet over time (Hodgkison et al. 2004). The apparent
63 flexibility of *C. brachyotis* in diet suggests a significant capacity to adapt to changing
64 environments. The flexible use of modified habitats may also bring fruit bats into conflict with
65 farmers in agricultural areas where bats may be perceived as foraging for food in cultivated
66 commercial crops and consequently targeted as crop pests (Fujita and Tuttle 1991).

67 One limitation with previous research into fruit bat foraging preferences has been the inability to
68 identify fruit pulp and fragmented material in their faeces. Taxonomic assessment of fruit bats’
69 food resources has been restricted to observations during behavioural studies of bats which are
70 difficult in low light conditions (Phua and Corlett 1989), or morphology-based species
71 identification of seeds in faeces or plant remnants in masticated pellets (Tan et al. 1998;
72 Hodgkison et al. 2004). One potential solution to this impediment is the use of molecular
73 methods such as DNA barcoding (Hebert et al. 2003; Wilson et al. 2016) which matches short
74 DNA sequences of standardised regions (e.g. *rbcL* and *ITS2* for plants; CBOL 2009; Chen et al.
75 2010) to taxonomically verified DNA sequences (Kuzmina et al. 2012). DNA barcoding has been
76 used to identify even the most degraded and digested material in the faeces of insectivorous
77 (Clare et al. 2009) and frugivorous bats (Hayward 2013; Aziz et al. 2017).

78 The objective of this study was to use DNA barcoding to identify the digested plant materials and
79 seeds in the faeces of frugivorous bats (*C. brachyotis*) and investigate whether (1) *C. brachyotis*

80 in urban and agricultural areas adapt to the changing landscapes to exploit cultivated and exotic
81 plants as a novel food resource and are thus are potential vectors of their dispersal or (2) whether
82 *C. brachyotis* exploit native plants and as a consequence potentially promote forest regeneration.

83

84 **METHODS**

85 **Ethics**

86 Faecal collection and bat sampling were conducted with authorization from Department of
87 Wildlife and National Parks, Peninsular Malaysia (JPHLandTN(IP)100-34/1.24 Jld. 4(34)) and
88 Institutional Animal Care and Use Committee, University of Malaya (ISB/10/06/2016/LVC (R)).

89 **Study sites and faecal sampling**

90 We conducted faecal sampling at three sites with either urban, agricultural or secondary forest
91 land use (Fig. 1). The urban site was an abandoned residential area located between University of
92 Malaya and MAHSA University in Kuala Lumpur city in close proximity to a busy hospital and
93 occupied residences. The agricultural site was located within a 2940 ha oil palm plantation
94 (*Elaies guineensis* x *Elaies oleifera*) at Bemban, Melaka. The secondary forest site was located at
95 the University of Malaya Field Studies Centre which is situated within 120 hectares of a
96 secondary forest selectively logged from 1956 to 1958 (Medway 1966; Sing et al. 2013).

97 We collected fresh faeces from individual bats (*C. brachyotis* sensu stricto identified following
98 Jayaraj et al. 2012) captured using mist nets at the urban site for eleven days between 10 June to
99 18 December 2015 and at the agricultural site for four days from 12 January to 15 January 2016.
100 Most of the bats defecated immediately when captured, but those that did not were kept in

101 individual cloth bags for one hour to produce faeces and were then released. The faeces collected
102 from one individual was considered as a single independent sample.

103 We located a roosting colony of *C. brachyotis* (identified by capturing and measuring four
104 individuals from the colony following Jayaraj et al. 2012) at the secondary forest site. The floor
105 below the roost was cleaned daily and fresh faeces from the colony were collected from the floor
106 non-invasively between 10 July and 25 September 2015. We treated each faecal sample (i.e.
107 collected into an individual Eppendorf tube) as an independent sample.

108 The faeces were kept in 1.5 ml Eppendorf tubes filled with 99.8% ethanol and stored at -20°C
109 prior to analysis. Ethanol is not normally used to preserve plant material, but is recommended to
110 prevent fungal and bacterial growth in bat faeces. The ethanol was evaporated from samples prior
111 to extraction. A total of 95 faecal samples were selected for plant DNA barcoding incorporating
112 approximately equal number of samples from each site: 32 samples from the urban site, 32
113 samples from the agricultural site and 31 samples from the secondary forest site.

114 **DNA extraction, amplification and sequencing**

115 We prioritised seeds over pulps to ensure the amplification of DNA and isolated the seeds from
116 the faecal samples. In cases where seeds were not found in the faecal samples, we used the pulps.
117 The seeds and pulps were sent to the Canadian Centre for DNA barcoding (CCDB) for DNA
118 extraction, PCR amplification, and Sanger sequencing of two gene regions (*rbcL*: ~550 bp and
119 *ITS2*: ~350 bp), following the standard plant protocols of the CCDB (Ivanova and Grainger 2008;
120 Ivanova et al. 2011; Kuzmina and Ivanova 2011a, 2011b).

121 **Plant species identification**

122 We searched GenBank (NCBI 2016) with both the *rbcL* and *ITS2* barcodes to assign taxonomic
123 names to the faecal samples. We prioritised the results of *ITS2* searches over *rbcL* due to the
124 greater taxonomic resolution of this gene fragment (Chen et al. 2010; Kuzmina et al. 2012). We
125 assigned species names based on *ITS2* and *rbcL* matches using a customised set of criteria (Fig.
126 2). Details of the assignment criterion used for specific samples are given in Online Resource 1.
127 We uploaded the DNA barcodes together with sample metadata to the Barcode of Life Data
128 Systems (BOLD; Ratnasingham and Hebert 2007) under project code VCCBD and the sequences
129 are also available in GenBank under accessions KY080541 to KY080613 and KY080617 to
130 KY080686.

131 An interaction figure between the bats and detected plants was created to compare the food
132 resource use of *C. brachyotis* at three sampling sites with different land use. The figure was
133 created using the bipartite package (Dormann et al. 2008) in R version 3.3.1. (R Core Team
134 2016).

135

136 **RESULTS**

137 Of the 95 faecal samples we analysed, 65 samples (68.4%; seeds=43; pulps=22) produced both
138 *rbcL* and *ITS2* DNA barcodes, 7 samples (7.4%; seeds=5; pulps=2) produced only *ITS2* barcodes,
139 8 samples (8.4%; seeds=1; pulps=7) produced only *rbcL* barcodes and the remaining 15 samples
140 (15.8%; seeds=2; pulps=13) failed to produce any DNA barcodes (See supplementary file). We
141 discarded two *ITS2* barcodes: one from the urban site due to the short length of usable sequence

142 (57 bp) and one from secondary forest which was suspected to be a contaminant due to its
143 similarity (96%) to algal sequences (*Chlorella angustoellipsoidea* and *Chloroidium ellipsoideum*).
144 We identified seventeen plant species in the faecal samples using DNA barcoding (Fig. 3; Table
145 1) of which eight plant species were detected from 26 samples at the urban site, six plant species
146 from 25 samples collected at the agricultural site and seven plant species from 28 samples
147 collected at the secondary forest site. Of the seventeen plant species, we identified nine as native
148 plants and four as exotic plants (Table 1). The status of the remaining four species are unknown
149 as we could not assign them with specific epithets (Table 1). We detected *Ficus fistulosa* at all
150 sampling sites with the highest detection frequency at agricultural and urban sites , and two plant
151 species at two sampling sites with lower detection frequency: *F. lepicarpa* at urban and
152 secondary forest sites, and *Durio zibethinus* at agricultural and secondary forest sites (Fig. 3).

153

154 **DISCUSSION**

155 Our study suggests that *C. brachyotis* feeds predominantly on pioneer and forest plants. The
156 pioneer plant genus *Ficus* which often dominates regenerating forest (Muscarella and Fleming
157 2007) emerged as the dominant component of the diet of *C. brachyotis* at all sampling sites with
158 *F. fistulosa* being the most frequently detected plant. Many *Ficus* species including *F. fistulosa*, *F.*
159 *lepicarpa* and *F. hispida* have multiple fruiting periods throughout the year (Phillipps and
160 Phillipps 2016), making *Ficus* a stable resource compared to more transient species (e.g.
161 *Syzygium jambos* and *Manilkara zapota*) (Corlett 1998; Tan et al. 1998; Fukuda et al. 2009),
162 consequently promoting stable population dynamics in consumers (Tan et al. 2000).

163 Native forest plants and cultivated plants were detected in faecal samples collected from urban
164 and agriculture sites although we did not observe all the plants at these locations. Seeds
165 belonging to *Ficus* were found in faecal samples collected from all sites and during our sampling
166 at the urban site, we captured an individual with a *Ficus* fruit in its mouth. This suggests that the
167 fruit bats are moving and depositing seeds away from parent plants, implying the role of fruit bats
168 in seed dispersal. In Thailand, *C. brachyotis* have been reported to travel up to 14.5 km per day
169 (Bumrungsri 2002) and by transporting seeds across habitats, *C. brachyotis* could promote plant
170 diversity, particularly in disturbed habitats (i.e, urban and agricultural areas) which often lack
171 seed resources and succession (Hodgkison et al. 2003; McConkey et al. 2012).

172 Exotic plants were detected in the pulps from the faecal samples at all sampling sites. The fairly
173 high detection rate of these exotic plants, particularly *P. aduncum* and *L. chinensis* shows that *C.*
174 *brachyotis* can exploit novel food resources and potentially could aid invasion of exotic plants
175 through dispersal activities (Muscarella and Fleming 2007). Although we did not visually
176 observe the seeds of exotic plants in the faecal samples nor visually assess the feeding behaviour
177 of *C. brachyotis* (i.e. carrying fruits away from parent trees to feeding perches), it would be a
178 compelling next step to determine the relative role of the fruit bats in facilitating the succession
179 of native species and/or promoting exotic plant invasions.

180 Our low detection of oil palm (*Elaies guineensis* x *Elaies oleifera*) in faecal samples collected at
181 agricultural site suggests that the fruit bats are not predominantly feeding on oil palm fruits and
182 their presence in oil palm plantations could be explained by other factors. The diverse diet of *C.*
183 *brachyotis* at the oil palm plantation (a monoculture) suggested that the bats may have used the
184 plantation as connecting flyway to travel to forest fragments and agricultural plantations nearby

185 which provide more diverse food resources. This is similar to the findings of Heer et al. (2015)
186 which detected a high number of frugivorous bats in rubber-cacao plantations that offered little
187 food resources to the bats, but obviously served as corridors. However, it is also possible we are
188 not detecting oil palm if it is ingested just before they depart from this area though the low
189 detection everywhere suggests this possibility is remote. Our detection of other cultivated plants
190 in faecal samples indicates *C. brachyotis* feed on other readily available food crops which
191 consequently may lead to conflict between fruit bats and fruit growers. Although the extent of the
192 damage to the food crops caused by *C. brachyotis* is significantly smaller than that of other larger
193 mammals (i.e. *Macaca nemestrina*, *Arctictis binturong*, *Cervus timorensis*, and *Sus barbatus*),
194 fruit bats are often killed in large numbers as they are generally of lower concern to the wildlife
195 authorities (Fujita and Tuttle 1991; Aziz et al. 2016).

196 We detected plants (i.e. *Ficus fistulosa*, *Szygium jambos*, and *Pellacalyx saccardianus*) which
197 have previously been reported in diet studies of *C. brachyotis* conducted at secondary forest and
198 urban areas (Phua and Corlett, 1989; Tan et al. 1998). However, we also failed to detect many
199 plants which were reported to be seasonally dominant in the diet of the fruit bats, most likely due
200 to our short sampling period. Nevertheless, our use of DNA barcoding detected cultivated plants
201 (i.e. *Parkia roxburghii*, *Elaies guineensis* x *Elaies oleifera*, and *Coccinia grandis*) and pioneer
202 plants (i.e. *Ficus hispida* and *F. lepicarpa*) which have not been reported in other studies of the
203 diet of *C. brachyotis* (Phua and Corlett, 1989; Tan et al. 1998; Hodgkison et al. 2004).

204 The advantage of using DNA barcoding to identify the diet of *C. brachyotis* is that we were able
205 to assign species names to most of the seeds and digested plant pulp in the faeces. With DNA
206 barcoding, most of the seeds were assigned with the species name *Ficus fistulosa* which also has

207 been reported by Phua and Corlett (1989) and Tan et al. (1998) as the most common *Ficus* eaten
208 by *C. brachyotis* at secondary forest and urban areas. Seeds belonging to *Ficus* can be easily
209 assigned to this plant genus based on the morphology of the seeds. However, assigning *Ficus*
210 seeds accurately to a species based on the morphology of the seeds is often time-consuming and
211 requires high level of plant taxonomic expertise. Phua and Corlett (1989) failed to assign species
212 name to six types of *Ficus* remains due to the difficulty in identifying the remnants of the seeds
213 and fruits while Hodgkison et al. (2004) germinated the seeds collected from faeces of bats for
214 species identification based on the morphology of the seedlings.

215 However, our reliance on existing databases and local floral records leaves these names as
216 provisional. We assigned most of the *ITS2* sequences with species names as the region is able to
217 distinguish closely related species within same genus when comprehensive reference libraries are
218 available (Braukmann et al. 2017). However, the region produces some ambiguous results in
219 rapidly radiating groups (e.g. *Ficus*) and in our case, local botanical records were used to refine
220 these cases. We observed that the *ITS2* region detected fewer plant families compared to *rbcL*. In
221 contrast, most of the *rbcL* sequences matched to sequences in GenBank recorded under multiple
222 species names with 100% similarity. High-throughput sequencing (HTS) could be utilized for
223 future diet studies of fruit bats, which may help distinguish mixed signals in individuals
224 consuming multiple species, although the smaller read length of HTS platforms may compromise
225 some plant identifications.

226 We detected plants (i.e. *Elaies guineensis* x *Elaies oleifera* and *Coccinia grandis*) with seeds that
227 are too large to be ingested by *C. brachyotis* and which consequently are not observed
228 morphologically in the faeces. Although the fruit bats may not be able to disperse large seeds

229 through defecation, *C. brachyotis* may still serve as important seed disperser by carrying the
230 heavy fruits with large seeds to feeding perches away from parent trees (Funakoshi and Zubaid
231 1997). Therefore, our findings highlight the importance of using DNA barcoding in dietary
232 studies of fruit bats, as the reliance on morphological identification of seeds in the faeces may
233 overlook plants with large seeds where only pulp is present and consequently overlook the
234 potential seed dispersal role of the fruit bats.

235 We preferentially selected seeds rather than fruit pulp for sequencing. If a bat had consumed a
236 large fruit (and dropped the large seed) along with a small fruit (and swallowed the small seeds),
237 it may potentially cause a bias in the detection of small seeded plants. However, the gut passage
238 time of most fruit bats is fast enough that we do not frequently see multiple fruit types in a
239 sample (E Clare personal observation) and thus the effect of the bias is likely minimal.

240 One limitation of using DNA barcoding to identify the species origin of plant pulp is that we
241 cannot determine which part of the plant the fruit bats are feeding on. For example, the most
242 important pollinator of economically important *Durio zibethinus* is *Eonycteris spelaea* which
243 feeds on nectar (Bumrungsri et al. 2009), whereas *C. bracyotis* is reported to feed on the flowers
244 (Funakoshi and Zubaid 1997). Although we detected *D. zibethinus* in the diet of *C. brachyotis*,
245 we could not determine whether the fruit bats feed on nectar and consequently pollinate the
246 economically important crops, or are consuming the fruits and/or flowers which would inhibit the
247 development of the crops.

248 **CONCLUSION**

249 The diet of *C. brachyotis* at secondary forest, urban and agricultural sites was compared using
250 DNA barcoding (i.e. Sanger sequencing). The high detection of *Ficus* seeds in the faeces of *C.*
251 *brachyotis* indicates that the bats rely heavily on this native food source in all habitats but the
252 fairly high detection of exotic and cultivated plants in the faeces suggests that *C. brachyotis* is
253 flexible and can exploit exotic and cultivated plants as novel food resource. The diverse diet of *C.*
254 *brachyotis* at the oil palm plantation indicated that the fruit bats are not predominantly feeding on
255 oil palm fruits but cultivated plants nearby the plantation. Together these observations suggest an
256 interesting dual role of *C. brachyotis* in dispersing (i) native pioneer plants which aid in forest
257 regeneration and (ii) non-native plants which potentially facilitate their invasion, consequently
258 suggesting a research avenue that deserves further investigation. The use of DNA barcoding in
259 this study enabled the detection of plant species that had not been reported in previous diet
260 studies of *C. brachyotis* but does not provide information regarding which part of the plant was
261 consumed by the bats. Nevertheless, this study demonstrated the utility of DNA barcoding in
262 dietary studies of frugivorous bats and the extent to which *C. brachyotis* is capable of adapting to
263 changing landscapes and plant resources.

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281 **CONFLICT OF INTEREST**

282 The authors declare that they have no conflict of interest.

283 **REFERENCES**

- 284 Aziz SA, Olival KJ, Bumrungsri S, Richards GC, Racey PA (2016) The conflict between
285 pteropodid bats and fruit growers: species, legislation and mitigation. In: Voigt CC, Kingston T
286 (eds) Bats in the Anthropocene: Conservation of Bats in a Changing World. Springer
287 International Publishing, New York, pp 377-426
- 288 Aziz SA, Clements GR, Peng LY, Campos-Arceiz A, McConkey KR, Forget PM, Gan HM (2017)
289 Elucidating the diet of the island flying fox (*Pteropus hypomelanus*) in Peninsular Malaysia
290 through Illumina Next-Generation Sequencing. PeerJ, 5, e3176
- 291 Braukmann TW, Kuzmina ML, Sills J, Zakharov EV, Hebert PD (2017) Testing the Efficacy of
292 DNA Barcodes for Identifying the Vascular Plants of Canada. PloS one, 12(1), e0169515
- 293 Bumrungsri S (2002) The foraging ecology of the short-nosed fruit bat, *Cynopterus brachyotis*
294 (Muller, 1838), in lowland dry evergreen rain forest, southeast Thailand. Doctoral dissertation,
295 University of Aberdeen Bumrungsri S, Sripaoraya E, Chongsiri T, Sridith K, Racey PA (2009)
296 The pollination ecology of durian (*Durio zibethinus*, Bombacaceae) in southern Thailand. J Trop
297 Ecol 25:85-92
- 298 Butler R (2013) 3.5 million ha of Indonesian and Malaysian Forest Converted for Palm Oil in 20
299 Years. Mongabay, California. [https://news.mongabay.com/2013/11/3-5-million-ha-of-](https://news.mongabay.com/2013/11/3-5-million-ha-of-indonesian-and-malaysian-forest-converted-for-palm-oil-in-20-years/)
300 [indonesian-and-malaysian-forest-converted-for-palm-oil-in-20-years/](https://news.mongabay.com/2013/11/3-5-million-ha-of-indonesian-and-malaysian-forest-converted-for-palm-oil-in-20-years/) Accessed 13 August 2016
- 301 Campbell P, Schneider CJ, Adnan AM, Zubaid A, Kunz TH (2004) Phylogeny and
302 phylogeography of Old World fruit bats in the *Cynopterus brachyotis* complex. Mol Phylogenet
303 Evol 33:764-781

304 CBOL Plant Working Group (2009) A DNA barcode for land plants. Proc Natl Acad Sci U S A
305 106:12794-12797

306 Chen S, Yao H, Han J et al (2010) Validation of the ITS2 region as a novel DNA Barcode for
307 identifying medicinal plant species. PLoS ONE 5:e8613

308 Clare EL, Fraser EE, Braid HE, Fenton MB, Hebert PD (2009) Species on the menu of a
309 generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect
310 arthropod prey. Mol Ecol 18:2532-2542

311 Dormann CF, Gruber B, Fruend J (2008) Introducing the bipartite Package: Analysing Ecological
312 Networks. R news Vol 8/2, 8 - 11.

313 Faeth SH, Warren PS, Shochat E, Marussich WA (2005) Trophic dynamics in urban
314 communities. BioScience 55: 399-407

315 Fitzherbert EB, Struebig MJ, Morel A et al (2008) How will oil palm expansion affect
316 biodiversity? Trends Ecol Evol 23:538-545

317 Fujita MS, Tuttle MD (1991) Flying foxes (Chiroptera: Pteropodidae): threatened animals of key
318 ecological and economic importance. Conserv Biol 5:455-463

319 Fukuda D, Tisen OB, Momose K, Sakai S (2009) Bat diversity in the vegetation mosaic around a
320 lowland dipterocarp forest of Borneo. Raffles Bull Zool 57:213-221

321 Funakoshi K, Zubaid A (1997) Behavioural and reproductive ecology of the dog-faced fruit bats,
322 *Cynopterus brachyotis* and *C. horsfieldi*, in a Malaysian rainforest. Mammal Study 22:95-108

323 Hebert PD, Cywinska A, Ball SL (2003) Biological identifications through DNA barcodes. Proc
324 R Soc Lond B Biol Sci 270:313-321

325 Hayward CE (2013) DNA barcoding expands dietary identification and reveals dietary similarity
326 in Jamaican frugivorous bats. Doctoral dissertation, The University of Western Ontario

327 Heer K, Helbig-Bonitz M, Fernandes RG, Mello MA, Kalko EK (2015) Effects of land use on bat
328 diversity in a complex plantation–forest landscape in northeastern Brazil. Journal of Mammalogy
329 96(4): 720-731

330 Hodgkison R, Balding ST, Zubaid A, Kunz TH (2003) Fruit bats (Chiroptera: Pteropodidae) as
331 seed dispersers and pollinators in a lowland Malaysian rain forest. Biotropica 35:491-502

332 Hodgkison R, Balding ST, Zubaid A, Kunz TH (2004) Temporal variation in the relative
333 abundance of fruit bats (Megachiroptera: Pteropodidae) in relation to the availability of food in a
334 lowland Malaysian rain forest. Biotropica 36:522-533

335 Ivanova N, Kuzmina M, Fazekas A (2011) CCDB Protocols: Manual Protocol Employing
336 Centrifugation: Glass Fiber Plate DNA Extraction Protocol for Plants, Fungi, Echinoderms and
337 Mollusks. Canadian Centre for DNA Barcoding. [http://ccdb.ca/site/wp-](http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_DNA_Extraction-Plants.pdf)
338 [content/uploads/2016/09/CCDB_DNA_Extraction-Plants.pdf](http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_DNA_Extraction-Plants.pdf) Accessed 10 August 2016

339 Ivanova NV, Grainger CM (2008) CCDB Protocols: Sequencing. Canadian Centre for DNA
340 Barcoding. http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_Sequencing.pdf Accessed 10
341 August 2016

342 Jayaraj VK, Laman CJ, Abdullah MT (2012) A predictive model to differentiate the fruit bats
343 *Cynopterus brachyotis* and *C. cf. brachyotis* Forest (Chiroptera: Pteropodidae) from Malaysia
344 using multivariate analysis. *Zool Stud* 51:259-271

345 Kuzmina M, Ivanova N (2011a) CCDB Protocols: Primer Sets for Plant and Fungi. Canadian
346 Centre for DNA Barcoding. [http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_PrimerSets-](http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_PrimerSets-Plants.pdf)
347 [Plants.pdf](http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_PrimerSets-Plants.pdf) Accessed 10 August 2016

348 Kuzmina, M, Ivanova N (2011b) CCDB Protocols: PCR Amplification for Plants and Fungi.
349 Canadian Centre for DNA Barcoding. [http://ccdb.ca/site/wp-](http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_Amplification-Plants.pdf)
350 [content/uploads/2016/09/CCDB_Amplification-Plants.pdf](http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_Amplification-Plants.pdf) Accessed 10 August 2016

351 Kuzmina ML, Johnson KL, Barron HR, Hebert PD (2012) Identification of the vascular plants of
352 Churchill, Manitoba, using a DNA barcode library. *BMC Ecol* doi:10.1186/1472-6785-12-25

353 McConkey KR, Prasad S, Corlett RT et al (2012) Seed dispersal in changing landscapes. *Biol*
354 *Conserv* 146:1-13

355 Medway L (1966) The Ulu Gombak Field Studies Centre. *Malayan Scientist* 2:1–16.

356 Muscarella R, Fleming TH (2007) The role of frugivorous bats in tropical forest succession. *Biol*
357 *Rev* 82:573-590

358 NCBI RC (2016) Database resources of the National Center for Biotechnology
359 Information. *Nucleic Acids Res* 44:D7

360 Phillipps Q, Phillipps K (2016) Phillipps' field guide to the mammals of Borneo and their ecology:
361 Sabah, Sarawak, Brunei, and Kalimantan. Princeton University Press, New Jersey

362 Phua PB, Corlett RT (1989) Seed dispersal by the lesser short-nosed fruit bat (*Cynopterus*
363 *brachyotis*, Pteropodidae, Megachiroptera). Malayan Nature Journal 42:251-256

364 Ratnasingham S, Hebert PDN (2007) Bold: The Barcode of Life Data
365 System(<http://www.barcodinglife.org>). Mol Ecol Notes 7:355-364

366 Schneider A, Mertes CM, Tatem AJ et al (2015) A new urban landscape in East-Southeast Asia,
367 2000-2010. Environ Res Lett doi:10.1088/1748-9326/10/3/034002

368 Sing KW, Syaripuddin K, Wilson JJ (2013) Changing perspectives on the diversity of bats
369 (Mammalia: Chiroptera) at Ulu Gombak since the establishment of the Field Study Centre in
370 1965. Raffles Bull Zool. S, 29, 211-217

371 Sing KW, Wang WZ, Wan T et al (2016) Diversity and human perceptions of bees
372 (Hymenoptera: Apoidea) in Southeast Asian megacities. Genome doi:10.1139/gen-2015-0159

373 Tan KH, Zubaid A, Kunz TH (1998) Food habits of *Cynopterus brachyotis* (Muller)(Chiroptera:
374 Pteropodidae) in Peninsular Malaysia. J Trop Ecol 14:299-307

375 Tan KH, Zubaid A, Kunz TH (2000) Fruit dispersal by the lesser dog-faced fruit bat, *Cynopterus*
376 *brachyotis* (Muller)(Chiroptera: Pteropodidae). Malayan Nature Journal 54:57-62

377 Wilson JJ, Sing KW, Halim MRA et al (2014). Utility of DNA barcoding for rapid and accurate
378 assessment of bat diversity in Malaysia in the absence of formally described species. Genet Mol
379 Res 13:920-925. doi:10.4238/2014.February.19.2

380 Wilson JJ, Sing KW, Lee PS, Wee AKS (2016) Application of DNA barcodes in wildlife
381 conservation in Tropical East Asia. Con Biol 30: 982-989

382 **SUPPORTING INFORMATION**

383 Additional supporting information can be found in the online version of this article.

384 **Online Resource 1.** Criteria used to assign species names to DNA barcodes.

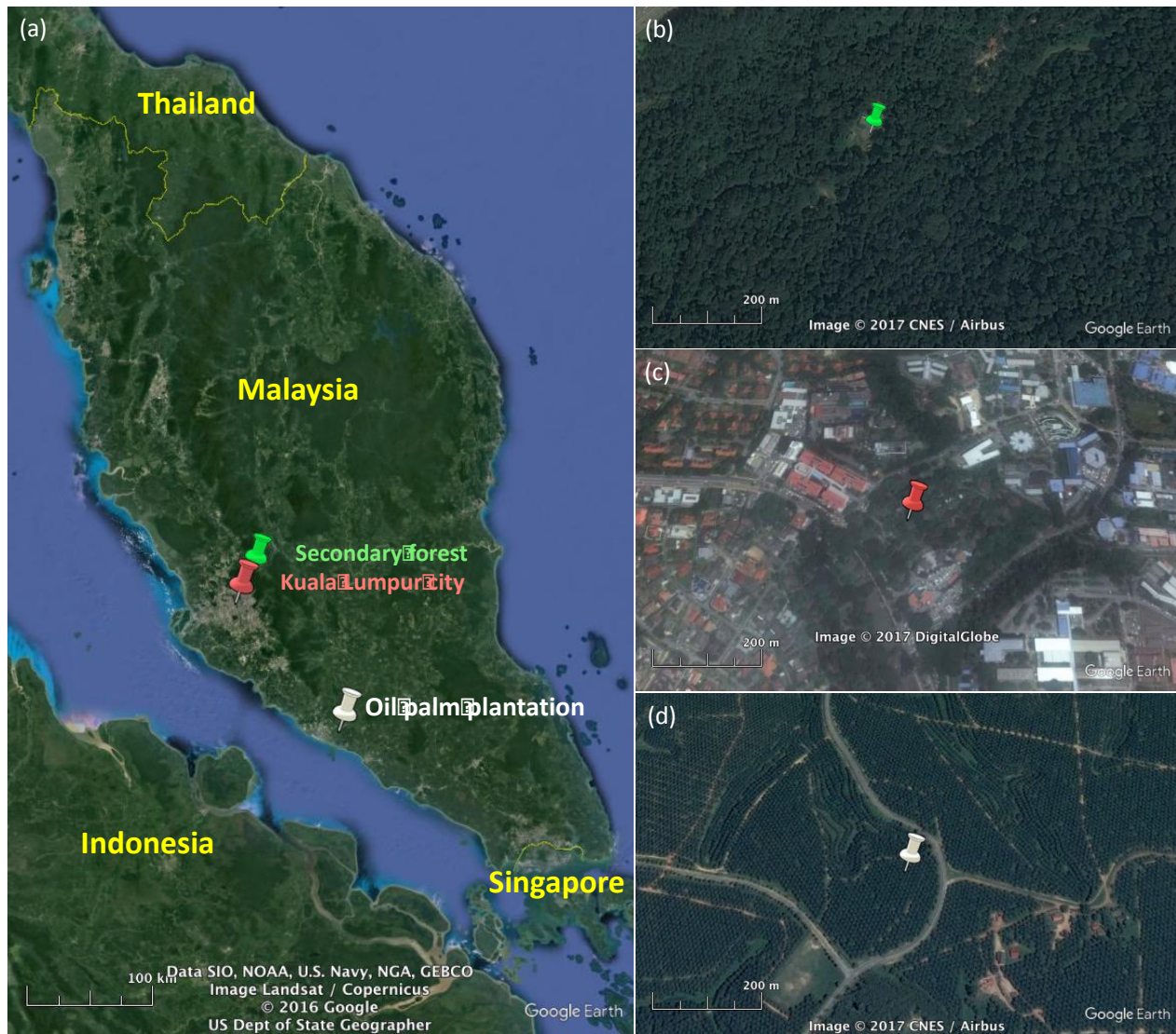
385 **DATA ACCESSIBILITY**

386 Morphological data and raw sequence data are provided in BOLD under project code VCCBD

387 and available in GenBank under accessions KY080541 to KY080613 and KY080617 to

388 KY080686.

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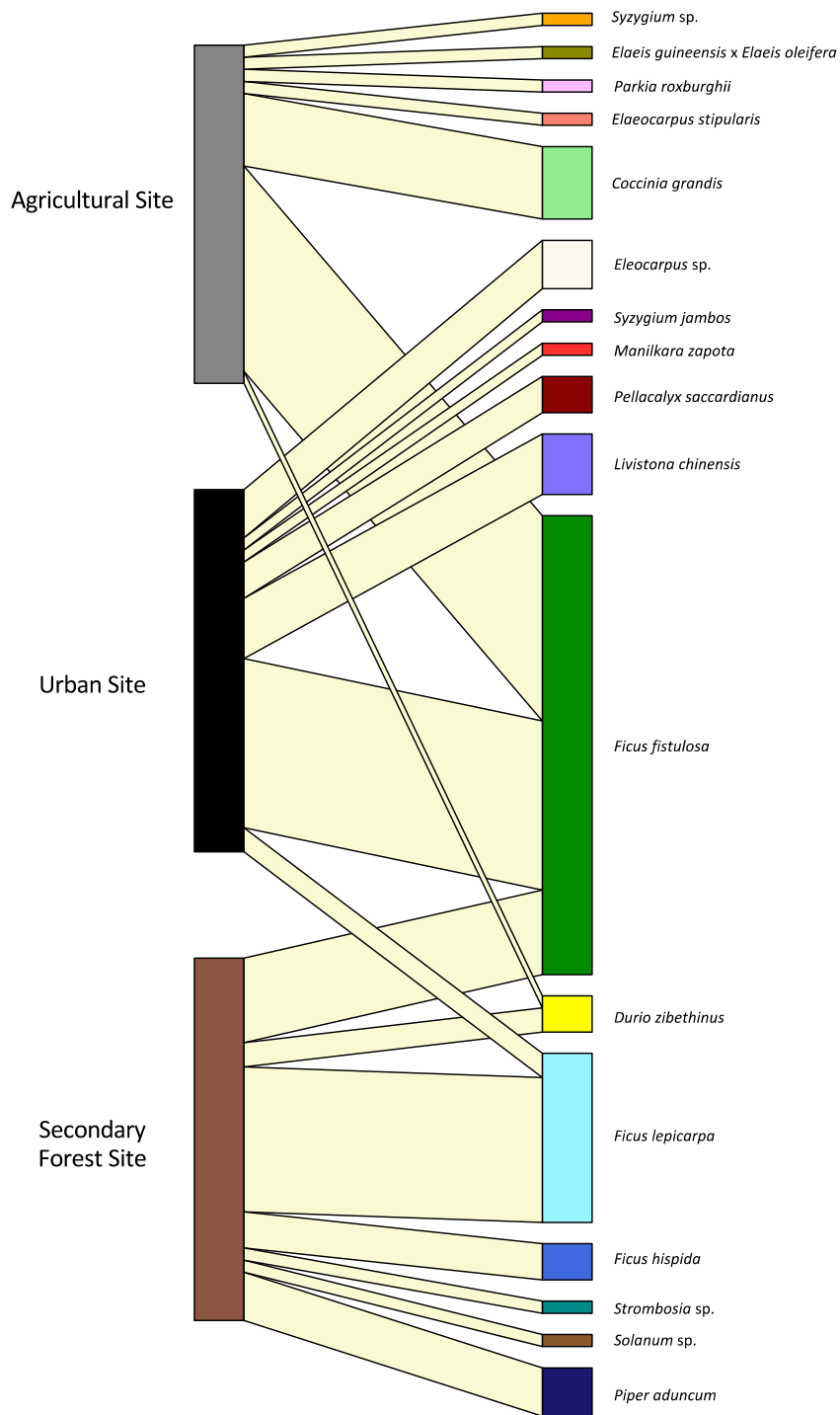
Fig. 1 The sampling location in Peninsular Malaysia. (a) The map of Peninsular Malaysia. (b) The sampling location at secondary forest. (c) The sampling location at urban area. (c) The sampling location at oil palm plantation.



396

397 **Fig. 2** Criteria used in assigning taxonomic names to the plant DNA barcodes based on matches

398 returned by BLAST searches on Genbank, NCBI database



400 **Fig. 3** The interaction between *C. brachyotis* and plant species detected from faecal samples
401 collected at three sites in Peninsular Malaysia. The width of the interaction bar corresponds to the
402 number of fruit bats and occurrence of plants in the faeces of fruit bats.

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404

405 **Table 1.** List of plants consumed by *C. brachyotis* in Peninsular Malaysia and Singapore. Phua &
 406 Corlett (1989) reported 21 plant species through observation of feeding behaviour of *C.*
 407 *brachyotis* at the botanical garden in Singapore. Tan *et al.* (1998) reported 53 plant species
 408 through morphological identification of the plant remnants in the masticated pellets of *C.*
 409 *brachyotis* at secondary forests in Peninsular Malaysia. Hodgkison *et al.* (2004) reported fifteen
 410 plant species through morphological identification of the plant remnants in the faeces, on the
 411 bodies and under the roosts of *C. brachyotis* at primary forest in Peninsular Malaysia.

| Family | Species | Status ^a | Phua & Corlett 1989 | Tan <i>et al.</i> 1998 | Hodgkison <i>et al.</i> 2004 | This study |
|-------------|--------------------------------|---------------------|---------------------|------------------------|------------------------------|------------|
| Moraceae | <i>Artocarpus fulvicortex</i> | N | | X | | |
| | <i>Artocarpus maingayi</i> | N | | X | | |
| | <i>Ficus fistulosa</i> | N | X | X | | X |
| | <i>Ficus benjamina</i> | N | | X | | |
| | <i>Ficus globosa</i> | N | | | X | |
| | <i>Ficus hispida</i> | N | | | | X |
| | <i>Ficus lepicarpa</i> | N | | | | X |
| | <i>Ficus magnoliifolia</i> | N | | | X | |
| | <i>Ficus religiosa</i> | E | | X | | |
| | <i>Ficus scortechinii</i> | N | | | X | |
| | <i>Ficus</i> (Unidentified) | | | X | | |
| Leguminosae | <i>Bauhinia purpurea</i> | E | | X | | |
| | <i>Cassia fistula</i> | E | | X | | |
| | <i>Parkia roxburghii</i> | N | | | | X |
| | <i>Peltophorum pterocarpum</i> | N | | X | | |
| | <i>Senna spectabilis</i> | E | | X | | |
| | <i>Erythrina subumbrans</i> | N | | X | | |
| | <i>Erythrina variegata</i> | N | | X | | |

| | | | | | | |
|------------|--|---|---|---|---|---|
| | <i>Erythrina fusca</i> | E | | X | | |
| | <i>Erythrina</i> (Unidentified) | | | X | | |
| Sapotaceae | <i>Manilkara zapota</i> | E | | X | | X |
| | <i>Mimusops elengi</i> | N | | X | | |
| | <i>Palaquium clarkeanum</i> | N | | X | | |
| | <i>Palaquium gutta</i> | N | X | X | | |
| | <i>Palaquium obovatum</i> | N | X | X | X | |
| | <i>Payena selangorica</i> | N | | X | | |
| | <i>Payena lucida</i> | E | | X | X | |
| | <i>Payena maingayi</i> | N | | X | | |
| | <i>Pouteria malaccensis</i> | N | | X | | |
| Myrtaceae | <i>Psidium guajava</i> | E | X | X | | |
| | <i>Syzygium jambos</i> | N | X | X | | X |
| | <i>Syzygium chloranthum</i> | N | | | X | |
| | <i>Syzygium grande</i> | N | X | X | | |
| | <i>Syzygium aqueum</i> | N | | X | | |
| | <i>Syzygium malaccense</i> | N | X | X | | |
| | <i>Syzygium lineatum</i> | N | X | | | |
| | <i>Syzygium</i> (Unidentified) | | | | | X |
| | <i>Eugenia</i> (Unidentified) | | | X | X | |
| Arecaceae | <i>Dyopsis lutescens</i> | E | | X | | |
| | <i>Elaies guineensis</i> x <i>Elaies oleifera</i> | E | | | | X |
| | <i>Ptychosperma macarthurii</i> | E | | X | | |
| | <i>Roystonea regia</i> | E | | X | | |

| | | | | | | |
|------------------|--------------------------------------|---|---|---|---|---|
| | <i>Saribus rotundifolius</i> | E | | X | | |
| | <i>Licuala grandis</i> | E | | X | | |
| | <i>Livistona chinensis</i> | E | | X | | X |
| Annonaceae | <i>Annona squamosa</i> | E | | X | | |
| | <i>Cyathocalyx scortechinii</i> | N | | | X | |
| | <i>Polyalthia longifolia</i> | E | | X | | |
| Anacardiaceae | <i>Camptosperma auriculatum</i> | N | X | | | |
| | <i>Mangifera indica</i> | E | | X | | |
| Pentaphylacaceae | <i>Adinandra dumosa</i> | N | X | | | |
| | <i>Adinandra sarosanthera</i> | N | | | X | |
| Elaeocarpaceae | <i>Elaeocarpus stipularis</i> | N | | X | X | X |
| | <i>Elaeocarpus</i> (Unidentified) | | | X | | X |
| Malvaceae | <i>Grewia tomentosa</i> | N | | X | | |
| | <i>Durio zibethinus</i> | N | | | | X |
| Clusiaceae | <i>Calophyllum inophyllum</i> | N | X | X | | |
| Combretaceae | <i>Terminalia catappa</i> | N | X | X | | |
| Cucurbitaceae | <i>Coccinia grandis</i> | N | | | | X |
| Euphorbiaceae | <i>Hevea brasiliensis</i> | E | | X | | |
| Gentianaceae | <i>Fagraea fragrans</i> | N | X | X | | |
| Lamiaceae | <i>Vitex pinnata</i> | N | X | | | |
| Melastomataceae | <i>Pternandra echinata</i> | N | | X | X | |
| Muntingiaceae | <i>Muntingia calabura</i> | E | X | X | | |
| Olacaceae | <i>Strombosia javanica</i> | N | | | X | |

| | | | | | | |
|----------------|--|---|---|---|---|---|
| | <i>Strombosia</i> (Unidentified) | | | | | X |
| Piperaceae | <i>Piper aduncum</i> | E | | X | | X |
| Podocarpaceae | <i>Podocarpus rumphii</i> | N | X | | | |
| Rhizophoraceae | <i>Pellacalyx</i> <i>saccardianus</i> | N | X | X | X | X |
| Rosaceae | <i>Prunus polystachya</i> | N | | | X | |
| Rubiaceae | <i>Nauclea officinalis</i> | N | | | X | |
| Salicaceae | <i>Flacourtia inermis</i> | E | | X | | |
| Sapindaceae | <i>Nephelium malaiense</i> | N | X | X | | |
| Urticaceae | <i>Cecropia peltata</i> | E | X | | | |
| Ebenaceae | <i>Diospyros</i> (Unidentified) | | X | X | | |
| Musaceae | <i>Musa</i> (Unidentified) | | X | X | | |
| Solanaceae | <i>Solanum</i> (Unidentified) | | | | | X |

412 a = Status of plants (N = native, E = exotic)

413