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4
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23 ABSTRACT

24 Frugivorous vertebrates, such as primates, are important dispersal agents in tropical forests,
25 although the role of folivorous colobines is generally not considered. However, recent
26 studies reported seed dispersal by endo- and epizoochory in colobine primates, including the
27 proboscis monkey (*Nasalis larvatus*), suggesting the role colobines play in seed dispersal
28 might have been underestimated. In the Lower Kinabatangan Floodplain, in Sabah,
29 Malaysian Borneo, we investigated whether seeds were still able to germinate after being
30 ingested by proboscis monkeys. Faecal samples (n = 201) from proboscis monkeys were
31 collected between 2015 and 2017. Intact seeds belonging to eight plant species were found
32 in 77% of the faecal samples. *Nauclea* spp. was the most abundant plant species, accounting
33 for 98% of all intact seeds. This study is the first to conduct germination trials on seeds
34 defecated by proboscis monkeys. Higher germination success was recorded in ingested
35 *Nauclea* spp. seeds than in control seeds, from both ripe and unripe *Nauclea orientalis* fruits
36 ($P < 0.001$). Therefore, we suggest that proboscis monkeys play a role in seed dispersal, by
37 enhancing the germination success of defecated seeds, for at least some plant species. Similar
38 to other colobines, although proboscis monkeys may provide a lower contribution to seed
39 dispersal (low seed diversity over short distances) than other sympatric frugivores, this study
40 emphasises that proboscis monkeys do contribute to the dispersal of intact seeds, such as
41 *Nauclea* spp., in potentially suitable riverine habitats.

42

43 **Keywords:** *Nauclea* spp., *Nasalis larvatus*, proboscis monkey, seed dispersal, seed
44 germination

45

46

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56 improved the manuscript.

57

58 INTRODUCTION

59 Many plant species across the globe rely on animals to disperse their seeds (Farwig and
60 Berens 2012). In tropical forests, up to 98% of plant species bear fruits and require
61 vertebrates, such as birds or mammals, for the dispersal of their seeds (Howe and Smallwood
62 1982; Terborgh et al. 2002). Largely because they represent a considerable proportion of the
63 frugivore biomass in tropical forests, primates have long been considered as seed dispersers
64 (Chapman 1995). When consuming fruits, primates can handle the seeds differently: feeding
65 on the pulp and dropping or spitting out seeds; swallowing and defecating intact seeds
66 (endozoochory); or chewing and destroying seeds (Corlett and Lucas 1990). Recent studies
67 highlighted the important role many primate species play in seed dispersal (Razafindratsima
68 et al. 2018), mostly by swallowing or dropping the seeds (Albert et al. 2013). However, some
69 primate taxa, such as Asian and African colobines, were generally not included and were
70 considered more as seed predators (Norconk et al. 1998), destroying most of the seeds they

71 consumed (Garber and Lambert 1998; Norconk et al. 1998). However, primate seed
72 predators can be involved in seed dispersal by swallowing small seeds intact without
73 masticating them, by removing pericarp and seed-coat before discarding seeds (Norconk et
74 al. 1998; Barnett et al. 2012), or by seeds attaching to the animal's fur and dropping off in a
75 different location (epizoochory) (Chen et al. 2018). Indeed, the dichotomy seed predator
76 versus seed disperser has been oversimplified (Norconk et al. 1998) and many primates play
77 a number of roles, depending on the plant consumed (McConkey 2018).

78 In colobines, seed dispersal has been recorded in five species, via endozoochory (*Nasalis*
79 *larvatus* (Matsuda et al. 2013), *Trachypithecus francoisi hatinhensis* (Nguyen et al. 2013),
80 *T. auratus* (Tsuiji et al. 2017) and *Presbytis* sp. (McConkey unpubl. in McConkey 2018))
81 and via epizoochory (*Rhinopithecus roxellana* (Chen et al. 2018)). Even though seed
82 germination trials were not conducted (Matsuda et al. 2013; Nguyen et al. 2013; Chen et al.
83 2018) or dispersed seeds showed low germination success (Tsuiji et al. 2017), authors
84 suggested that the role colobines play in seed dispersal might have been underestimated. In
85 regard to the wide distribution and biomass of Asian and African colobines, colobine
86 primates might play a considerable role in seed dispersal of some plant species (Matsuda et
87 al. 2013; Corlett 2017; Tsuiji et al. 2017).

88 Proboscis monkey (*Nasalis larvatus*) is an endangered colobine primate endemic to the
89 island of Borneo, where the species inhabits riverine, swamp and mangrove forests
90 (Meijaard et al. 2008). In riverine forest, proboscis monkey home range averages 81 ha
91 (Stark et al. 2017) and the mean daily distance travelled is 940 m (Stark 2018). Proboscis
92 monkeys highly use areas close to rivers, select sleeping sites along water and avoid sleeping
93 at the same location on consecutive nights (Stark 2018). Similar to other colobines, proboscis
94 monkeys have a large sacculated forestomach wherein food fermentation occurs (Nijboer

95 and Clauss 2006), and therefore, they usually avoid feeding on ripe fleshy fruits which are
96 rich in sugar susceptible to ferment in gas, and therefore, can be deleterious for their
97 digestive system (Waterman and Kool 1994). Proboscis monkeys mostly consume young
98 leaves, unripe fruits and seeds (Matsuda et al. 2009) and display long gut retention times
99 (mean retention times (MRTs) of 40 hours (Matsuda et al. 2015)).

100 The role of proboscis monkeys as seed dispersers has previously been suggested, based on
101 the presence of intact seeds in 11% of monkey analysed faeces (Matsuda et al. 2013).
102 However, until now, no known germination tests have been conducted to determine how the
103 ingestion of seeds by proboscis monkeys would affect seed germination. We focused on seed
104 dispersal by endozoochory and aimed to investigate whether seeds were still able to
105 germinate after being ingested by proboscis monkeys. We predicted that (1) small intact
106 seeds would be found in proboscis monkey faeces, as reported by Matsuda et al. (2013) and
107 because small seeds might be less damaged by mastication and pass intact through the animal
108 gut (Corlett and Lucas 1990; Norconk et al. 1998), and (2) due to their feeding habits
109 (preferring unripe fruits over ripe fruits), the seeds defecated by proboscis monkeys would
110 experience lower germination rates than control seeds (collected directly from fruits), such
111 as reported in the colobine Javan lutung (*T. auratus*) (Tsuji et al. 2017).

112

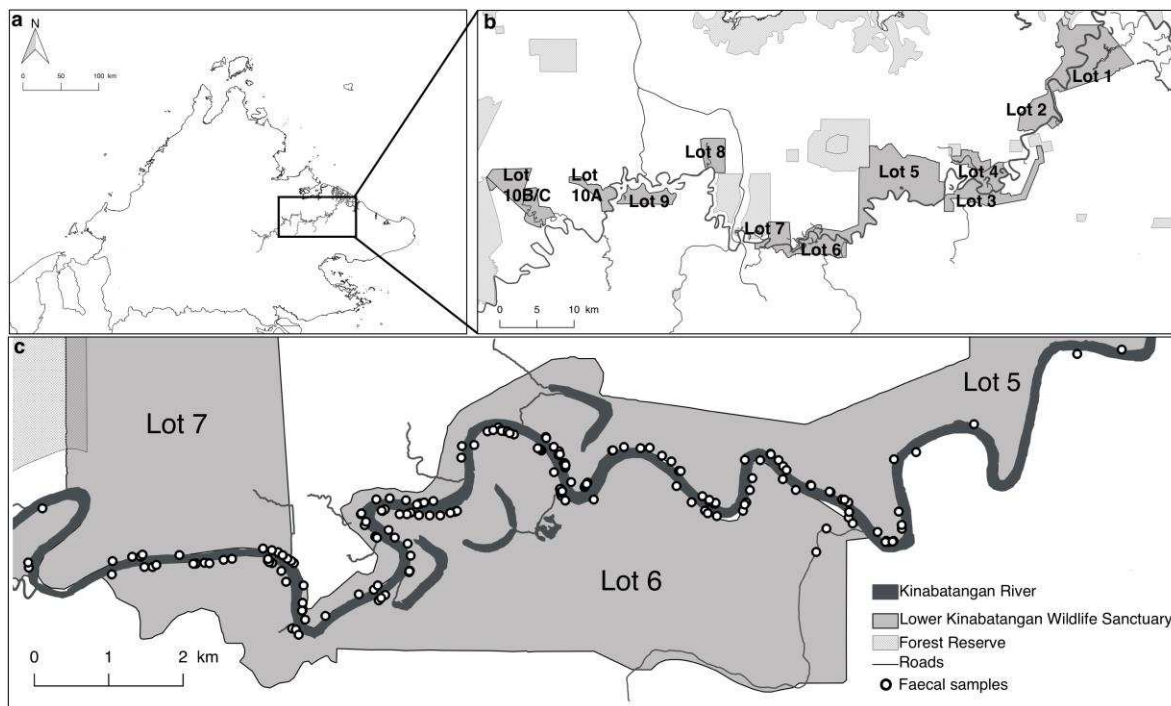
113 METHODS

114 **Study site**

115 This study took place over 14 months between May to August 2015, January to May 2016,
116 and November 2016 to March 2017, in Lots 5, 6, and 7 of the Lower Kinabatangan Wildlife
117 Sanctuary (LKWS, 5°10'-05°50'N, 117°40'-118°30'E), Sabah, Malaysian Borneo (Fig. 1).
118 The major forest types in the LKWS include dry lowland forests, semi-inundated and

119 permanent swamps, and mangrove forests (Abram et al. 2014). Forest vegetation is
120 dominated by *Dillenia excelsa*, *Mallotus muticus*, *Colona serratifolia*, *Antidesma*
121 *puncticulatum* and *Vitex pinnata* tree species. The five most abundant vine species are
122 *Lophopyxis maingayi*, *Entada rheedii*, *Bridelia stipularis*, *Dalbergia stipulacea* and *Croton*
123 *triqueter* (6.25 ha botanic plot survey, Thiry unpubl.). Daily temperatures and rainfall were
124 measured at the research station. During the study, mean monthly rainfall was 177 (\pm SD =
125 121) mm. Mean minimum and maximum temperatures were 24.5 (\pm SD = 0.7) and 30.4 (\pm
126 SD = 1.8) $^{\circ}$ C, respectively.

127



129 **Fig. 1** Maps of the study area: **a** the State of Sabah in Malaysian Borneo with the location of
130 the Kinabatangan River; **b** the Lower Kinabatangan Wildlife Sanctuary, with its 10 protected
131 forest lots (dark grey) and the Forest Reserve (light grey); **c** zoom on the study site in lots 5,
132 6 and 7, with faecal sampling locations

133

134 **Faecal sampling**

135 In riverine habitats, proboscis monkeys generally spend their nights in trees along riverbanks
136 (Matsuda et al. 2011). We conducted boat-based surveys in the late afternoon to look for
137 proboscis monkeys along the Kinabatangan River. To avoid sampling the same group
138 multiple times within the same month, we searched for proboscis monkey groups along
139 different sections of a pre-established 21 km river transect in a month. We selected the first
140 proboscis monkey group that we found and conducted behavioural observations until dark
141 (instantaneous scan and ad libitum sampling). The following morning, we returned to the
142 same trees where the group was located the previous evening and continued our observations
143 on the same group. This allowed us to ascertain that no other diurnal primate species (long-
144 tailed and pig-tailed macaques (*Macaca fascicularis* and *M. nemestrina*); silvered, maroon
145 and Hose's langurs (*Trachypithecus cristatus*, *Presbytis rubicunda* and *P. hosei*); Bornean
146 gibbons (*Hylobates muelleri*) and Bornean orangutans (*Pongo pygmaeus*)) spent the night in
147 or neighbouring (within 10 m) proboscis monkeys' sleeping trees. In the morning, once the
148 proboscis monkeys had left the riverside, we approached the bank to search for fresh faecal
149 samples under the sleeping trees. Although some faeces might have fallen in the water when
150 a sleeping tree canopy was overhanging the river, we were still able to find faecal samples
151 most (if not all) mornings. Proboscis monkey faeces were easily identifiable, generally
152 properly shaped and soft (Nijboer 2006) and spread out on leaves, on the forest ground or
153 on the river bank. One single large faecal sample assumed to belong to an adult individual
154 was collected per group and stored in an empty Falcon tube until analysis. An extra sample
155 was collected to supply faecal matter in germination trial experiments (see treatment F2
156 below). When sympatric primates were within 10 m of the group, we did not collect faecal

157 samples. During the study, we collected a total of 201 proboscis monkey faecal samples
158 (mean \pm SD = 14 ± 6 faeces month⁻¹). Most faeces sampled within the same month were
159 considered to belong to distinct proboscis monkey groups that ranged in different areas along
160 the study transect.

161

162 **Faecal analysis**

163 Within four hours of collection, the sample was weighed and cleaned with water using a 0.4
164 mm mesh strainer until all faecal matter was removed (Trolliet et al. 2016). Using a
165 stereoscope, intact seeds were extracted and grouped according to their shape, size and
166 colour, and the exact numbers of intact seeds were counted. Finally, ingested seeds were
167 identified by comparing them to a pre-established seed reference collection, containing seeds
168 from identified fruit species found in the study area. When identification was not possible
169 on site, seeds were sent to the Forest Research Centre (Sandakan, Sabah) to be identified by
170 botanical experts.

171

172 **Germination trials**

173 *Preliminary germination trials*

174 For each seed species found intact in proboscis monkey faeces, cleaned seeds (F1) were
175 placed in Petri dishes (maximum 16 seeds / Petri dish) filled with filter paper (Serio-Silva
176 and Rico-Gray 2003). Filter paper was chosen for the ease to visualise small seeds. Petri
177 dishes were placed outside at ambient temperature and in continuous shade for 60 days.
178 Seeds were checked every four days and germination event was defined as the emergence of
179 the radicle (Trolliet et al. 2016).

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Advanced germination trials: Nauclea spp.

As *Nauclea* spp. seeds were found in abundance in proboscis monkey faeces, further germination trials were conducted, considering different seed treatments. To assess the effect of proboscis monkey gut passage on *Nauclea* spp. seed germination, we conducted experiments to compare the germination of ingested seeds and control seeds from fruits. *Nauclea orientalis* and *N. subdita* trees are present throughout the study site, however the seeds are morphologically indistinguishable: the fruits are brown with an irregular globular shape and contain thousands of tiny ovoid seeds measuring 1 mm long (Lim 2013). Even though fruits from both species are consumed by proboscis monkeys (Matsuda et al. 2009; Thiry unpubl.), fruit-feeding behaviour in the current study was mostly on *N. orientalis* (88% of feeding occurrences recorded at the riverside by scan sampling method). Therefore, seeds from *N. orientalis* fruits were used as experimental controls.

Based on Samuels and Levey (2005), we investigated five different treatments, which included the effect of being discarded from fruit pulp (manually extracted seeds from *N. orientalis* unripe (FR1) and ripe fruits (FR2) vs. seeds from ripe fruits with fleshy pulp (FR3)); and the chemical/mechanical effect of being ingested by proboscis monkeys (gut passed seeds, without (F1) or with (F2) faecal matter vs. manually extracted seeds (FR1 and FR2)) (Table 1). We considered two treatments for gut passed seeds to better represent natural conditions, F1 consists of defecated seeds that are freed from faecal matter (i.e., after secondary seed dispersal by dung beetles, or heavy rain washing the faecal matter away) and F2 where seeds lay inside faecal clumps.

203 **Table 1.** Categories (gut passed and control) and treatments (F1, F2, FR1, FR2 and FR3) of
 204 *Nauclea* spp. and *N. orientalis* seeds used during germination trials, set up in Petri dishes
 205 filled with forest soil.

Seed categories and treatments	Seed code in survival analyses
Gut passed seeds: <i>Nauclea</i> spp.	
F1: without faecal matter (n = 109)	F (n = 201)
F2: with faecal matter (n = 92)	
Control seeds: <i>N. orientalis</i>	
FR1: from unripe fruit (n = 104)	UF (n = 104)
FR2: from ripe fruit without pulp (n = 75)	RF (n = 153)
FR3: from ripe fruit with pulp (n = 78)	

206 ⁱ n = number of seeds per treatment.

207

208 Petri dishes were filled with forest soil (depth: 0.5 – 1 cm) and 16 *Nauclea* seeds of the same
 209 treatment (F1, F2, FR1, FR2 or FR3) were deposited on the soil surface of each dish. All
 210 Petri dishes were placed outside at ambient temperature and in continuous shade for 60 days
 211 (Trolliet et al. 2016) and subsequently the Petri dishes were moved into direct sunlight for
 212 20 more days (i.e., sun conditions) (Hodgkison et al. 2003). We checked the seeds and added
 213 water every four days. To investigate the effect of the five treatments on *Nauclea* spp. seed
 214 germination, we conducted survival analyses (McNair et al. 2012) by measuring initial
 215 germination delay (the time it took for the first seed to germinate), germination speed (how
 216 many seeds germinated within four days) and total percentage of seeds that germinated at
 217 the end of the experiment (McNair et al. 2012). Survival analyses were only performed for
 218 *Nauclea* spp. (see below), as sample sizes of other seed species were too small.

219

220 **Statistical analyses**

221 *Advanced germination trials: Nauclea spp.*

222 Analyses were conducted with R (version 3.4.3) with a statistical significance of $P < 0.05$
223 (R Development Core Team 2017). We performed time-to-event analyses (survival analysis)
224 on *Nauclea* spp. germination data (McNair et al. 2012), using the *survival* package
225 (Therneau 2015) to fit Kaplan-Meier survival functions. Survival curves were then plotted
226 using the *survimer* package (Kassambara et al. 2017). Finally, log-rank tests were performed
227 to assess the homogeneity of multiple survivor functions and to conduct pair-wise
228 comparisons of different seed treatments (McNair et al. 2012).

229

230 **RESULTS**

231 A high proportion (77.1%) of the analysed faecal samples (155 out of the 201) contained
232 intact seeds, with seeds being recorded each month of the study. There were 28,452 intact
233 seeds from at least eight different plant species found in 77.1% of the faecal samples (Table
234 2). All species were small-seeded, with most seeds measuring less than 2 mm in length,
235 except for *Antidesma puncticulatum* (2-5 mm), Unknown sp. B (2-5 mm) and *Bridelia*
236 *stipularis* (5-8 mm). The number of seeds ranged from 0 to 2,408 per sample, with an average
237 of 141 ± 262 seeds per sample. Accounting for 98% of all intact seeds and found in 70.1%
238 of the faecal samples, *Nauclea* spp. were the most abundant seed species, followed by an
239 unidentified species (Unknown sp. A, recorded in 14.4% of faeces). Other species were
240 observed less frequently, on average in $3.2 \pm 2.3\%$ of the faeces (Table 2). Chewed (broken)
241 seeds were not considered: due to the small seed size we would not have been able to
242 distinguish or identify them.

243

244 **Table 2.** List of intact seeds recorded in proboscis monkey faeces (n = 155) collected between
 245 2015 and 2017

Intact seeds (family and species)		Number of faecal samples containing intact seeds	Range of number of seeds per faecal sample	Seed length (mm)
Moraceae	<i>Ficus racemosa</i>	15	1 - 23	< 2
Moraceae	<i>Ficus septica</i>	8	1 - 21	< 2
Phyllanthaceae	<i>Antidesma puncticulatum</i>	3	2 - 7	2 - 5
Phyllanthaceae	<i>Bridelia stipularis</i>	5	1 - 12	5 - 8
Rubiaceae	<i>Nauclea</i> spp.	141	1 – 2408	< 2
Unknown	Unknown sp. A	29	1 - 120	< 2
Unknown	Unknown sp. B	2	1 - 4	2 - 5
Unknown	Unknown spp. ⁱ	6	1 - 55	NA

246 ⁱ Unknown spp. refers to a combination of several indistinguishable seeds, different from
 247 Unknown sp. A and B.

248

249 **Germination trials**

250 ***Preliminary germination tests***

251 Seeds of six plant species germinated after passing through the gut of proboscis monkeys
 252 (Table 3, no germination tests could be conducted with *A. puncticulatum* and Unknown
 253 spp.).

254

255 **Table 3.** Germination fate of intact seeds after ingestion by proboscis monkeys.

Seed species	Yes	No	NA	Total
<i>Bridelia stipularis</i>	1	0	35	36
<i>Ficus racemosa</i>	19	43	0	62
<i>Ficus septica</i>	8	0	0	8
<i>Nauclea</i> spp.	114	53	8	175

Unknown sp.A	12	46	5	63
Unknown sp.B	1	0	2	3

256 ⁱ NA refers to failure occurring during germination experiments (i.e., insect damage,
 257 humidity conditions, etc.)

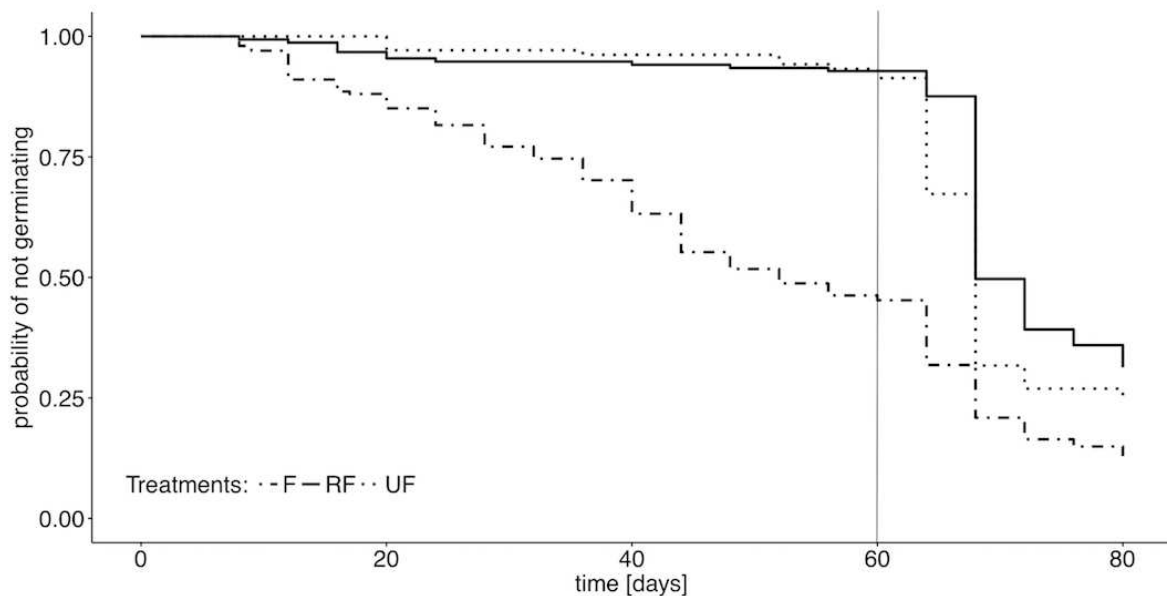
258

259 ***Advanced germination tests: Nauclea spp.***

260 For reasons of clarity, different seed treatments were grouped when homogeneity tests were
 261 not significantly different and did not influence differently the fate of tested seeds. F is the
 262 combination of the treatments F1 and F2 (Log Rank: $\chi^2 = 0$, $df = 1$, $P = 0.993$), RF is the
 263 combination of FR2 and FR3 (Log Rank: $\chi^2 = 0.3$, $df = 1$, $P = 0.592$) and UF refers to FR1
 264 (Table 1).

265 Homogeneity tests showed that the different treatments influenced the fate of seeds exposed
 266 to shade and sun conditions (Log Rank: $\chi^2 = 79.5$, $df = 2$, $P < 0.001$). We observed that
 267 ingested seeds (F) had a higher probability of germination (day 80: 87% of seeds had
 268 germinated) than control seeds from unripe fruits (UF) (Log Rank: $\chi^2 = 29$, $df = 1$, $P < 0.001$;
 269 day 80: 76%) and ripe fruits (RF) (Log Rank: $\chi^2 = 63$, $df = 1$, $P < 0.001$; day 80: 69%).
 270 Moreover, we observed that UF seeds had a higher percentage of germination than RF seeds
 271 (Log Rank: $\chi^2 = 6.3$, $df = 1$, $P < 0.05$). Finally, ingested seeds (F) showed a shorter initial
 272 germination delay (mean \pm SD: 46 ± 21 d) than RF (65 ± 15 d) and UF (65 ± 11 d) seeds, as
 273 well as an increased germination speed (at day 60: 108 F seeds had already germinated, as
 274 opposed to only 10 RF and 7 UF seeds) (Fig. 2).

275



276
 277 **Fig. 2** Germination curves of seeds growing under shade conditions (60 d) followed by sun
 278 exposure (20 d) for three treatments; F = *Nauclea* spp. seeds ingested by proboscis monkeys
 279 (n = 201), RF = seeds from ripe *N. orientalis* fruits (n = 153), and UF = seeds from unripe
 280 *N. orientalis* fruits (n = 104); Each dip in the survival curve represents a germination event.

281
 282 **DISCUSSION**

283 Our study demonstrated that proboscis monkeys do play a role in seed dispersal. We
 284 recorded the presence of intact seeds in their faeces each month, with 77.1% of analysed
 285 faecal samples containing seeds. As proboscis monkeys avoid ripe fruits and select unripe
 286 fruits (Matsuda et al. 2009), we suggest the small intact seeds are from unripe fruits. Our
 287 results contrast with previous studies that reported a low presence (only 11% of faeces in
 288 three months out of a 13-month study) (Matsuda et al. 2013) or an absence of seeds in
 289 proboscis monkey faeces entirely (Ranglack and Yeager 1986). Moreover, our study

290 reported higher seed diversity (eight species) in comparison to Matsuda et al. (2013), who
291 identified seeds belonging to only three plant species (*Ficus globosa*, *Antidesma*
292 *thwaitesianum* and *N. subdita*). We reported similar densities of *Ficus* and *A. puncticulatum*
293 (the updated name for *A. thwaitesianum*) seeds in proboscis monkey faeces (1 - 23 and 2 - 7
294 seeds per sample, respectively) as Matsuda et al. (2013) in Sukau, a study site located 30 km
295 downstream from ours. However, we recorded remarkably higher densities of *Nauclea* spp.
296 seeds (1-2,408 seeds per faecal sample) in comparison to the low *N. subdita* seed densities
297 reported in Sukau (1-23 seeds per sample) (Matsuda et al. 2013). Finally, we recorded
298 *Bridelia stipularis* seeds in five faecal samples, while Matsuda et al. (2013) did not. The
299 observed disparities in seed species diversity, density and monthly presence in proboscis
300 monkey faeces between both studies might be the result of differences in plant (fruit)
301 composition and availabilities. High numbers of plant species are reported in both study sites
302 (n=180 in Matsuda et al. (2009), and n=201 in this study). However, as vegetation survey
303 methods differed between studies (trail transects (Matsuda et al. 2009) and botanic plots
304 (Thiry unpubl.)), plant availabilities are difficult to compare. Results more probably differ
305 between studies because the methods used to examine the content of faecal samples were
306 different. Indeed, in Matsuda et al. (2013), faecal samples were analysed manually with
307 forceps and a magnifying glass, without draining faecal matter away, and this method may
308 have missed seeds, while our approach with a strainer may be more appropriate to record the
309 presence of small seeds. Although proboscis monkeys are also known as seed predators (e.g.,
310 consumed seeds of the unripe *Ficus binnendijkii* fruit were never found in faeces) (Matsuda
311 et al. 2013), we did not monitor proboscis monkeys the whole day, and therefore were not
312 able to record whether other consumed seeds were absent from their faeces (i.e., chewed
313 seeds).

314 Even though the role of proboscis monkeys in seed dispersal has previously been suggested
315 (Matsuda et al. 2013), the present study is the first to detail the germination of seeds ingested
316 by this colobine species. We found that six of the eight seed species examined were able to
317 germinate after passage through the gut of proboscis monkeys. While statistical tests could
318 not be performed on the germination data of five of the seed species, survival analyses
319 conducted with the most abundant small-seeded species, *Nauclea* spp., indicated that seeds
320 ingested by proboscis monkeys exhibit higher germination success (87%) than seeds from
321 control fruits (both ripe and unripe). This result differs from a previous study on another
322 colobine, the Javan lutung (*T. auratus*), where lower germination success (only 5%) was
323 observed in defecated *Ficus* spp. seeds than in control seeds (10%), suggesting that lutungs
324 are not efficient fig dispersers (Tsuji et al. 2017). In our study, differences are particularly
325 striking under shade conditions (first 60 days) where the seeds ingested by proboscis
326 monkeys started to germinate earlier and faster than the seeds extracted from fruits: at d 60,
327 54% of ingested seeds had already germinated, as opposed to only 7% of the control seeds
328 (from ripe and unripe fruits). A possible explanation for this germination pattern could be
329 that seeds ingestion increases seed-coat permeability and enhances gas exchange and water
330 absorption (Barnea et al. 1990). Pulp removal is also reported to be beneficial, as fruit flesh
331 may inhibit germination (Evenari 1949), although we did not observe any difference between
332 FR2 and FR3 treatments (control seeds without and with pulp). Additionally, faecal matter
333 surrounding ingested seeds has been suggested to have a fertilising effect and to promote
334 seedling growth (Traveset et al. 2001). On the other hand, faecal matter may promote fungal
335 and/or bacterial growth, that could in turn prevent seedling establishment (Meyer and
336 Witmet 1998). The presence of faecal matter appeared to be neutral in our study, as F1 and

337 F2 treatments (ingested seeds without and with faecal matter, respectively) did not differ
338 significantly.

339 We observed that UF seeds (control seeds from unripe fruits) had a higher probability of
340 germination than RF seeds (control seeds from ripe fruits) by the end of the germination
341 experiment (d 80: 60 days of shade followed by 20 days of sun exposure). Although
342 counterintuitive (Arthur 1985), higher or similar germination success of seeds from unripe
343 fruits, compare with mature fruits, has been reported (Foster 1977; Arditti et al. 1981; Cruz-
344 Tejada et al. 2018). In the case of *N. orientalis* (used for control treatments), this could be
345 an advantage to benefit from seed dispersal by animals that preferentially feed on unripe
346 fruits, such as colobines (Davies et al. 1988; Dasilva 1994; Matsuda et al. 2009; Hanya and
347 Bernard 2012; Ehlers Smith et al. 2013).

348 Seed density has been reported to negatively affect germination success (Barnea et al. 1992).
349 Therefore, when high density of seeds are found in faecal samples, such as in our present
350 study, intra- and interspecific competition may occur between seeds, affecting seedling
351 survival and establishment (Loiselle 1990; Traveset et al. 2007). However, being consumed
352 by frugivores might still benefit plant species bearing multi-seeded fruits, such as *Nauclea*
353 or *Ficus* species. Indeed, faecal clumps may contain lower seed densities than the multi-
354 seeded fruit itself, reducing seed competition and potentially enhancing germination (Barnea
355 et al. 1992).

356 The presence of *N. orientalis* seeds has already been recorded in the faeces of another
357 colobine primate, the Javan lutung (*T. auratus*) in Indonesia (Tsuji et al., 2017). In our study
358 site, silvered langurs (*T. cristatus*) are also known to consume *N. orientalis* fruits. Colobines
359 are not the only mammals consuming *N. orientalis* fruits, as other frugivores, such as the
360 Bornean bearded pig (*Sus barbatus*), large flying fox (*Pteropus vampyrus*), long-tailed

361 macaque or Bornean orangutan, also feed on these fruits, underlying the potential existence
362 of a wide community of *N. orientalis* dispersers (Bhasin, unpubl.). The relative contribution
363 of colobines to seed dispersal may be lower than that of other sympatric frugivorous animals
364 (i.e., macaques, gibbons, hornbills, etc.), mostly as they disperse low diversity of seeds over
365 short distances (Tsuji et al. 2017, McConkey 2018). However, in regard to the wide
366 distribution and large biomass of colobines in Asian and African forests (Matsuda et al.
367 2013), they could potentially be important seed dispersers for some plant species. Moreover,
368 as colobines mostly target unripe fruits and disperse seeds over shorter distances (i.e., < 100
369 m in Javan lutung (Tusji et al. 2017)), we believe they can provide a complementary service
370 to other frugivores (i.e., gibbons) consuming ripe fruits and dispersing seeds over longer
371 distances (i.e., up to 1.3 km in gibbon (McConkey 2018)). As *N. orientalis* naturally occurs
372 along riverbanks (Azmi 1998, Boland et al. 2006), we suggest that riverine seed deposition
373 by proboscis monkeys is suitable for *Nauclea* spp. In addition, as proboscis monkey sleeping
374 trees are often located along water (Stark, 2018), secondary seed dispersal might also occur
375 by hydrochory: when branches overhang the river, proboscis monkey faeces may fall in the
376 water, float away until reaching a bank and potentially establish seedlings in a distant
377 suitable riverine habitat. *N. orientalis* seed dispersal by water has already been reported
378 along the Ord River in Australia (Pettit & Froend 2001). These results suggest that *N.*
379 *orientalis* has more than one dispersal mechanism (polychory).

380 It is of interest to note that we often recorded large numbers of faecal samples under
381 proboscis monkey sleeping trees, which may attract a vast guild of secondary seed dispersers
382 (e.g., dung beetles) (Shepherd and Chapman 1998) or seed predators (e.g., rodents)
383 (Andresen and Levey 2004). Although faecal samples were collected shortly after being
384 defecated, dung beetles were often already observed in proboscis monkey faeces. Therefore,

385 further research should investigate the post-dispersal fate of proboscis monkey dispersed-
386 seeds.

387 To conclude, our study emphasises that the role proboscis monkeys play in seed dispersal
388 has been underestimated thus far. Proboscis monkeys should be considered as seed
389 dispersers, at least of some plant species. First, they prefer to feed on unripe fruits over young
390 leaves during certain times of the year (Matsuda et al. 2009). They then defecate large
391 numbers of tiny intact seeds, which can improve the germination success of some plant
392 species, such as those of *Nauclea* spp. And finally, in regard to their long MRTs (Matsuda
393 et al., 2015), daily ranging movements and sleeping site selection close to rivers (Stark,
394 2018), we suggest that proboscis monkeys contribute to the dispersal of intact seeds, away
395 from the parent plant, in potentially suitable riverine habitats.

396

397 COMPLIANCE WITH ETHICAL STANDARDS

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402

403 **Conflict of Interest**

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

405

406 **Ethical approval**

407 No direct contacts with animals were conducted during this research and ethical aspects of
408 the research protocols have been approved by the FNRS committee and Sabah Biodiversity
409 Council.

410

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