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Characterization and comparison of poorly known moth communities through DNA barcoding in two Afrotropical environments

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1 **Characterization and comparison of poorly known moth communities through DNA**
2 **barcoding in two Afrotropical environments**

3

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39 **Abstract**

40 Biodiversity research in tropical ecosystems – popularized as the most biodiverse habitats on
41 Earth – often neglects invertebrates, yet representing the bulk of local species richness. Insect
42 communities in particular remain strongly impeded by both Linnaean and Wallacean
43 shortfalls, and identifying species often remains a formidable challenge inhibiting the use of
44 these organisms as indicators for ecological and conservation studies.

45 Here we use DNA barcoding as an alternative to traditional taxonomic approach for
46 characterizing and comparing the diversity of moth communities in two different ecosystems
47 in Gabon. Though sampling remains very incomplete, as evidenced by the high proportion
48 (58%) of species represented by singletons, our results reveal an outstanding diversity. With
49 about 3500 specimens sequenced and representing 1385 BINs (Barcode Index Numbers, used
50 as a proxy to species) in 23 families, the diversity of moths in the two sites sampled is higher
51 than the current number of species listed for the entire country. Both seasonal and spatial
52 turnovers are strikingly high (18.3% of BINs shared between seasons, and 13.3% between
53 sites), emphasizing the need to account for these when running regional surveys. Our results
54 also highlight the richness and singularity of savannah environments and emphasize the status
55 of Central African ecosystems as hotspots of biodiversity.

56

57 **Keywords:** Community Ecology, DNA barcodes, Lepidoptera, Tropical Africa, Taxonomic
58 deficit

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64 **Introduction**

65 Tropical ecosystems host unrivalled species richness (Kier *et al.* 2005; Myers 1984; Myers *et*
66 *al.* 2000), a fact that has long captivated public attention and raised concerns about the way to
67 conserve this immense biodiversity (Wilson 1988). Understanding of tropical biodiversity has
68 historically been biased toward the largest organisms such as angiosperms and vertebrates
69 (May 2011), leaving considerable gaps in our knowledge of hyperdiverse groups of smaller
70 animals, especially arthropods. These organisms are nevertheless key to ecosystem
71 functioning (Erwin 1983; Zhang 2011) and the shortfalls in our taxonomic, biogeographic and
72 ecological knowledge are strong impediments against the integration of these organisms in
73 conservation and management strategies (Miller & Rogo 2002; Whittaker *et al.* 2005).
74 Because the few studies addressing this topic predict high extinction numbers for insects
75 (Fonseca 2009; Stork & Habel 2013), it is urgent to lift “the curse of ignorance” (Diniz-Filho
76 *et al.* 2010) by developing multi-scale studies on insect diversity that benefit from the
77 technological revolution of the ‘genomic era’ (Godfray 2006; Wilson 2003) and its recent
78 developments in biodiversity sciences (Hebert *et al.* 2003a).

79 The Afrotropical region is one of the Major Tropical Wilderness Areas on earth (Myers 1990;
80 Wilson 2002), i.e. a large and highly diverse area that has seen little impact from human
81 activities until recently (i.e. < 5 inhab km⁻² and $> 75\%$ of the original vegetation still present)
82 (Mittermeier *et al.* 1998). However, recent estimates indicate that annual net deforestation of
83 African tropical rainforests, although less dramatic than in Latin America or Southeast Asia,
84 approached 0.3 million ha/year for the 2000-2010 decade (Achard *et al.* 2014), which could
85 have led to dramatic biodiversity loss. As many as 100,000 insect species have been reported
86 from the area, but Miller and Rogo (2002) suggest that species richness could exceed 600,000
87 species. In Gabon, a central-African country which is still covered by 80% of tropical
88 rainforests, insect inventories have only considered butterflies (vande Weghe 2010), a few

89 groups with limited number of species such as Mantodea (Roy 1973), Lucanidae (Maes &
90 Pauly 1998) or Apoidae (Pauly 1998), and groups with specific economical and/or
91 agronomical importance such as Pseudococcidea (Hemiptera) and their parasitoids
92 (Boussienguet *et al.* 1991). A few studies have also targeted terrestrial arthropod assemblages
93 along human disturbance gradients (Basset *et al.* 2004, 2008).

94 Several authors emphasized the potential of using highly diverse groups, such as Lepidoptera,
95 as environmental indicators (Axmacher *et al.* 2004a, 2004b; Beck *et al.* 2013; Kitching *et al.*
96 2000; Ricketts *et al.* 2001). They are indeed key herbivores and an important link within
97 foodwebs as prey or as hosts for parasitoids. Variation in the diversity and structure of
98 Lepidopteran communities is thus likely to be representative of changes at other trophic
99 levels. For instance, lepidopteran species depend on their host plant species (or a few closely
100 related plants), and in turn play a fundamental role as pollinators; this connects them closely
101 to plant community structure and composition (Ehrlich & Raven 1964; Novotny *et al.* 2002b).
102 On the other hand, trophic cascades in food webs are likely to link both host plant and
103 primary consumer assemblages to associated higher trophic levels of predators and
104 parasitoids. Surprisingly however, only a few studies have examined this group in the
105 Afrotropics. The taxonomic deficit and the high number of species that occur in those
106 environments are certainly important causes for this deficit, because they impede reliable
107 inventories and the description of community patterns. In a recent study based on a substantial
108 sampling effort in Papua New Guinea (over 30,000 specimens collected over several years),
109 Ashton *et al.* (2014) found that no asymptote was reached by species accumulation curves.
110 These authors, however, also suggested that more limited sampling could be efficient in
111 highlighting differences in the diversity and composition of moth communities among distant
112 localities.

113 In this study, we use DNA barcodes to document and compare communities of moths in two
114 differing ecosystems of Gabon. Several recent studies have demonstrated the effectiveness of
115 DNA barcoding – a tool for species identification based on a short standardized DNA
116 fragment (Hebert *et al.* 2003b) – in documenting species diversity of lepidopteran
117 communities in regions where species assemblages are very diverse and when many species
118 are undescribed (Janzen *et al.*, 2009; Lamarre *et al.* 2016; Lees *et al.* 2014; Zenker *et al.*
119 2016). With this approach we aim at evaluating the sampling effort required to produce
120 relevant census of these communities, to document seasonal variation in community
121 composition, and if species-turnover (β -diversity) as revealed from our data is reflecting
122 significant differences in richness and composition that can be linked to the different habitats
123 sampled. Finally, we discuss the contribution of our study to the current knowledge of moth
124 diversity in Gabon and in the Afrotropical region.

125 **Material and Methods**

126 ***Study sites***

127 Moths were collected at two locations (named *Lopé 2* and *Ipassa* research station) in the
128 province of Ogooué-Ivindo, in Gabon (Figures 1 and 2):

129 - *Lopé 2* site is situated in the northern part of Lopé National Park, about 12 km south from
130 Lopé village and the Dr. Alphonse Mackanga Missandzou Training Centre (CEDAMM,
131 Wildlife Conservation Society; coordinates: S0°13'9.699" / E11°35'5.6394"; altitude: 300m).
132 Vegetation comprises a mosaic of forest and shrub savannah (Figure 2A). Shrub savannah is
133 dominated by Poaceae and Cyperaceae like *Anadelphia arrecta*, *Andropogon pseudapricus*,
134 *Schizachyrium platyphyllum*, *Hyparrhenia diplandra* or *Ctenium newtonii* and by a shrub
135 layer with *Crossopteryx febrifuga* and *Nauclea latifolia* (White & Abernethy 1997). Forest
136 patches are mainly secondary to mature okoumé rainforests, the dominant forest type in

137 western Gabon, dominated by *Aucoumea klaineana* (“okoumé”), *Lophira alata*, *Desbordesia*
138 *glaucescens*, *Scyphocephalum ochochoa*, *Dacryodes buttneri*, *Santiria trimera*, *Sindoropsis*
139 *le-testui* and *Uapaca guineensis* (Ben Yahmed & Pourtier 2004; White & Abernethy 1997).

140 - The *Ipassa* research station (Institut de Recherches en Ecologie Tropicale) is situated in the
141 northern part of Ivindo National Park, 12 km from the city of Makokou (coordinates:
142 N0°30'38.1456" / E12°48'1.2594"; altitude: 500m). The site is mainly surrounded by mature
143 Guineo-Congolese rainforest showing both Atlantic and continental influences (Doumenge et
144 al. 2004; Nicolas 1977; White 1983), with *Baphia leptobotrys* and *Millettia laurentii*
145 dominating the tree cover, as well as *Scorodophloeus zenkeri*, *Plagiostyles africana*,
146 *Dichostemma glaucescens*, *Santiria trimera*, *Polyalthia suaveolens* and *Poncovia pedicellaris*
147 (Figure 2B).

148 The two sites are 160 kilometers apart and share a similar seasonal cycle typical of the
149 equatorial transition zone, with short (January-February) and long (June-September) dry
150 seasons. The average monthly temperature is 24°C while mean annual precipitation is 1500
151 mm at Lopé and 1700 mm at Ipassa.

152 ***Moth sampling***

153 Sampling was conducted at both sites in November 2009 and at *Lopé 2* in February-March
154 2011 during a field class organized in the Lopé National Park (ECOTROP field class -
155 <http://www.ecotrop.com/ECOTROP>). We used a standard light trap technique consisting of a
156 250W UV (mercury vapor) bulb placed 4-5 meters above the ground to attract insects
157 (Figures 2A and 2C). Two low voltage lamps (80W) were positioned on both sides of a
158 vertical white sheet positioned below the UV bulb. Specimens were collected during dark-
159 moon phases from dusk to dawn (6pm to 6am, local time) in order to collect species with
160 varying flight times (Lamarre *et al.* 2015). Overall, four collecting nights were carried out in

161 each site in 2009 (10th to 14th of November at *Lopé 2* and 14th to 18th of November at
162 *Ipassa*), and three additional nights at the end of the short dry season at *Lopé 2* in 2011 (27th
163 February, 1st and 4th March). Our sampling design was therefore relevant to compare observed
164 communities between sites from the samples collected during the rainy season in 2009, and to
165 investigate seasonal turnover at *Lopé 2*.

166 Our study focuses on Macroheterocera, i.e. macromoths whose wingspan were >1 cm (Figure
167 2D). Each night and in all families of Macroheterocera, we sampled as many species as could
168 be distinguished morphologically when collecting. Specimens were killed using a cyanide jar
169 or by an injection of ammonia into the thorax for larger species. Moths were placed in
170 glassine envelopes marked with a code unique to each sampling event. Specimens were
171 subsequently sorted into morphospecies, i.e. groups of specimens that were readily
172 distinguishable from their external morphology. A maximum of four specimens per
173 morphospecies and per collecting night were selected for molecular analyses. Specimens are
174 currently deposited in the Museum national d'Histoire Naturelle in Paris, where they are
175 available for further taxonomic study.

176 ***DNA barcoding and taxonomic assignments***

177 A small piece of tissue (generally a complete leg or its tarsus for the largest species) was
178 sampled for each specimen selected in the field (Figure 2D). DNA extraction was carried out
179 at the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph following a
180 standard automated protocol (Ivanova et al. 2006; Hajibabaei *et al.* 2005). Tissue lysis
181 occurred in 50µl of lysis buffer and proteinase K [0.02 mg/µL] incubated at 56°C overnight.
182 A 658 bp segment of the 5' region of the COI mitochondrial gene used as a standard DNA
183 barcode was amplified through PCR using the primer pair LepF1/LepR1 (Hebert *et al.* 2004).
184 Samples failing to amplify after this first PCR pass were re-processed using the primer sets
185 LepF1/MLepR1 and MLepF1/LepR1 that target 307 bp and 407 bp overlapping fragments,

186 respectively (Hajibabaei *et al.* 2006). A standard PCR reaction protocol was used for all PCR
187 amplifications and products were checked on a 2% E-gel 96 Agarose (Invitrogen). Unpurified
188 PCR amplicons were sequenced in both directions using the same primers as those used for
189 the initial amplification, and following standard CCDB protocols (<http://ccdb.ca/resources/>)
190 (Hajibabaei *et al.* 2005). Trimming of primers, sequence editing and contig assembly were
191 carried out at CCDB using CodonCode software (CodonCode Corporation, Centerville, MA,
192 USA). All sequences were aligned and inspected for frame-shifts and stop codons for removal
193 of editing errors and possible pseudogenes, and then uploaded in the Barcode of Life Data
194 systems (BOLD, Ratnasingham & Hebert 2007). All records – including specimen and
195 sequence data – can be accessed publicly in BOLD and GenBank, and were assembled within
196 BOLD dataset DS-LOPELEP1.

197 Species assignments of specimens using morphology, either as named species through formal
198 identification or as provisionally delineated morphospecies, could not be achieved for all the
199 specimens, because of the lack of taxonomic expertise for many of the moths collected and
200 because processing the large number of specimens (spreading of wings and often genitalic
201 dissections) was intractable. Because morphospecies are unreliable for a thorough assessment
202 of observed species diversity (Zenker *et al.* 2016), we used DNA barcodes to delineate
203 molecular taxonomic units (MOTUs) as a proxy for species. More specifically, we used
204 Barcode Index Numbers (BINs) derived from the automated MOTU delineation tool
205 implemented in BOLD (Ratnasingham & Hebert 2013), and which have already been used to
206 consistently approximate species in Lepidoptera (Hausmann *et al.* 2013; Kekkonen & Hebert
207 2014). In two families, Saturniidae and Sphingidae, species were carefully identified (by RR
208 and TD) on the basis of morphology and the results were used to test their correspondence
209 with BINs.

210 For most specimens, we were able to provide a family-level identification based on their
211 general morphology during tissue sampling, or, subsequently using DNA barcode results
212 coupled with the BOLD identification tool as well as the topology of the NJ tree. For this
213 second approach, the richness of the BOLD DNA barcode library, with records for more than
214 100,000 species of Lepidoptera – proved very useful using a simple query for best close
215 matches in the database. Instead of applying a (necessarily subjective) threshold to generate
216 family (or occasionally subfamily and genus) assignment, we verified the proposed
217 assignments by comparing images and, where relevant, by examining the specimens and
218 confirming the proposed taxon on the basis of its morphology.

219 ***Community data analyses***

220 The α -diversity at each site was assessed by plotting rarefaction curves and their
221 extrapolations for both species richness and sample coverage, using specimen numbers as a
222 measure of sampling intensity. These analyses were carried out using the *iNEXT* package
223 (Hsieh *et al.* 2014) for R 3.0.2 (R Development Core Team 2004). We then used the *Vegan*
224 package (Oksanen *et al.* 2013) to calculate several diversity indices: observed richness
225 (defined as the total number of observed BINs at a given sampling site or on a given date),
226 Chao1, ACE and second order jackknife diversity estimators, and Fisher α -diversity index. We
227 also used *iNEXT* to calculate the number of species observed given a constant level of
228 sampling coverage, and *Vegan* for the estimation of species richness rarefied to a constant
229 level of sampling intensity (i.e. a constant number of specimens collected). We finally used
230 *fisherfit*, *prestonfit* and *prestondistr* functions of *Vegan* to plot rank-abundance diagrams and
231 fit Fisher's logseries, Preston's lognormal and truncated lognormal models to abundance data
232 for each sampling site.

233 To assess β -diversity among sampling sites (for samples collected in 2009) and seasons (in
234 *Lopé 2* site only), we calculated an average Sørensen's index of dissimilarity using the
235 package *Vegan* (Oksanen *et al.* 2013):

$$236 \beta_{BC} = (b+c)/(2 a + b + c)$$

237 where a is the number of species (here BINs) shared between two sites B and C, and b and c
238 are the numbers of unique BINs for sites B and C.

239 We used the *betapart* package to decompose β -diversity into two components (Baselga 2010):
240 nestedness (i.e. when the composition of communities with a smaller species number is a
241 subset of a richer community) which reflects non-random processes of species loss, and
242 spatial turnover which results from species replacement as a consequence of environmental
243 sorting or spatial and historical constraints (Qian *et al.* 2005; Ulrich *et al.* 2009; Wright &
244 Reeves 1992). Analyses of β -diversity were carried out with and without singletons (i.e. BINs
245 represented by a single specimen in the dataset), as their inclusion can lead to overestimation
246 of β -diversity.

247 **Results**

248 *Species richness at the regional scale*

249 We obtained 3494 (97.7%) sequences from the 3576 specimens selected for DNA barcoding.
250 These sequences included representatives of 1385 BINs representing 23 families of
251 Lepidoptera (Table 1) and only 6 specimens (6 BINs) could not be identified to family level.
252 Noctuidae, Erebidae and Geometridae represented about one third of the BINs and sampled
253 individuals, whereas 10 other families were each represented by less than 10 specimens. More
254 than half of the BINs (786 in total, 57%) were represented by a single individual in our data
255 set (i.e. singleton).

256 Morphological examination of specimens in the families Saturniidae (177) and Sphingidae
257 (267), led to the distinction of 42 and 63 species, respectively, of which only two (in family
258 Saturniidae) could not be identified to species and were given a provisional name
259 (*Orthogonioptilum* mgab_RR01 and *Dogoia* mgab_RR01). The correspondence between
260 morphologically assigned species and BINs was nearly perfect: 42 species versus 43 BINs in
261 Saturniidae (98%) and 63 versus 66 in Sphingidae (95%) (see DNA barcode NJ trees in SI
262 Figures 1 and 2). In other families, 112 species (representing 121 BINs) were formally
263 identified by taxonomic experts (see acknowledgments) or through DNA barcode matches in
264 BOLD. Overall, with Saturniidae and Sphingidae included, these species represent about 16%
265 of all BINs (230/1385).

266 Comparison between the number of BINs observed in our study and the list of recognized
267 species and subspecies for Gabon, as derived from the AfroMoths online database (De Prins
268 & De Prins 2017), revealed the strong taxonomic deficit that characterizes moth diversity in
269 the Afrotropics. AfroMoths is based on the survey of 7355 published sources for the whole
270 Afro-tropical region (as of August 8th, 2017) and the authors' own studies. It lists 1,301 moth
271 species and subspecies for Gabon, belonging to 36 families. Our survey (Figure 4), limited to
272 macro-moths collected during only 11 nights at two sites, revealed 1385 BINs in just 25
273 families. Three families (Bombycidae, Brahmaeidae, and Lecithoceridae) detected in our
274 study lack published records for Gabon in the AfroMoths database. For 10 of the 22 other
275 families, the number of BINs recorded in our study exceeded the number of known species
276 (Table 1). Large differences were observed for Cossidae (1 species in AfroMoths versus 11
277 BINs), Crambidae (9 vs. 52), Erebidae (309 vs. 369), Geometridae (184 vs. 220),
278 Lasiocampidae (68 vs. 101), Noctuidae (71 vs. 224), and Pyralidae (6 vs. 70), which may
279 represent the most understudied families or those yet incompletely surveyed in the AfroMoths
280 database.

281 In the few families that are well-studied for this region, we collected approximately half the
282 known number of species (48.2%, $sd=6.9$, $N=4$ – including Saturniidae (43 BINS vs. 110
283 species listed in AfroMoths, 39%), Eupterotidae (15 vs. 32, 47%), Sphingidae (66 vs. 124,
284 53%) and Lasiocampidae (101 vs. 188 as listed by P. Basquin, personal communication,
285 54%).

286 *Species richness and diversity patterns between sampling sites*

287 Our survey revealed a total of 823 BINs (1604 specimens analyzed) and 782 BINs (1890
288 specimens analyzed) in the *Ipassa* and *Lopé 2* sites, respectively (Table 1). Sampling resulted
289 in a high proportion of singletons at both sites (64% in *Ipassa*, 59% in *Lopé 2*; 57% when
290 combining both sites), and the distributions of BIN abundance are a strong fit to a log-series
291 model (SI Figure 3). While observed richness was similar between the sites, we collected
292 fewer BINs in *Lopé 2*, despite collecting three additional nights in this site during the dry
293 season.

294 For comparison of the two sites, we only considered specimens collected during the wet
295 season when sampling efforts were identical. The four collecting nights at each site resulted in
296 the capture of 1604 and 1110 specimens, which belonged to 823 and 481 BINs for *Ipassa* and
297 *Lopé 2*, respectively (Table 2). Richness estimators indicate that species richness ranged
298 between 1250 and 1850 species at *Ipassa* and between 700 and 1200 species at *Lopé 2*.
299 Rarefaction curves clearly show a higher richness in *Ipassa* (Figure 5a), while sampling
300 coverage rate was slightly higher at *Lopé 2* (73% versus 68% at *Ipassa*) (Table 2, Figures 4b
301 and 4c). Overall, the moth communities at both sites showed a similar relative abundance of
302 the different families, both in terms of specimen numbers and BINs, although observed
303 richness in the most diverse families was consistently higher in *Ipassa*, with the exception of
304 Crambidae and Pyralidae, which had more BINs at *Lopé 2* (Table 1).

305 Comparison of BINs collected during the wet season at *Lopé 2* and *Ipassa* revealed only 158
306 BINs shared by the two sites, 13.8% of the total number analyzed. Sørensen's index of β -
307 diversity calculated between the two sites was 0.76 for the whole dataset and 0.42 after
308 singletons were removed (Table 3). In both cases, β -diversity was mainly explained by spatial
309 turnover (71.0% and 67.6%, respectively) and to a lesser extent by nestedness (29.0% and
310 32.4%).

311 ***Seasonal changes in moth assemblages at Lopé 2***

312 We generated DNA barcodes for 1110 and 780 specimens from *Lopé 2* during the rainy and
313 the dry seasons, respectively. Observed richness during the wet season was slightly higher
314 (478 BINs versus 441 BINs during the dry season), but this trend was reversed after rarefying
315 richness to a constant sampling effort or a constant sampling coverage. Rarefaction curves
316 and diversity estimators were also quite similar, the later ranging between 650 and 1100 for
317 both seasons (Figure 5).

318 During the dry season, we collected moths belonging to 17 families versus 21 families during
319 the wet season. Seven families were not shared between the two sampling seasons, but all
320 were represented by few BINs (maximum 2) and individuals (maximum 2), excepting one
321 BIN in the family Thyrididae for which 18 specimens were collected in the wet season.
322 Overall, the diversity for each family was similar for the two sampling periods (Table 1) with
323 a few exceptions: the Crambidae (31 vs. 16 BINs), Pyralidae (37 vs. 18), and Saturniidae (31
324 vs. 9), which were all more diverse during the wet season, and the Sphingidae (40 vs. 28) that
325 was more diverse during the dry season. Out of a total of 782 BINs, 144 (i.e. 18.5%) were
326 found during both the rainy and the dry seasons. Sørensen's index of dissimilarity between
327 seasons was 0.69, largely explained by temporal turnover (95.4%), but it dropped to 0.23 and
328 was evenly explained by turnover and nestedness after removing singletons from the data set
329 (Table 3).

330 **Discussion**

331 *DNA barcodes for the study of moth diversity in the tropics*

332 Of the 25 moth families identified among more than 3500 collected specimens, three (namely
333 Bombycidae, Brahmaeidae and Lecithoceridae) have no record from Gabon in the AfroMoths
334 database (De Prins & De Prins 2017) and ten have a number of BINs equal or higher than the
335 number of species currently reported therein. Overall, we documented 1385 species-level
336 molecular units (BINs), a number slightly higher than the 1301 species listed for the country
337 in AfroMoths. Considering the relatively shallow geographical range and temporal extent of
338 our study, this result highlights the weakness of the current knowledge of moth diversity in
339 the Afrotropics, despite the remarkable efforts by De Prins & De Prins (2017) to synthesize
340 and centralize this knowledge in the AfroMoths database. Our results clearly highlight the
341 value of DNA barcoding for producing a rapid and accurate census of moth diversity in a
342 poorly studied tropical region. Because this approach facilitates comparisons between
343 sampling campaigns through barcode matches (as exemplified here between sites, but it can
344 also be applied between countries as currently in progress with a similar campaign in Central
345 African Republic), its systematic implementation would represent a powerful mean to address
346 both the Linnean and Wallacean shortfalls (Lomolino 2004), i.e. the inadequacies in
347 taxonomic and distributional knowledge that characterise most invertebrate taxa in poorly
348 studied regions such as the Congo basin (Whittaker *et al.* 2005).

349 In our study, the large number of BINs without taxonomic assignation at species level (1155
350 out of 1385) corresponds both to already known species not yet documented in the BOLD
351 libraries and to species that are new to science. The number and proportion of the later
352 remains unclear and further study by expert taxonomists of the specimens collected is needed,
353 as well as continued efforts to populate DNA barcode reference libraries. In addition, the
354 inflation of species numbers in many families may reflect an incomplete census of Gabonese

355 records in past studies, a considerable task initiated in the AfroMoths database, but certainly
356 suffering from the absence of recent dedicated efforts to synthesize Lepidopteran diversity
357 data for this country. The bombycid *Amusaron kolga* (Druce, 1887) and brahmaeid
358 *Dactyloceras lucina* (Drury, 1782) for instance represent new records for their respective
359 families in Gabon, but are species known to occur in neighbouring countries of the Congo
360 basin (De Prins & De Prins 2017). In Lasiocampidae the number of species listed in
361 AfroMoths (68) is identical to the number of species reported from an independent literature
362 survey by a specialist of this family on the African continent (P. Basquin, personal
363 communication). Furthermore, this same taxonomic authority (unpublished results) has
364 recorded approximately 188 Gabonese lasiocampid species in natural history collections
365 worldwide, which clearly demonstrates how insufficient the published data are for this family
366 at the regional scale and is consistent with the number of BINs (101) reported in our study.

367 Molecular data can reveal cases of cryptic species, i.e. species that cannot be distinguished
368 from morphological characters, or that present subtle morphological and/or ecological traits
369 previously ascribed to intra-specific variation or thought to be insignificant for species-level
370 recognition (Janzen *et al.* 2009; Janzen *et al.* 2013; Rougerie *et al.* 2014). In our study, such
371 discrepancies between morphologically identified and molecular species were found in four
372 cases within Saturniidae and Sphingidae, corresponding to supposedly morphological well-
373 defined species that appeared to be split into two or three distinct BINs. This is also likely to
374 be the case in more speciose and less studied families such as Erebidae, Geometridae and
375 Noctuidae, leading to an increase of species numbers in these groups compared to available
376 checklists that are only based on morphologically recognized species.

377 ***Moth diversity at Ivindo and Lopé National Parks***

378 Among the 1385 BINs found in our samples, 796 (i.e. 58% of the total) were represented by a
379 single specimen, which is a high singleton proportion compared to the average of 32% found

380 by Coddington *et al.* (2009) in a review of tropical arthropod studies. There are little or no
381 biological explanations for the high proportion of rare species usually found in tropical insect
382 surveys (Novotný & Basset 2000). Rather, this pattern can be attributed to undersampling of
383 highly diverse communities (Coddington *et al.* 2009), suggesting that caution should be taken
384 when interpreting the observed patterns of community composition and structure. It also
385 suggests that the estimates of species richness derived from our results probably represent a
386 low estimation of the actual diversity of these ecosystems. Both rarefaction curves and
387 sampling coverage indices (Figure 5, Table 2) support this idea, suggesting that at least twice
388 the number of collected species may occur in the study area.

389 We found only a few studies that assessed moth local richness in tropical rainforest or
390 savannah ecosystems and that can be readily compared with our own results. Ashton *et al.*
391 (2014) sampled 791 to 2795 species and produced Chao1 estimates ranging from 1478 to
392 3666 among three rainforest locations in Malaysia. In Costa Rica, Janzen *et al.* (2009)
393 published a census of 2349 species using a DNA barcode-based assessment of macro-moth
394 assemblages in the Area de Conservación Guanacaste. On the other hand, Hawes *et al.* (2009)
395 reported 98 species of Arctiinae (Erebidae), 43 of Saturniidae and 5 of Sphingidae in a
396 primary forest area of Brazilian Amazonia, which is well below our findings in the present
397 study. Variations in the number of species observed among studies are however difficult to
398 interpret, because they can both reflect real differences in species diversity, but can also be
399 biased by differences in sampling efforts and/or sampling performed in different seasons. In
400 fact, the moth sampling by Ashton *et al.* (2014) and Hawes *et al.* (2009) was done through
401 264 and 30 collecting nights per study site, respectively, while the survey of Janzen *et al.*
402 (2009) was conducted over decades and involved additional sampling methods (in particular,
403 the mass rearing of caterpillars). Comparing the results obtained in different studies and with
404 different sampling intensity requires standardization through rarefaction procedure (Gotelli &

405 Colwell 2001). Applying this approach to the data from Ashton *et al.* (2014) produces a result
406 different from what can be directly deduced from observed richness (Nakamura A., Ashton
407 L.A., Kitching. R.L., personal communication). For instance, species numbers in their
408 Malaysian sites ranged from 290 to 475 after standardization to a constant sampling effort of
409 1000 individuals, and between 100 to 270 at a constant sampling coverage of 50%, which was
410 lower to what we found in our two study sites (Table 2). This suggests that Central African
411 rainforests may represent an important hotspot for moth diversity.

412 ***Variation in moth diversity and composition among study sites***

413 Our analyses of moth assemblages during the rainy season in the rainforest of *Ipassa* and the
414 savannah/forest landscape of *Lopé 2* unveiled significant differences in both species diversity
415 and composition. As expected from differences in vegetation coverage, the observed and
416 estimated richness were both higher in *Ipassa*. Plant diversity is indeed higher in the rainforest
417 landscape of *Ipassa* than in the shrubland savannahs and peaty marshes that dominate the
418 landscapes of the northern part of Lopé National Park (White & Abernethy 1997). In addition,
419 despite presenting a comparable structure, forests at *Ipassa* are more humid and present
420 higher tree diversity when compared with the gallery forests of *Lopé 2*. These features
421 presumably offer a broader diversity of ecological niches in terms of trophic resources and
422 microhabitats, in particular via the important diversity of epiphytes and lianas (Ben Yahmed
423 & Pourtier 2004).

424 Difference in species assemblage composition among sites was high, with only 13.3% of
425 BINs found in both. This high β -diversity was mainly attributed to spatial turnover, meaning
426 that undersampling may only weakly account for this variation. This is in contrast with other
427 studies that reported relatively low β -diversity of insect herbivores in comparable tropical
428 rainforest habitats (Basset *et al.* 2012; Novotny *et al.* 2007). This also concords with other
429 studies having reported high species turnovers among sites as long as these comprised enough

430 variability in vegetation types (Beck & Chey 2007; Ødegaard 2006). In fact, contrasted
431 composition of dominant forest tree species among our study sites may have selected for
432 different assemblages of herbivorous species, as leaf-chewing insects are usually specialized
433 on a single genus of host plants (Novotny *et al.* 2002a, 2002b). Similarly, the presence of
434 herbaceous ecosystems and secondary forests at *Lopé 2* may have also driven the presence of
435 specific species assemblages associated with these open habitats. The high diversity of
436 Crambidae and Pyralidae observed at this site compared to *Ipassa* could for instance be linked
437 to species preferences within these groups for herbaceous host-plants (Kitching *et al.* 2000).

438 Even if additional sampling is necessary to confirm this finding, these preliminary results
439 suggest that landscapes dominated by a savannah-forest patchwork may host substantial levels
440 of herbivore insect diversity with a high compositional specificity at species level compared
441 to typical tropical rainforests. This argues in favor of a better consideration of savannah
442 ecosystems in both global estimates and conservation strategies of insect biodiversity.

443 ***Seasonal variation of moth assemblages***

444 At *Lopé 2* we found little difference in species richness of moth assemblages collected during
445 the rainy and the dry seasons. In contrast, BINs compositions clearly differed from one season
446 to the other, with only 18.3% of the BINs collected being observed in both seasons, and this
447 temporal β -diversity being clearly explained by seasonal turnover rather than by nestedness
448 (Table 3). Composition may simply be influenced by the level of vegetation development
449 during the seasonal cycle, which is well known to influence the phenology of lepidopteran
450 species, or by different climatic preferences linked to the feeding and/or reproductive activity
451 of the moths.

452 From a methodological point of view, these results highlight the importance of standardizing
453 the period of sampling to provide fully comparable results among different localities. They

454 also suggest the need of sampling different seasons to obtain a reliable inventory of species at
455 a given study site, as the assemblages observed at the rainy season (the usually preferred
456 period for moth collecting) clearly do not provide a representative overview of the actual
457 species composition of the focal community.

458 ***Conclusion***

459 Our study highlights the usefulness of utilizing DNA barcodes for performing rapid analyses
460 of taxonomic diversity and composition of moth assemblages in poorly studied areas. It also
461 stresses the need to accelerate biodiversity inventories in those areas that have been
462 insufficiently explored regarding moths and other poorly studied invertebrates. Central Africa
463 clearly is one of those areas and our results represent the first robust assessment of moth
464 diversity in Gabonese forests and savannahs, highlighting a strongly understudied fauna. The
465 material collected and the DNA barcode library released with this study are thus important
466 contributions and we expect that they will serve the development of knowledge on the
467 diversity and distribution of African moths. In general, studies combining molecular data and
468 traditional taxonomic expertise are critically needed to better document invertebrate
469 communities in tropical areas, especially in the regions where anthropogenic pressures are
470 high and where species extinctions remain unaccounted for because species simply remain
471 undocumented.

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703 **Tables**

704 **Table 1.** Number of individuals and number of BINs collected for different families/sub-
 705 families of macro-moths at the two study sites and for two seasons at *Lopé 2*, and number of
 706 species listed in the AfroMoths online database (grey bars; De Prins & De Prins 2017) for the
 707 same families/sub-families (WS= wet season; DS= dry season).

	Ipassa (WS)		Lopé (WS)		Lopé (DS)		Lopé		Total	
	# ind	# BINs	# ind	# BINs	# ind	# BINs	# ind	# BINs	# ind	# BINs
Bombycidae	2	2							2	2
Brahmaeidae	2	1	2	1			2	1	4	1
Cossidae	6	5	5	4	8	4	13	8	19	11
Crambidae	17	14	73	31	19	16	92	43	109	52
Drepanidae	6	4			1	1	1	1	7	5
Erebidae (Arctiinae)	198	71	131	41	75	37	206	65	404	113
Erebidae (Erebinae)	75	38	71	24	47	34	118	46	193	72
Erebidae (Lymantriinae)	220	103	47	32	79	54	126	73	346	164
Other Erebidae	61	33	102	18	22	18	124	32	185	60
Eriocottidae			2	2			2	2	2	2
Eupterotidae	13	10	22	3	5	3	27	5	40	15
Euteliidae			1	1	4	2	5	2	5	2
Geometridae	293	153	130	63	117	62	247	107	540	220
Lasiocampidae	88	55	80	42	74	36	154	61	242	101
Lecithoceridae			1	1	2	1	3	2	3	2
Limacodidae	31	15	20	14	8	7	28	18	59	30
Noctuidae	199	125	103	66	95	69	198	124	397	224
Nolidae			2	2			2	2	2	2
Notodontidae	155	77	72	31	45	27	117	49	272	104
Psychidae	1	1	2	2	6	4	8	4	9	5
Pyralidae	65	29	82	37	25	18	107	49	172	70
Saturniidae	62	32	79	31	36	9	115	33	177	43

Sphingidae	98	44	58	28	111	40	169	47	267	66
Thyrididae	4	4	18	1			18	1	22	5
Tineidae			2	1			2	1	2	1
Tortricidae	5	4			1	1	1	1	6	5
Uraniidae			1	1			1	1	1	1
Zygaenidae	1	1					0		1	1
Not identified	2	2	4	4			4	4	6	6
Total	1604	823	1110	481	780	443	1890	782	3494	1385

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710 **Table 2.** Summary of macro-moth data sets collected at the two study sites and for two
 711 seasons in *Lopé 2* (numbers in parentheses represent the 95% confidence intervals based on a
 712 bootstrap method with 200 replications).

	Ipassa (WS)	Lopé (WS)	Lopé (DS)	Lopé
Number of individuals collected	1604	1110	780	1890
Observed richness	823	481	443	782
Proportion of singletons (%)	63.85	63.61	64.93	59.31
Sampling coverage (%)	67.32 (± 2.95)	72.44 (± 2.27)	63.14 (± 3.02)	75.54 (± 1.77)
Richness at constant sampling coverage of 50%	469.2 (± 13.8)	197.6 (± 7.0)	313.7 (± 12.9)	330.4 (± 7.6)
Richness at constant sampling intensity of 1000 indiv.	599.1 (± 8.9)	449.9 (± 4.4)	511.4 (± 25.6)	521.5 (± 9.5)
Chao1 estimated richness	1837.0 (± 130.6)	1011.6 (± 107.9)	869.5 (± 70.9)	1513.4 (± 96.5)
ACE estimated richness	1849.4 (± 27.6)	1120.6 (± 20.7)	1054.4 (± 22.9)	1629.5 (± 26.2)
				1211.4 (\pm
First order jackknife estimated richness	1269.2 (± 286.2)	728.5 (± 169.7)	663.7 (± 166.9)	197.0)
Fisher alpha	678.3 (± 36.5)	318.2 (± 21.0)	419.3 (± 32.3)	491.5 (± 25.0)

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714

715 **Table 3.** Comparison of macro-moth species assemblages between the two study sites and for
 716 two seasons in *Lopé 2* showing the Sørensen index of dissimilitude (with singletons removed
 717 or not from the dataset) and its partitioning into geographical/seasonal turnover and
 718 nestedness.

	Sørensen	Turnover (%)	Nestedness (%)
Ipassa vs Lopé 2	0.75	70.97	29.03
Same without singletons	0.40	67.57	32.43
Wet vs dry season (Lopé 2)	0.69	95.39	4.61
Same without singletons	0.23	54.76	45.24

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721 **Figures**

722 **Figure 1.** Location of the study sites; Dark grey areas on the upper right map represent
723 National Parks in Gabon. The *Ipassa* site is located near Ivindo.

724 **Figure 2.** Photos of the two study sites and sampling methods: A) View of the savannah-
725 forest patchwork in Lopé National Park, showing the position of the light trap (*Lopé 2*,
726 November 2009); B) Rainforests at *Ipassa* research station at the edge of the Ivindo river
727 (November 2009); C) Light trapping at *Lopé 2* in March 2011; D) Tissue sampling for DNA
728 barcoding during the ECOTROP field class in March 2011.

729 **Figure 3.** Diversity and composition of the macro-moth sample at the two locations (*Lopé 2*
730 and *Ipassa*): the circular phylogram represents the results of a Neighbor Joining analysis in
731 BOLD of 3,494 COI sequences clustering into 1,385 BINs; barcodes obtained for specimens
732 from *Ipassa* are in green while those from *Lopé 2* are in grey. The pie chart represents the
733 relative contribution (ordered) of the different families and sub-families (for Erebidae) of
734 moths collected in the two sites; numbers within brackets indicate the number of BINs and
735 number of specimens sampled, respectively.

736 **Figure 4.** Comparisons between the numbers of BINs observed in this study for 28 families
737 and sub-families of macro-moths (dashed bars) and the numbers reported from Gabon in the
738 AfroMoths online database (grey bars; De Prins & De Prins 2017).

739 **Figure 5.** Individual-, sample-, and coverage-based rarefaction and extrapolation curves for
740 the two study sites and for two seasons at *Lopé 2* (DS: dry season, WS: wet season): A) Size-
741 based rarefaction/extrapolation curves; B) Sample coverage plotted against the number of
742 individuals; C) Coverage-based rarefaction/extrapolation (rarefaction curves are represented
743 in solid lines, extrapolation curves in dashed lines; shaded areas represent a 95% confidence
744 intervals based on a bootstrap method with 200 replications).

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747 **Supplementary material**

748 **SI Table 1.** List of members of the ECOTROP team and their affiliations.

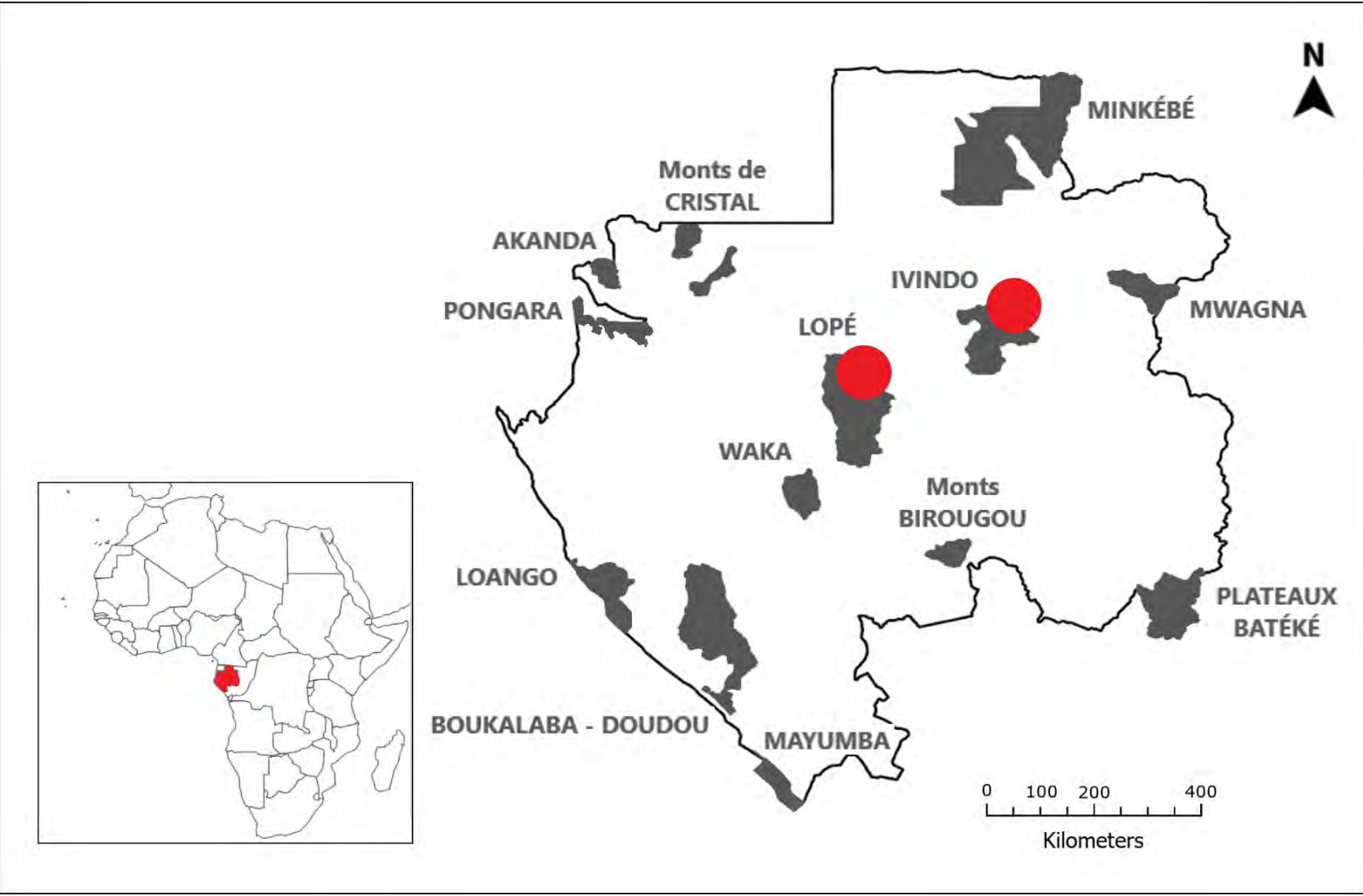
749 **SI Fig. 1.** Neighbour Joining (NJ) tree based on K2P distances for the Sphingidae and
750 Saturniidae moths collected in *Ipassa* (terminals labelled as ‘makokou’ in the tree) and *Lopé 2*
751 (labelled as ‘La Lope’). The tree was produced with records in BOLD dataset DS-LOPELEP1
752 using BOLD-alignment and default settings.

753 **SI Fig. 2.** Images of specimens in NJ tree of SI Fig. 1; numbers of images correspond to
754 numbers of terminals in SI Fig. 1 tree.

755 **SI Fig. 3.** Rank-abundance diagrams for *Ipassa* (left panels) and *Lopé 2* (right panels): (A)
756 and (C) represent Fisher's logseries functions fitted on abundance data; (B) and (D) represent
757 Preston's lognormal (red lines) and truncated lognormal (blue lines) models.

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759



A



B



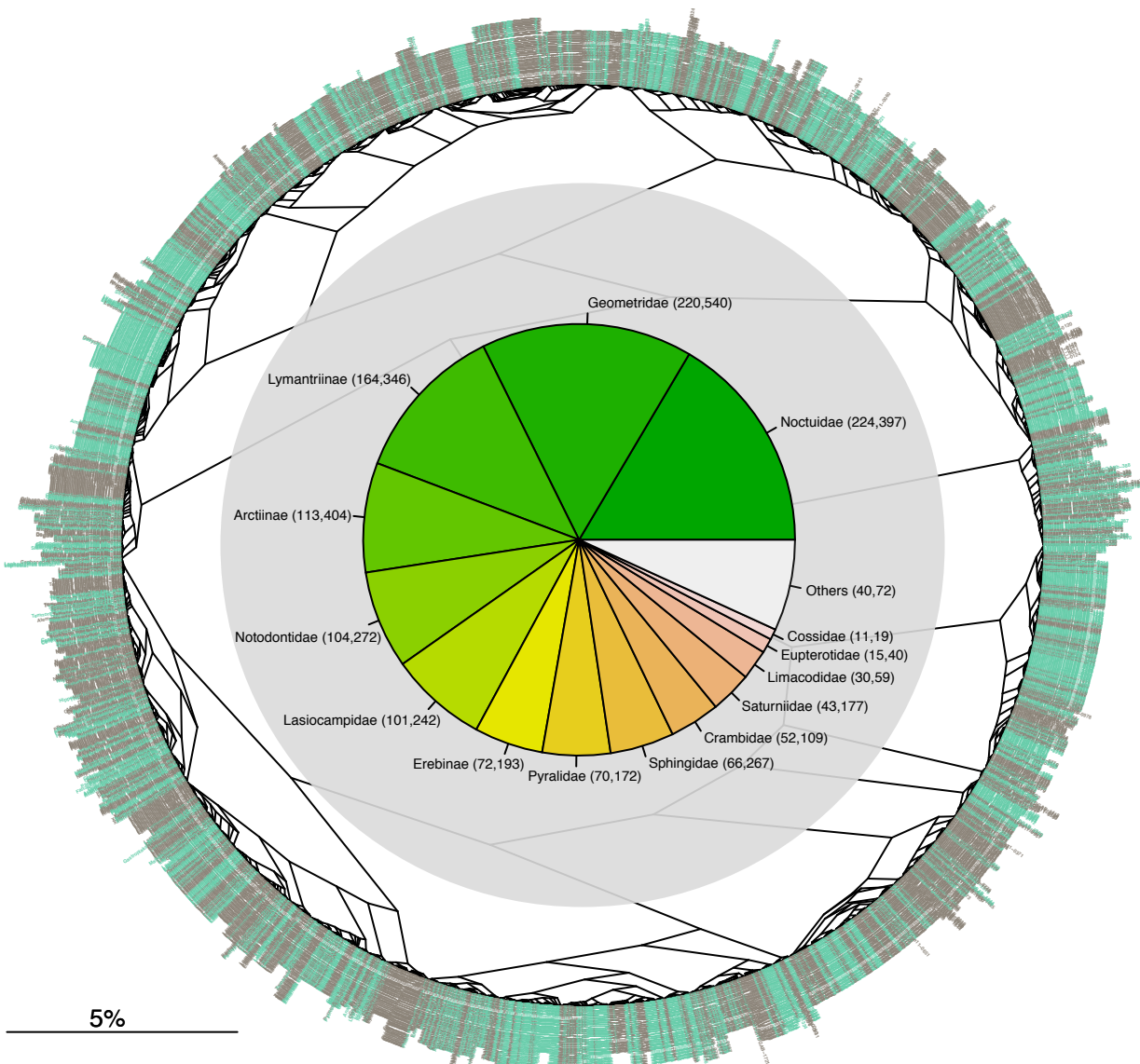
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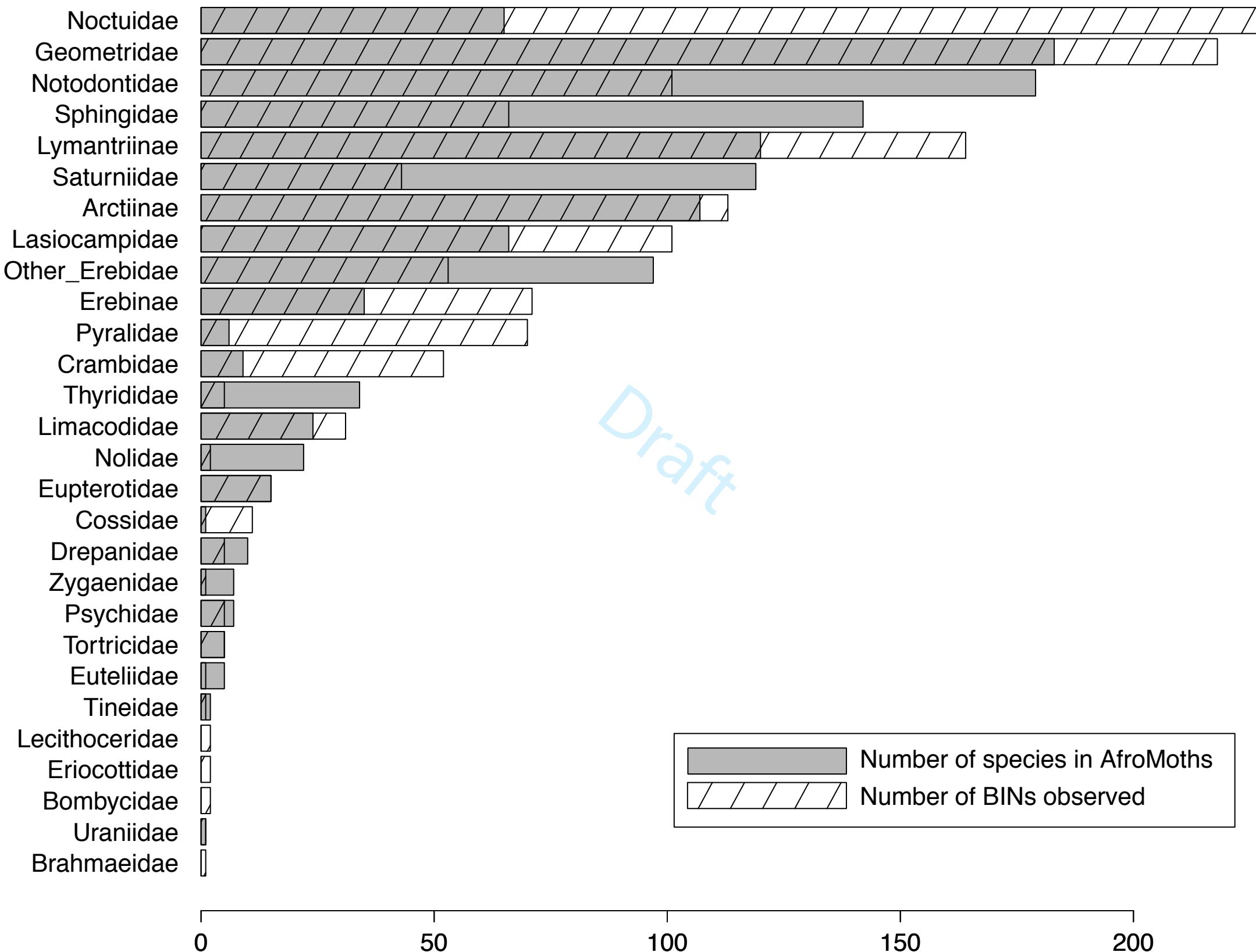
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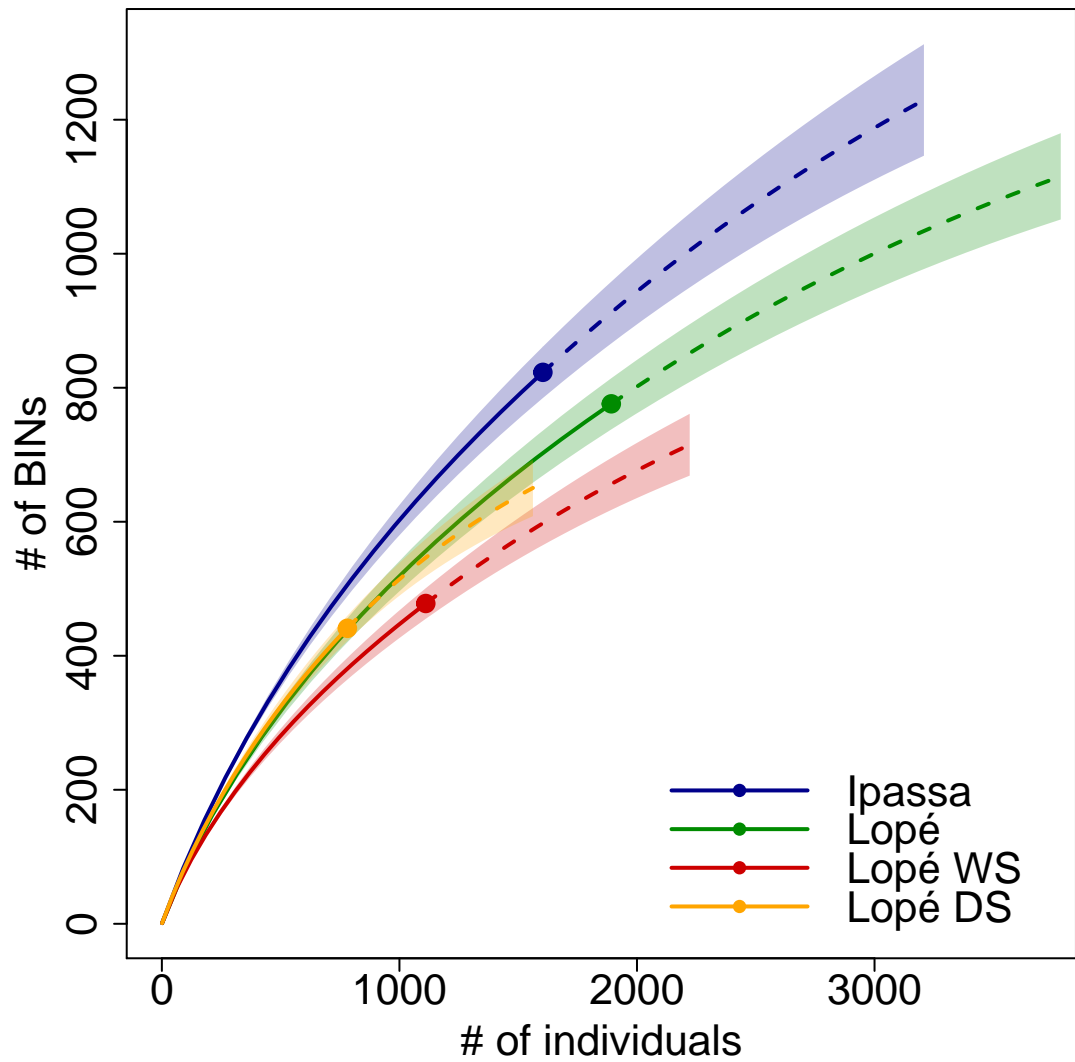
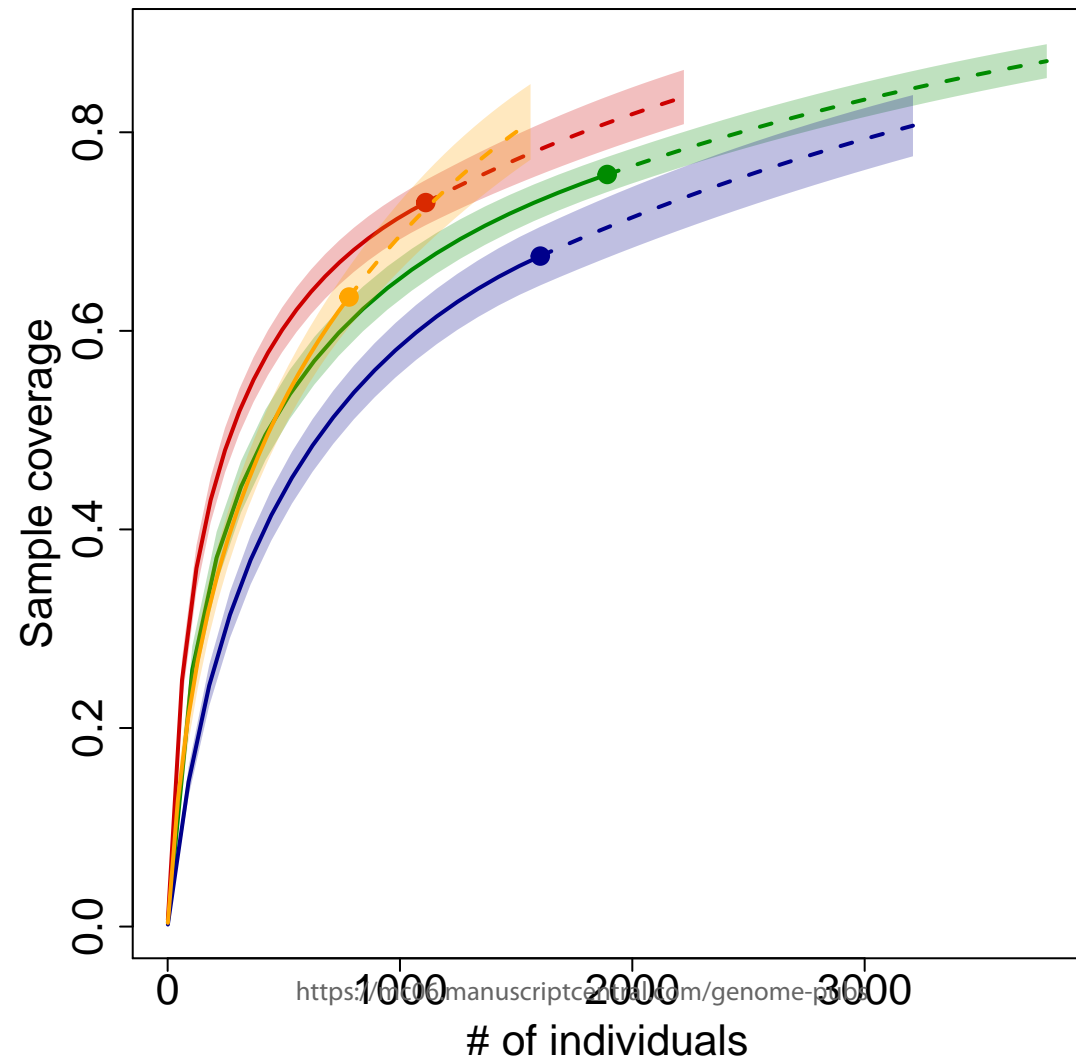
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A**B****C**