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1 Which frugivory-related traits facilitated historical long-distance 2 dispersal in the custard apple family (Annonaceae)?

3
4 **Running title: Long-distance dispersal traits in Annonaceae**

5
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36

37 **Abstract**

38

39 **Aim**

40 Long-distance dispersal has contributed to the disjunct biogeographical distribution of rain
41 forest plants – something that has fascinated biogeographers since Humboldt’s time.
42 However, the dispersal ‘agent’ for these tropical plant lineages remains puzzling. Here, we
43 investigate which frugivory-related traits may have facilitated past intercontinental long-
44 distance dispersal in the custard apple family (Annonaceae), a major vertebrate-dispersed
45 tropical plant family. We hypothesize that long-distance dispersal was associated with the
46 evolution of traits related to dispersal by large-bodied mammals (e.g. large, dull-coloured,
47 ‘megafaunal’ fruits) and strong-flying, ocean-crossing birds and bats (e.g. dehiscent,
48 moniliform or cauliflorous fruits).

49

50 **Location**

51 Global

52

53 **Taxon**

54 Annonaceae

55

56 **Methods**

57 We used a fossil-calibrated phylogenetic framework to infer the biogeographic history of 234
58 Annonaceae species (10%, covering nearly all genera) in relation to the evolution of 15
59 frugivory-related traits, using maximum likelihood and Bayesian inferences. Furthermore, we
60 used linear and generalized linear models and phylogenetic simulations to test whether
61 ancestral fruit traits during intercontinental dispersal were different from those of other
62 lineages not involved in long-distance dispersal.

63

64 **Results**

65 We inferred the ancestral Annonaceae fruits to be small with a single or few small seeds and a
66 small number of fruitlets. These fruits were most probably apocarpous, indehiscent and/or
67 moniliform (i.e. long beads of fruitlets). Furthermore, most of the long-distance dispersal
68 events in Annonaceae occurred via the expanded tropical forests in the Early Cenozoic
69 (‘geodispersal’), and were significantly associated with large (c. 3 cm long), dull-coloured
70 fruits, and short stipes. Additionally, long-distance dispersal was also facilitated by dehiscent,
71 moniliform and non-cauliflorous fruits.

72

73 **Main conclusions**

74 We suggest that the evolution of frugivory-related traits associated with dispersal by
75 frugivores that frequently move across large distances and/or barriers, such as large-bodied
76 mammals and strong-flying birds, has contributed to the disjunct tropical biogeographical
77 distribution of Annonaceae, and probably of tropical rain forest plants more generally.

78

79 **Keywords:** Annonaceae, frugivory, functional traits, fruit size, geodispersal, historical
80 biogeography, long-distance dispersal, megafauna, rain forest, vicariance.

81

82 Introduction

83

84 Since the travels of Alexander von Humboldt (1889), the tropical rain forest biome, its
85 diversity, and the disjunct biogeographic distribution of rain forest families, has fascinated
86 biogeographers. Indeed, rain forests comprise an extraordinary plant diversity even though
87 they cover only ca. 7% of the Earth's surface (Morley, 2000). Numerous major plant lineages,
88 such as palms (Arecaceae), custard apples (Annonaceae) or laurels (Lauraceae), are distributed
89 across all major tropical rain forest realms of the world. These lineages are often
90 physiologically dependent on the humid and hot climatic rain forest conditions (Morley,
91 2003). Traditionally, it was thought that these intercontinental disjunct distributions of rain
92 forest lineages were the result of 'vicariance' – i.e. the divergence of a once widespread
93 lineage due to the break-up of the Gondwanan supercontinent (Raven & Axelrod, 1974).
94 However, estimates of divergence times using fossil-calibrated phylogenetic trees have shown
95 that the timing of continental break-up often preceded the timing of lineage divergence
96 associated with the colonization of new continents (Davis *et al.*, 2002; Renner *et al.*, 2010;
97 Couvreur *et al.*, 2011; Baker & Couvreur, 2013). Long-distance dispersal (e.g. overland
98 dispersal of distances > c. 1000 km and/or across oceans, Pires *et al.*, 2017) therefore seems
99 the most plausible scenario to explain much of the disjunct distributions of tropical plant
100 lineages. However, it is often unclear by which dispersal mode (e.g. wind, water or animals)
101 tropical plants historically colonized new continents and their rain forests.

102 Up to 94% of extant woody tropical rain forest plant species depend on fruit-eating
103 animals ('frugivores') for their seed dispersal (Jordano, 2000), in which frugivores are
104 rewarded by obtaining important nutrients from the fruits they feed on. Rain forest taxa often
105 show high degrees of continental endemism (at species and genus levels) (e.g. palms, Kissling
106 *et al.*, 2012), because intercontinental dispersal by animals, as compared to wind or water, is
107 relatively rare. Indeed, seed dispersal distances, the geographic pattern of seed rain, and the
108 frequency of long-distance dispersal of animal-dispersed taxa are determined by frugivores
109 and their movement behaviours (Nathan *et al.*, 2008; Pires *et al.*, 2017), which may in turn
110 influence the diversification of fleshy-fruited plants (Onstein *et al.*, 2017). Long-distance
111 dispersal is typically rare ("fat-tailed dispersal kernels", Nathan & Muller-Landau, 2000) and
112 mostly depends on dispersal by large-bodied and wide-ranging frugivorous megafauna (e.g.
113 tapirs [Tapiridae], elephants [Elephantidae]) or strong-flying volant frugivores (e.g. hornbills
114 [Bucerotidae], toucans [Ramphastidae] and fruit bats [Pteropodidae]) (Shanahan *et al.*, 2001;
115 Holbrook *et al.*, 2002; Nathan *et al.*, 2008; Schurr *et al.*, 2009; Pires *et al.*, 2017). It is
116 therefore expected that the largest and most widespread frugivores have been most important
117 for the historical dispersal of tropical plant lineages across continents, but this remains largely
118 untested.

119 We take a trait-based approach to identify frugivory-related plant traits associated
120 with long-distance seed dispersal, in order to understand which fruits and frugivores may
121 have been most important for historical long-distance dispersal. Large-bodied frugivores, for
122 example, are more likely to perform long-distance seed dispersal and often prefer the largest
123 fruits (Jordano, 2000; Herrera, 2002), thereby increasing the probability of long-distance
124 dispersal for plant species with large fruits compared to those with smaller fruits. Similarly,
125 primates [Primates] disperse seeds over long distances and in multiple habitats due to their
126 large home ranges, and often prefer dull-coloured fruits (Gautier-Hion *et al.*, 1985;
127 Lomáscolo & Schaefer, 2010), thus increasing the probability of long-distance dispersal for
128 plants with dull-coloured fruits compared to those with bright-coloured fruits. Furthermore,

129 strong-flying, volant frugivores, such as certain species of birds (e.g. hornbills) and bats (e.g.
130 fruit bats), are able to cross barriers (e.g. water) and thereby increase the chance for long-
131 distance dispersal (Onstein *et al.*, 2017). Small-seeded fruits with a smooth exocarp, dehiscent
132 or moniliform fruits (i.e. fruits constricted between the seeds, like a necklace) or cauliflory,
133 have been associated with bird- or bat-dispersal, potentially increasing the probability of long-
134 distance dispersal for these fruits (van der Pijl, 1972; Janson, 1983; Gautier-Hion *et al.*, 1985;
135 Lomáscolo *et al.*, 2010). Thus, certain frugivory-related ‘trait syndromes’ (i.e. sets of
136 correlated traits) (van der Pijl, 1972; Janson, 1983; Gautier-Hion *et al.*, 1985; Lomáscolo *et*
137 *al.*, 2010) may have predisposed plants for long-distance seed dispersal. However, the role of
138 these traits in relation to the disjunct biogeographical distribution of vertebrate-dispersed
139 plant lineages remains puzzling.

140 Here, we investigate which frugivory-related functional traits may have facilitated
141 intercontinental long-distance dispersal (i.e. overland dispersal across large distances > c.
142 1000 km and/or across oceans, Pires *et al.*, 2017). First, we hypothesize that long-distance
143 dispersal was associated with relatively large fruits with dull colours (i.e. brown, green), and /
144 or large, (pseudo-)syncarpous fruits (in some species described as "megafaunal fruits",
145 Guimarães *et al.*, 2008) (see Figure 1 for examples). These ‘mammal’ trait syndromes (Figure
146 2, Janson, 1983) may have enhanced overland long-distance seed dispersal by large-bodied
147 megafauna (e.g. primates, elephants, tapirs, or extinct giant sloths [Megatheriidae] and
148 gomphotheres [Gomphotheriidae]) and thus increase the probability of long-distance
149 dispersal, as compared to small, bright-coloured and / or apocarpous fruits. Second, we expect
150 small seeds, a smooth exocarp, conspicuous, dehiscent or moniliform fruits and / or
151 cauliflorous fruits to be associated with long-distance dispersal (see Figure 1 for examples).
152 These ‘bird/bat’ trait syndromes (Figure 2, Janson, 1983) may have increased the opportunity
153 for long-distance dispersal by strong-flying, ocean-crossing, volant frugivores as compared to
154 large-seeded, warty or pubescent, indehiscent, non-moniliform and / or non-cauliflorous
155 fruits. As some of these traits may be correlated to other plant traits (e.g. plant height) or
156 present a resource-allocation trade-off (e.g. number vs. size of fruits/seeds and stipe length,
157 Table 1) we also included plant height, growth form, stipe length, fruit and seed number in
158 our analysis.

159 We test these hypotheses in the custard apple family (Annonaceae, Magnoliales), a
160 major vertebrate-dispersed, globally-distributed plant family, typically confined to the rain
161 forest biome (Keßler, 1993). Annonaceae comprises c. 2400 species and has been well
162 studied from phylogenetic (Chatrou *et al.*, 2012; Guo *et al.*, 2017a), evolutionary (Couvreur *et*
163 *al.*, 2011; Pirie *et al.*, 2018) and biogeographical (Doyle *et al.*, 2004; Couvreur *et al.*, 2011;
164 Zhou *et al.*, 2012; Thomas *et al.*, 2015) points of view. Furthermore, almost all Annonaceae
165 species are dispersed by animals, ranging from birds, bats, primates, small-bodied mammals
166 (e.g. rodents), and, in some cases, fish (Keßler, 1993). Consequently, the family shows a
167 spectacular diversity of fruit types (Figure 1). We use a newly generated fossil-calibrated
168 phylogenetic framework of Annonaceae, on which we identify long-distance dispersal events
169 using historical biogeographical inference. Furthermore, we reconstruct the ancestral states of
170 fifteen frugivory-related functional traits and use linear and generalized linear models and
171 phylogenetic simulations to identify which fruit traits are significantly associated with long-
172 distance seed dispersal events, as compared to other lineages not involved in long-distance
173 dispersal.

174 **Materials and Methods**

175

176 **Molecular dataset**

177 We sampled 234 Annonaceae species (c. 10% of total) representing 105 (c. 97% of total)
178 genera and all four subfamilies, and seven outgroup taxa from related families (Annonaceae
179 was constrained to be monophyletic). For these species, we generated a molecular
180 supermatrix of eight plastid markers as assembled by Chatrou et al. (2012) supplemented with
181 157 additional sequences for 37 species. For details on the methods, see Chatrou et al. (2012),
182 GenBank numbers for the new sequences are provided in Appendix S1.

183 **Phylogeny and dating**

184 We reconstructed and dated the Annonaceae phylogenetic tree in BEAST 1.8.2 (Drummond
185 & Rambaut, 2007). We used the uncorrelated lognormal clock model and a Yule birth prior,
186 but both the Yule and birth-death models inferred similar divergence times (Figure S1).
187 Substitution rates of markers were linked, because all markers are located on the chloroplast
188 and linkage allowed the analyses to reach convergence. We used the GTR + gamma +
189 invariant sites substitution rate model. To date the Annonaceae, we used the fossils
190 *Endressinia* (most-recent common ancestor of Magnoliaceae and Annonaceae; 113 Ma, Mohr
191 & Bernardes-de-Oliveira, 2004; Massoni *et al.*, 2015a) for the split between Magnoliaceae
192 and Annonaceae, and *Futabanthus* (89 Ma, Takahashi *et al.*, 2008) for the Annonaceae crown
193 node (for more details, see Appendix S2).

194 **Fruit traits**

195 We collected data on 15 frugivory-related functional traits (six continuous traits and nine
196 binary traits) for the same Annonaceae species for which sequence data were available,
197 excluding seven species because of lack of fruit trait data (i.e. total trait data for $n = 228$
198 species, $n = 3680$ data records scored from 197 sources). These species cover the taxonomic
199 and functional variation in the family (Table 1). Data were gathered from scientific articles,
200 monographs, books, and herbaria in Zurich and Paris (Z, ZT and P) and in the field (Sabah,
201 Borneo, Malaysia, for details see Appendix S1). All traits were assembled in the PROTEUS
202 database (Sauquet, 2016), and the data and data sources are available from Appendix S1 and
203 Dryad XXX.

204 The fruit measurements relate to the dispersal unit of the plant (Figure 1), because we
205 are interested in the functional role of the fruit in relation to seed dispersal (Table 1), i.e. how
206 the fruit appears to frugivores. For most Annonaceae the dispersal unit is the separate fruitlet
207 ('monocarp'), developed from a carpel that is free in flower (i.e. $n = 205$ apocarpous
208 Annonaceae species in our dataset). In some genera (*Annona*, *Anonidium*, and genera in tribe
209 Duguetieae) the carpels that are free in flower fuse postgenitally during fruit development
210 (Chatrou & He, 1999). In *Isolona* and *Monodora*, carpels are fused both in flower and fruit
211 stage (Couvreur, 2009). Despite the distinction in morphology and development, we treat the
212 latter two fruit types similarly, as both produce fleshy fruits that are equivalent from the
213 perspective of fruit dispersal (i.e. $n = 23$ [pseudo-]syncarpous Annonaceae species in our
214 dataset). We calculated species averages when more than one observation for a trait was
215 recorded. Furthermore, fruit length, fruit number, seed number and maximum plant height
216 were log-transformed to gain normality in the data and to reduce the effect of outliers in the
217 analyses. We performed a principal component analysis (PCA) to evaluate whether the
218 correlation between frugivory-related traits allows us to define trait-syndromes potentially

219 associated with particular frugivores using the ‘mix.dudi’ function in the ‘ade4’ package in R
220 (Dray & Dufour, 2007). In addition, Phylogenetic Generalized Least Squares (PGLS)
221 regressions were performed to further evaluate correlations between these traits (Martins &
222 Hansen, 1997), resulting in 45 pairwise trait PGLS regressions. The PGLS method estimates
223 phylogenetic signal and regression parameters simultaneously, adjusted for the phylogenetic
224 signal in the model residuals. This was done using the ‘pgls’ function in the ‘caper’ R
225 package (Orme *et al.*, 2018).

226 **Biogeographical inference**

227 Ancestral geographic ranges of Annonaceae were inferred using the maximum-likelihood
228 approach implemented in BioGeoBEARS (Matzke, 2013). An unconstrained dispersal-
229 extinction-cladogenesis model (DEC), which allows for probabilistic inference of models of
230 range expansion (Ree *et al.*, 2005; Ree & Smith, 2008), as well as a model which additionally
231 allows for cladogenetic dispersal outside the parental areas (‘founder-event speciation’,
232 DEC+J) were fit to the Annonaceae maximum clade credibility (MCC) tree, and compared
233 using a likelihood ratio-test. All species were coded as present / absent in six geographical
234 regions (Couvreur *et al.*, 2011): North America (including Central America), South America,
235 Asia, Afrotropics, Madagascar and Oceania (including the Pacific islands). We set the
236 maximum number of areas to three to reflect the maximum number of areas occupied by any
237 extant Annonaceae species. The dispersal probability through time between areas was set to
238 equal, to avoid making subjective assumptions on the historical connectivity between
239 continents (but see section on ‘classification of long-distance dispersal routes’). The
240 biogeographical reconstructions obtained from the best model were visualized on the MCC
241 tree. These reconstructions were used to identify long-distance dispersal events, i.e. changes
242 in the most likely ancestral area between nodes in the tree. By only evaluating
243 biogeographical reconstructions in the Cenozoic, we excluded vicariance as a possibility to
244 explain biogeographical shifts between ascending and descending nodes.

245 **Classification of long-distance dispersal routes**

246 We classified long-distance dispersal events of Annonaceae based on the timing and the
247 direction of the events into five routes (for more details see Appendix S2): (1) ‘Laurasian
248 geodispersal’ (ca. 66–45 Ma) across land bridges during times when the climate was
249 favourable for rain forest taxa at higher latitudes (van Steenis, 1962; Thorne, 1972; Morley,
250 2000; Morley, 2003). ‘Geodispersal’ refers to the process whereby species can expand their
251 geographical ranges due to geological or climatic changes (Lieberman, 2005); (2) ‘Afro-
252 Asian land bridge’ enabling migration from Africa to Asia across Arabia via the expanded
253 tropical forests during the Miocene (23–5.3 Ma) (Zachos *et al.*, 2001); (3) ‘South America-
254 Africa (SA-A) stepping stones’ supporting stepping stone dispersal across islands of the Rio-
255 Grande Rise and the Walvis Ridge, connecting South America and Africa up until the
256 Oligocene (66–33.9 Ma) (Morley, 2003); (4) ‘Pacific island hopping’ enabling dispersal
257 between Southeast Asia, Australia and the Pacific islands from the late Miocene-Pliocene
258 onward (ca. 10 Ma), when Australia and the Asian Plate collided; (5) ‘remaining long-
259 distance dispersal’ events that could not be assigned to any of the dispersal routes.

260 Importantly, long-distance dispersal of Annonaceae lineages may have occurred any
261 time along a branch, and the uncertainties in estimated divergence times were therefore taken
262 into account when assigning long-distance dispersal events to dispersal routes (Table S1).

263 **Ancestral fruit trait reconstructions**

264 To evaluate which traits may have facilitated historical long-distance dispersal in
265 Annonaceae, we reconstructed ancestral states for the fifteen frugivory-related traits on 100
266 phylogenetic trees randomly sampled from the posterior of the BEAST analyses, and
267 summarized them on the MCC tree. Inferred probabilities for trait states therefore take
268 topological and divergence time uncertainties into account.

269 For the binary traits, we used maximum likelihood as well as Bayesian inferences to
270 perform the ancestral state reconstructions. We used the ‘multistate’ function in BAYESTRAITS
271 v.2 (Pagel & Meade, 2006) using the ‘addMRCA’ command followed by a list of species
272 descending from a particular node for which we aimed to infer the ancestral trait state. These
273 descending species do not necessarily have to form a clade in all 100 trees. For the maximum
274 likelihood analysis, we evaluated support for an equal or unequal transition rate model; the
275 unequal transition rate model was supported for six out of nine discrete traits (Table S2). For
276 the Bayesian Markov chain Monte Carlo (MCMC), we used a reversible jump (rjMCMC)
277 hyper prior with an exponential prior between 0 and 100 for 10×10^6 iterations, with a 10%
278 burn-in. The rjMCMC approach integrates results over the model space and visits models
279 (e.g. equal and unequal transition rate models) and parameters (e.g. RateDev parameter, to get
280 an acceptance rate of changes between 20 – 40%) in proportion to their posterior probability.
281 The hyper prior provides a distribution from which values are drawn to seed the exponential
282 prior (Pagel *et al.*, 2004; Pagel & Meade, 2006; see Sauquet *et al.*, 2015; and Onstein &
283 Linder, 2016 for empirical examples).

284 For the continuous traits, we used the two-step approach in BAYESTRAITS, in which
285 ‘random-walk’ models (i.e., Brownian motion) for each trait were run on 100 Annonaceae
286 phylogenetic trees, and the average trait value was estimated subsequently for each internal
287 node. Again, the ‘addMRCA’ command indicated the common ancestor of a group of species,
288 and the MCMC was run for 10×10^6 iterations, with a 10% burn-in.

289
290 **Inferring trait-related long-distance dispersal**

291 To evaluate whether frugivory-related traits involved in historical long-distance dispersal of
292 Annonaceae deviated from ancestral fruits not involved in long-distance dispersal, we
293 combined the data from the biogeographical inference with the reconstructed traits. First, we
294 tested whether fruit traits were different for ancestral lineages involved in long-distance
295 dispersal compared to those not involved in long-distance dispersal, using Welch Two Sample
296 t-tests. It is important to note that the geographical distribution of some of our characters is
297 highly skewed. For example, (pseudo-)syncarpous fruits do not occur in Asia, some occur in
298 Africa, whereas they are abundant in the Neotropics. Moniliform fruits only occur in Africa,
299 Madagascar and Asia, and are totally absent in the Neotropics. Furthermore, as nodes (and
300 inferred trait values on nodes) are correlated in phylogenies, we expect long-distance
301 dispersal events to be non-randomly distributed across the Annonaceae tree (conditional on
302 the presence of frugivory-related character states as opposed to, for example, ‘random’
303 opportunities for dispersal). In addition, it is possible that we detect an association between
304 traits and long-distance dispersal using our method when it is not truly there (i.e. type II error)
305 (Maddison & FitzJohn, 2015). To address these issues, we compared the empirical results
306 against 1000 simulations of long-distance dispersal events across the Annonaceae tree. In
307 each simulation we randomly assigned long-distance dispersal events to branches within the
308 Annonaceae tree (without replacement, and keeping the total number of long-distance
309 dispersal events the same as empirically inferred), and repeated the t-test as described above
310 (i.e. to test for an association between traits and long-distance dispersal). For these ‘random’

311 long-distance dispersals we expected no significant association with frugivory-related traits,
312 and thus we evaluated whether our observed t-value fell outside the 95% distribution of
313 simulated t-values.

314 Second, we tested whether fruit traits were different for ancestral lineages involved in
315 the different dispersal routes (Laurasian geodispersal, Afro-Asian land bridge, SA-A stepping
316 stones, Pacific island hopping and the remaining long-distance dispersal). To this end, linear
317 models (LM) were performed, regressing the continuous fruit traits against the dispersal
318 routes. We evaluated whether the model met the model requirements, i.e. normality of
319 residuals and presence or absence of a relationship between the fitted values and, respectively,
320 the observed and residual values. For the discrete traits, generalized linear models (GLM)
321 using a quasibinomial family with a logit link function were performed, using the probability
322 (between 0 and 1) of presence of the traits as the response variables. The quasibinomial
323 family was used in order to adjust the dispersion parameter to match the degrees of freedom
324 in the data. Similarly, as done for the t-tests, we evaluated the effect of type II error rates on
325 our results by repeating the LMs and GLMs after simulating random long-distance dispersal
326 events 1000 times on the Annonaceae phylogeny.

327 **Results**

328 **Phylogeny**

329 The Annonaceae crown node was estimated to be 96.8 Ma (89.0 – 104.6 Ma [ranges reflect
330 the 95% HPD]), the Ambavioideae 69.9 (50.6 – 86 Ma), the Annonoideae 74.6 Ma (65.6 –
331 84.3 Ma) and the Malmeoideae 38.2 Ma (27.9 – 49.8 Ma). More details are available from the
332 MCC tree (Dryad XXX).

333 **Biogeographical inference and dispersal routes**

334 The DEC+J model fitted the data significantly better than the DEC model (Log Likelihood of
335 -233 for DEC+J vs. -259 for DEC), indicating that (founder-event) jump dispersal may have
336 been an important process in the biogeographical history of Annonaceae. However, due to
337 issues related to estimation of the +J parameter (Ree & Sanmartín, 2018), we present results
338 consistent across both models here (both models gave qualitatively similar results and are
339 presented in the Supporting tables and figures). Both models identified 24 long-distance
340 dispersal events during Annonaceae evolution, which took place throughout the Late
341 Cretaceous and Cenozoic, possibly from c. 70 Ma until as recent as 0.5 Ma (Figure 3, Figure
342 S2, Table S1). These long-distance dispersals took place within all four Annonaceae
343 subfamilies (Anaxagoreoideae, Ambavioideae, Annonoideae and Malmeoideae), and include
344 shifts within the tribes Bocageae, Xylopieae, Duguetieae, Guatterieae, Annoneae, Uvarieae,
345 Malmeeae and Miliuseae. Our biogeographical inference also shows that the ancestral area for
346 the Annonaceae crown node cannot be reconstructed with confidence, but Africa is the most
347 probable ancestral area for Annonaceae excl. Anaxagoreoideae (Figure 3, Figure S2).

348 **Ancestral fruit trait reconstruction and trait ordination**

349 The maximum likelihood and Bayesian ancestral state reconstructions of the frugivory-related
350 traits gave qualitatively similar results, and only the Bayesian reconstructions were therefore
351 used in the subsequent analyses. Based on these reconstructions, we inferred the ancestral
352 Annonaceae fruits to be small, placed on short stipes, with a single or a few small seeds and a
353 small number of fruitlets. These fruits were most probably apocarpous, indehiscent and/or

354 moniliform. Colour, conspicuousness, exocarp, pubescence and cauliflory showed ambiguous
355 (equivocal) reconstructions at the ancestral nodes. All ancestral state reconstructions are
356 illustrated in Figure S3.

357 The PCA shows that the frugivory-related traits we hypothesized to be associated to
358 bird, bat or mammal ‘syndromes’ (see hypotheses) are clustered in trait space, and thus
359 explain distinct parts of the total frugivory-related functional trait variation in Annonaceae
360 (Figure 2). Similar frugivory-related trait syndromes were also recovered with PGLS, i.e.
361 mammal syndromes of (1) few, syncarpous fruits with many seeds, large fruits, large seeds;
362 bird syndromes of (2) many, bright-coloured, small fruits with few, small seeds and long
363 stipes, and (3) dehiscent fruits with small seeds; and a bat syndrome of (4) dull-coloured,
364 cauliflorous fruits (Table S3). This suggests that frugivory-related traits do not evolve
365 independently from each other and that ‘dispersal syndromes’ may indeed exist, i.e. sets of
366 co-occurring fruit traits that match the behaviour, feeding preferences and morphology of
367 particular types of frugivores.

368

369 **Trait-related long-distance dispersal**

370 Taking all Annonaceae long-distance dispersal events together, we found that five (out of 15)
371 of the frugivory-related traits were significantly associated with long-distance dispersal as
372 compared to those lineages not involved in long-distance dispersal (based on $p < 0.05$, for test
373 statistics see Table S4). These long-distance dispersal fruits were, on average, relatively large
374 (c. 3 cm long) and were subtended by short stipes (c. 0.6 cm long). Fruits were also more
375 likely to be dull-coloured, moniliform and/or non-cauliflorous (Figure 4, Table S4). Several
376 of these traits are associated with our hypothesized dispersal syndromes (i.e. a mammal
377 syndrome of large fruits, short stipes, dull colours and a bird syndrome consisting of
378 moniliform fruits). Our simulations show that these results are unlikely explained by type II
379 error rates, because our observed t-value linking frugivory-related traits to long-distance
380 dispersal (for those traits that showed empirical significance) falls outside the 95%
381 distribution of simulated t-values (i.e. after randomly reshuffling long-distance dispersal
382 events across the Annonaceae phylogenetic tree) (Figure S4).

383 When testing fruit trait associations with any of the five Cenozoic dispersal routes in
384 particular (Laurasian geodispersal, Afro-Asian land bridge, SA-A stepping stones, Pacific
385 island hopping and remaining long-distance dispersal events), we found that nine (out of 15)
386 traits showed a significant association with at least one of these routes (Figure 4, Tables S5
387 and S6). These traits were few, viz. large fruits on short stipes and a high probability of being
388 syncarpous (associated with Laurasian geodispersal, SA-A stepping stones), dehiscent or
389 moniliform fruits (associated with Laurasian geodispersal, Afro-Asian land bridge, SA-A
390 stepping stones), non-cauliflorous fruits (associated with Pacific island hopping) and dull-
391 coloured fruits (associated with remaining long-distance dispersal events, but only in the
392 DEC+J inference). Seed length and seed number may also be associated with long-distance
393 dispersal, but results were inconsistent between biogeographical models (i.e. DEC+J vs.
394 DEC). For example, small seeds were associated with Pacific island hopping when following
395 inferences from the DEC+J model, but the DEC model associated seed length with Afro-
396 Asian land bridge and SA-A stepping stone dispersal. Similarly, seed number was positively
397 associated with Early Cenozoic geodispersal and stepping stone dispersal following the
398 DEC+J model, but was associated with remaining long-distance dispersal events when
399 following inferences from the DEC model (Figure 4, Table S5).

400 Again, several of these traits could be linked to the hypothesized dispersal
401 syndromes (i.e. a mammal syndrome of large, [pseudo-]syncarpous fruits and dull colours; a

402 bird syndrome consisting of dehiscent or moniliform fruits, Figure 2). Furthermore, these
403 results are unlikely to be driven by ‘random’ opportunities for long-distance dispersal as
404 shown by our simulations, although some uncertainty exists for seed number, seed length,
405 fruit colour, and dehiscent fruits (Figure S5).

406 **Discussion**

407

408 In this study, we combined a comprehensive trait database with a comparative phylogenetic
409 approach to identify frugivory-related traits associated with past intercontinental long-
410 distance dispersal in Annonaceae, a major vertebrate-dispersed tropical plant family (Figure
411 1). We show that most long-distance dispersal occurred in the Early Cenozoic (Figure 3)
412 when climates were warm and rain forests were extended beyond their current ranges
413 (Morley, 2003). These long-distance dispersal events may have been facilitated by the
414 evolution of few, large fruits, short stipes, dull colours, (pseudo-)syncarpy, dehiscent and/or
415 moniliform fruits (Figures 2 and 4). The recurrent evolution of these traits may have
416 predisposed Annonaceae to colonize (new) continents or islands throughout the Cenozoic,
417 through their interactions with wide-ranging and/or ocean-crossing frugivores.

418

419 **Frugivory-related traits influence long-distance dispersal**

420 We show that (pseudo-)syncarpous fruits have been associated with long-distance dispersal of
421 Annonaceae in the Early Cenozoic (c. 45–65 Ma), with dispersals from Africa to South
422 America and Asia (Figure 1d, e, Figure 3, Figure 4f). Some of these (pseudo-)syncarpous
423 fruits have been described as ‘megafaunal’ fruits, as they are >10 cm in size and contain
424 several seeds in a juicy, fleshy pulp (Guimarães *et al.*, 2008), similar to for example durians
425 and jackfruits. These species supposedly rely on large-bodied mammals (‘megafauna’, >40
426 kg) such as elephants, primates and tapirs for their seed dispersal (Rogers *et al.*, 2004;
427 Guimarães *et al.*, 2008). This is in line with the idea that megafauna had a central role in the
428 dispersal of early angiosperm seeds (Eriksson *et al.*, 2000; Tiffney, 2004), facilitating Early
429 Cenozoic dispersal of fleshy-fruited plants via the boreotropical route in Laurasia (Morley,
430 2003; Couvreur *et al.*, 2011). Indeed, palms likely also possessed megafaunal fruits during
431 their Late Cretaceous/Early Cenozoic dispersals across Africa, Asia and South America
432 (Baker & Couvreur, 2013; Onstein *et al.*, 2018). Furthermore, Asian elephants (*Elephas*
433 *maximus*) and their ancestors were proposed as important seed dispersers of several of the
434 megafaunal-fruited *Artocarpus* (Moraceae) species in South-East Asia (Williams *et al.*, 2017).
435 Many megafaunal seed dispersers, such as South American proboscideans, xenarthrans, and
436 Notoungulata, went extinct in the Late Quaternary (Barnosky *et al.*, 2004), and present-day
437 plants with megafaunal fruits therefore suffer from dispersal limitation, small geographical
438 ranges, and, possibly, extinction (Guimarães *et al.*, 2008; Pires *et al.*, 2017; Onstein *et al.*,
439 2018).

440 Generally, our results suggest that large fruits (minimum c. 3 cm long) growing on
441 short stipes and often dull-coloured (e.g. brown or orange) may have contributed to Cenozoic
442 long-distance dispersal (Figure 1c, f, h and Figure 4a, e, g). These large fruits are found, for
443 example, in extant *Uvaria*, *Monoon* or *Trigynaea* species, or in the Central American species
444 in tribe Miliuseae (especially in the genera *Sapranthus* and *Tridimeris*). These fruits are
445 associated with dispersal by relatively large mammals (e.g. primates as suggested for *Uvaria*,
446 Zhou *et al.*, 2012) and likely contributed to the overland dispersal of Annonaceae throughout

447 the Cenozoic, for example via vegetation corridors from Africa to Asia during the Neogene
448 (Figure 3), which coincided with the ‘out-of-Africa’ dispersal of primates (Zhou *et al.*, 2012;
449 Thomas *et al.*, 2015). Only few extant, frugivorous bird species, such as cassowaries
450 (*Casuarius*), have gape widths large enough to swallow these fruits.

451 We also find strong support that moniliform fruits (long strings of bead-like fruitlets,
452 found in e.g. extant *Dasymaschalon*, *Desmos* and *Monanthes*, Wang *et al.*, 2012; Guo *et al.*
453 *et al.*, 2017b, Figure 1a, Figure 4j), dehiscent fruits (e.g. found in extant *Xylopi*a and
454 *Cymbopetalum*, Figure 1j, Figure 4i), and (relatively) small seeds (Figure 4c) are traits
455 associated with long-distance dispersal. Both moniliform and dehiscent fruits have been
456 shown to be particularly attractive to birds (Gautier-Hion *et al.*, 1985; Lomáscolo & Schaefer,
457 2010; Guo *et al.*, 2017b), although *Xylopi*a has been observed to be fed on by both birds and
458 primates (Johnson & Murray, 2018). These vertebrate dispersers facilitate germination in
459 these species by removing the aril, the sarcotesta, or both (i.e. structures that inhibit
460 germination) (Stull *et al.*, 2017). The disjunct tropical African/Asian distributions of
461 *Dasymaschalon* and suggests that dispersal may have occurred via the Afro-Asian land bridge
462 in the Neogene (Figure 3). During this time, frugivorous songbirds (Passeri) showed waves of
463 expansion from Australia through Asia to the rest of the globe (Moyle *et al.*, 2016), possibly
464 facilitating these plant dispersals. Similar bird-dispersed traits may have assisted long-
465 distance dispersal in other tropical families, such as the fleshy berries of Melastomataceae
466 (Renner, 2004b), and the dehiscent fruits of Meliaceae (Koenen *et al.*, 2015). Furthermore,
467 seasonally migratory frugivorous birds, or large-bodied and strong-flying fruit pigeons
468 (*Ptilinopus*) and frugivorous hornbills (*Penelopides*) (Holbrook *et al.*, 2002; Kissling *et al.*,
469 2009) may have assisted historical ‘island hopping’ of Annonaceae and other tropical plants
470 lineages across Asia, Oceania and the Pacific (e.g. in *Cinnamomum*, Lauraceae, Huang *et al.*,
471 2016). Against our expectation, we found that cauliflorous fruits (sometimes associated with
472 bat-dispersal, Table 1), may hinder long-distance dispersal (Figure 4h). Possibly, by growing
473 in the understorey, these fruits may be dispersal limited due to the sedentary behaviour of
474 understory frugivores (Givnish, 2010; Onstein *et al.*, 2017).

475 Although some of the long-distance dispersal traits identified in this study (e.g. few,
476 large fruits) have been recognized as specialized dispersal syndromes associated with
477 specialized frugivores (i.e. large-bodied animals, highly dependent on fruits, efficient
478 dispersers, feeding on few plant species) (Howe, 1993), some traits (e.g. small seeds,
479 dehiscent fruits) may instead reflect a more generalized, opportunistic dispersal strategy
480 (Howe, 1993). It is therefore expected that a mix of specialized and generalized dispersal
481 agents have been important for the dispersal of vertebrate-dispersed plants (incl. Annonaceae)
482 across continents.

483

484 **Phylogeny, biogeography and traits: some of the limitations in this study**

485 Our sampling of Annonaceae (234 / 2400) is sparse and raises the question whether these taxa
486 are sufficiently representative of their distribution and traits to make a meaningful inference.
487 Most (but not all, e.g. *Anaxagorea*, *Xylopi*a) genera occupy a single area, and most traits
488 show phylogenetic signal (e.g. fruit length, dehiscence, syncarpy and moniliform fruits, Table
489 S7), indicating that most biogeographical and trait transitions happened relatively early during
490 Annonaceae evolution, i.e. throughout the Paleogene (66–23 Ma) and Neogene (c. 23–2.6
491 Ma), and our sampling (97% of genera were sampled) would be able to capture these events.
492 Undoubtedly, we underestimated the real number of long-distance dispersal events and
493 functional trait transitions, especially during more recent times. Biogeographical studies that
494 target Annonaceae sampling within genera (e.g. Zhou *et al.*, 2012; Guo *et al.*, 2017b; Pirie *et*

495 *al.*, 2018) therefore provide a more comprehensive understanding of Annonaceae dispersal at
496 finer temporal and spatial scales. Importantly, phylogenetically labile traits (e.g. exocarp
497 traits, Table S7, Figure S3) which did not appear significant in the Annonaceae-wide analysis,
498 could provide valuable insights into trait-related long-distance dispersal within genera.
499 Unfortunately, functional trait data for most Annonaceae taxa is currently lacking.

500 Besides the sampling, we acknowledge uncertainty with respect to divergence times
501 and biogeographic reconstruction, in part due to lack of suitable internal calibrations and
502 informative priors, and the models used to infer ancestral areas and traits remain very
503 simplistic (Ree *et al.*, 2005; Pagel & Meade, 2006; Drummond & Rambaut, 2007; Ree &
504 Smith, 2008; Matzke, 2013). For example, among-lineage and through-time rate
505 heterogeneity (Couvreur *et al.*, 2011; Massoni *et al.*, 2015b) was not taken into account, and
506 analyses could be improved by including fossils directly in the inference, especially to
507 account for, or estimate, extinction. Our phylogenetic reconstructions show that lineages of
508 major Annonaceae clades are inferred to be slightly older than those obtained in previous
509 studies (Su & Saunders, 2009; Couvreur *et al.*, 2011; but see, Massoni *et al.*, 2015b), although
510 95% HPDs overlap, and phylogenetic relationships are similar to previous results (Chatrou *et*
511 *al.*, 2012; Guo *et al.*, 2017a). The reason for these older ages is likely our use of uniform (as
512 compared to lognormal) distributions for the calibration priors (Sauquet *et al.*, 2012).
513 Furthermore, our biogeographical inference (Figure 3) is consistent with previous
514 biogeographical reconstructions in Annonaceae (for a detailed discussion on historical
515 biogeography in Annonaceae, see Doyle *et al.*, 2004; Couvreur *et al.*, 2011; Zhou *et al.*, 2012;
516 Thomas *et al.*, 2015), but we obtained some differences between the DEC+J and DEC
517 biogeographical scenarios (Figures 3 and S2), with moderate consequences for trait-
518 associated long-distance dispersal (i.e. inconsistencies for seed size, seed number and fruit
519 colour). Nevertheless, even though the Annonaceae fossil record is poorly understood, our
520 results are consistent with fossil woods from the Paleocene-Eocene of England (Crawley,
521 2001) and Oregon (Wheeler & Manchester, 2002), supporting the Laurasian boreotropical
522 dispersal route.

523 We cannot discard the possibility that historical dispersal happened by modes other
524 than frugivores, as it has previously been argued that long-distance dispersal shows little
525 correlation with fruit trait syndromes (Thomas *et al.*, 2015). Indeed, long-distance dispersal
526 could have happened by rare rafting events on vegetation floats, diaspores in the mud
527 adhering to the feet of birds, or extreme meteorological events. Furthermore, some fruits may
528 be able to float and remain viable for a longer time in salt water (e.g. in invasive mangrove-
529 associated *Annona* species on Fiji), and may thus have been dispersed by sea currents
530 (Renner, 2004a). Nevertheless, if long-distance dispersal would have been independent from
531 frugivory-related traits, we would expect a random distribution of traits across dispersal
532 events, whereas we detected a significant association between Annonaceae long-distance
533 dispersal and ten out of fifteen frugivory-related traits (Figure 4). Furthermore, our simulation
534 study indicates that this is unlikely driven by neutral / random evolution of these traits on the
535 Annonaceae phylogenetic tree (Figures S4 and S5).

536 Last, we lack a mechanistic model linking traits and dispersal rates. Although we
537 have taken a trait-based approach to infer the potential frugivores important for long-distance
538 dispersal (Figure 2), actual, present-day dispersers remain unknown for most plant species
539 (incl. Annonaceae) and data availability tends to be localized. Further confidence and
540 precision could therefore be gained in future work addressing these specific limitations.

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543

544 **Conclusion**

545

546 Since Humboldt's travels (e.g. 1889), an important finding in the field of historical
547 biogeography has been that long-distance dispersal (vs. vicariance) may have been the rule
548 rather than the exception to explain the pantropical distribution of many plant families (e.g.
549 Myristicaceae, Doyle *et al.*, 2004; Melastomataceae, Renner, 2004b; Monimiaceae, Renner *et*
550 *al.*, 2010; Arecaceae, Baker & Couvreur, 2013; Moraceae, Williams *et al.*, 2017).

551 Nevertheless, it has remained puzzling by which mechanism these long-distance dispersal
552 events have occurred (van Steenis, 1962; Thorne, 1972; Morley, 2003). This study contributes
553 to our understanding of the plant traits (such as fruit size, type and colour) and dispersal
554 agents that may have facilitated past intercontinental long-distance dispersal of Annonaceae
555 across the tropical realms. These frugivory-related traits are not unique for this family, and
556 similar traits may thus explain the disjunct distributions of tropical plants more generally.

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Table 1: Fifteen frugivory-related traits measured for Annonaceae species ($n = 228$) and their hypothesized functionality with respect to frugivory.

	Trait	Description	Functionality	References
1	Fruit length (cm)	Length of monocarp or fruit.	Positively correlated with frugivore body size, so that large fruits rely on large-bodied frugivores for their dispersal.	(van der Pijl, 1972; Jordano, 2000; Herrera, 2002; Onstein <i>et al.</i> , 2017; Onstein <i>et al.</i> , 2018)
2	Fruit(let) number	Number of monocarps / fruits attached to fruit stalk.	Allometric constraint and / or increase of dispersal probability. Trade-off between fruit size and number.	(Howe, 1993)
3	Seed length (cm)	Length of single seed.	Positively correlated with frugivore body size, so that large seeds rely on large-bodied frugivores for their dispersal.	(van der Pijl, 1972; Jordano, 2000; Herrera, 2002; Onstein <i>et al.</i> , 2017; Onstein <i>et al.</i> , 2018)
4	Seed number	Number of seeds per monocarp / fruit.	Allometric constraint and / or increase of dispersal probability. Trade-off between seed size and seed number.	(Howe, 1993)
5	Stipe length (cm)	Length of stalk bearing monocarp.	Allometric constraint and / or increase of dispersal probability. Trade-off between fruit size and stipe length.	(Howe, 1993)
6	Fruit type (Apocarpous / Syncarpous)	Apocarpous fruits consist of 'monocarps' developed from a gynoeceum that comprises more than one free carpel; in (pseudo-)syncarpous fruits the carpels are fused either before or after fertilisation.	Several syncarpous 'megafaunal' fruits rely on primates and (extinct) megafaunal mammals for their dispersal, as these animals are able to tear apart the fruits.	(Gautier-Hion <i>et al.</i> , 1985; Guimarães <i>et al.</i> , 2008)
7	Fruit colour (Dull / Bright)	Dull monocarps / fruits are green or brown, bright fruits are yellow, red, purple, black or orange	Dull-coloured fruits have been associated with dispersal by mammals, and primates in particular, as well as with dispersal by bats.	(van der Pijl, 1972; Janson, 1983; Gautier-Hion <i>et al.</i> , 1985; Lomáscolo & Schaefer, 2010)
8	Conspicuousness of fruit display	Present when the stipe has a different colour from the monocarp, when seeds are shiny black against a light-coloured monocarp (i.e. not brown / black) or colourful pulp, or when	Conspicuousness of fruit display has been associated with bird dispersal.	(van der Pijl, 1972; Lomáscolo <i>et al.</i> , 2010; Lomáscolo & Schaefer, 2010)

		the aril (surrounding the seed) has a different colour from the seed or monocarp.		
9	Fruit exocarp 'defence'	Present when fruit exocarp was described as sruphy, warty, woody, verrucose and / or pubescent. Absent when fruit surface was described as smooth.	Smooth exocarps have been associated with bird dispersal, warty/woody fruits rely on dispersal by mammals.	(van der Pijl, 1972; Janson, 1983)
10	Pubescence	Hairs or tomentum on fruit exocarp.	Smooth exocarps have been associated with bird dispersal.	(van der Pijl, 1972)
11	Cauliflory	Flowers and fruits grow on the main trunk.	Cauliflory has been associated with dispersal by bats and understorey frugivores.	(Lomáscolo <i>et al.</i> , 2010)
12	Dehiscence	Fruit breaks open to display the (arillate) seeds.	Dehiscence has been associated with bird and sometimes primate dispersal.	(Gautier-Hion <i>et al.</i> , 1985; Johnson & Murray, 2018)
13	Moniliform	Monocarps constricted between the seeds, like a necklace.	Moniliform fruits have been associated with bird dispersal.	(Wang <i>et al.</i> , 2012; Guo <i>et al.</i> , 2017b)
14	Habit (Shrub / Tree / Liana)	Trees were defined as plants that have one main trunk, shrubs as plants that may have several trunks, and lianas as climbers.	Habit relates to display of fruits in the canopy (i.e. tree and liana, unless cauliflorous) or understorey (i.e. shrub and liana). This may relate to dispersal by e.g. sedentary understorey frugivores.	(Givnish, 2010; Onstein <i>et al.</i> , 2017)
15	Maximum plant height (m)	Maximum-recorded plant height (except for lianas).	Plant height is positively related to dispersal distance.	(Thomson <i>et al.</i> , 2011)

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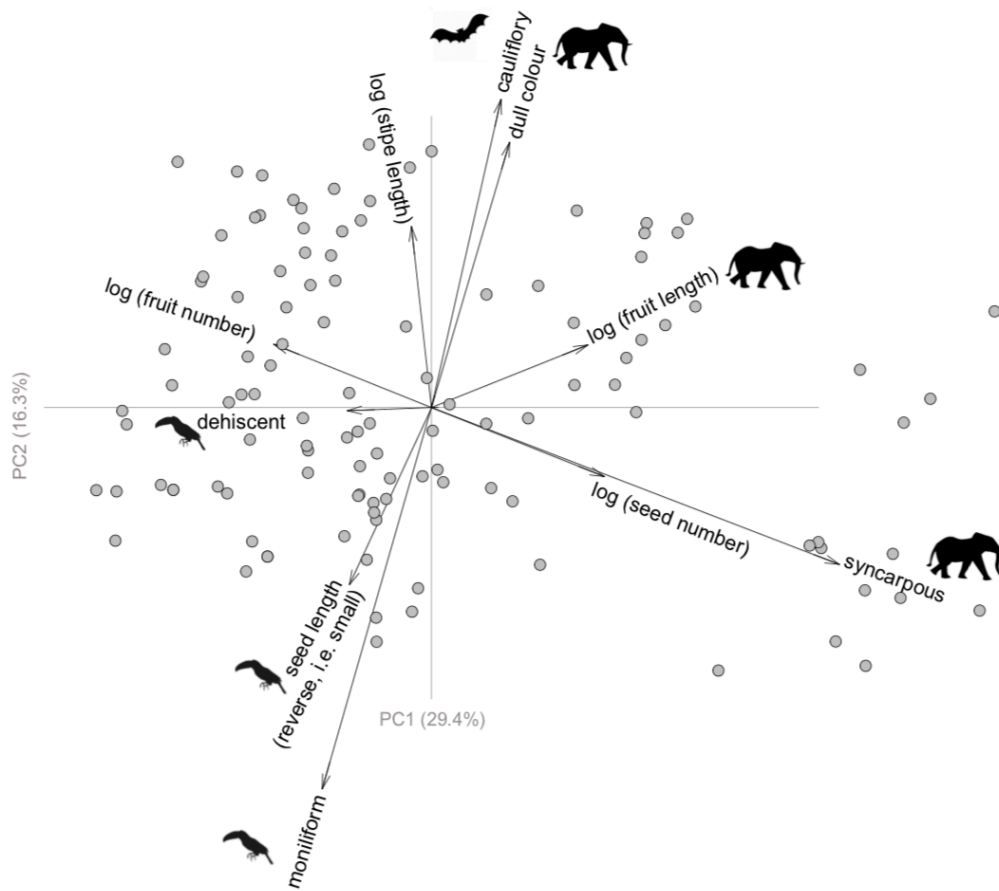
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564 **Figure 1: Diversity of vertebrate-dispersed Annonaceae fruit types.** Illustration of
 565 frugivory-related traits: (a) moniliform fruits, *Monanthes* sp; (b) conspicuous fruit
 566 display, *Malmea manausensis*; (c) large apocarpous fruits on short stipes, *Uvaria muricata*;
 567 (d) large (pseudo-)syncarpous fruit with several seeds, *Duguetia confinis*; (e) large
 568 syncarpous fruit, *Monodora myristica*; (f) large apocarpous fruits on short stipes, *Uvaria*
 569 *klainei*; (g) dehiscent fruits, *Xylopia Africana*; (h) large apocarpous fruits on short stipes with
 570 several seeds, *Uvaria klainei*. Photos were taken from World Annonaceae
 571 (<http://annonaceae.myspecies.info/>); photo credit: L.W. Chatrou (a), T.L.P. Couvreur (b, c, e-
 572 h), J. van der Maesen (d).
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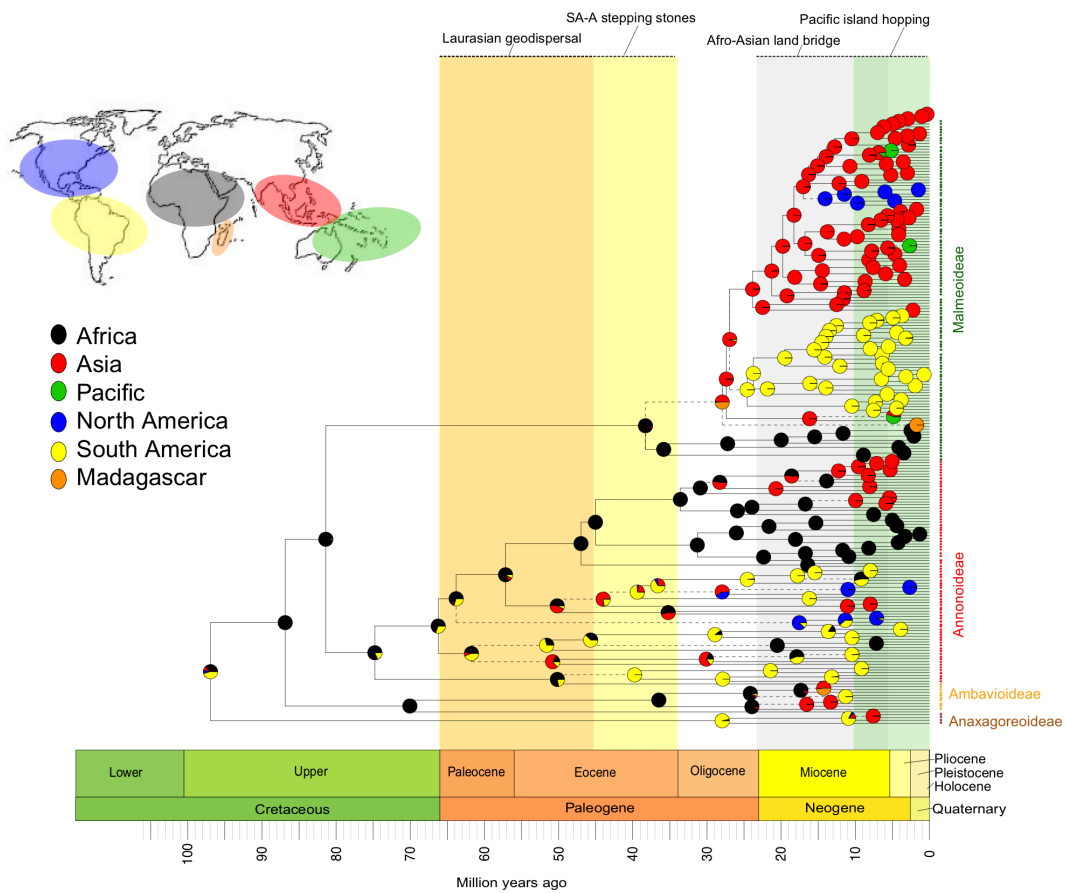
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575 **Figure 2: Ordination illustrating the frugivory-related trait syndromes in Annonaceae.**
 576 Principal component analysis (PCA) of the 10 functional traits which were significantly
 577 associated with long-distance dispersal in Annonaceae. Grey dots indicate the position of the
 578 species in the ordination ($n = 133$ species without any missing trait data). The animal
 579 silhouettes (toucan, bat, and elephant) illustrate our expectation with respect to fruit traits and
 580 primary seed dispersers, i.e. bird, bat or (large) mammal fruit traits (see introduction). The
 581 ordination shows that traits are correlated and explain distinct variation in trait space,
 582 potentially related to bird, bat or mammal trait 'syndromes'. The amount of total variation
 583 explained by principal components PC1 and PC2 is indicated at the axes.
 584
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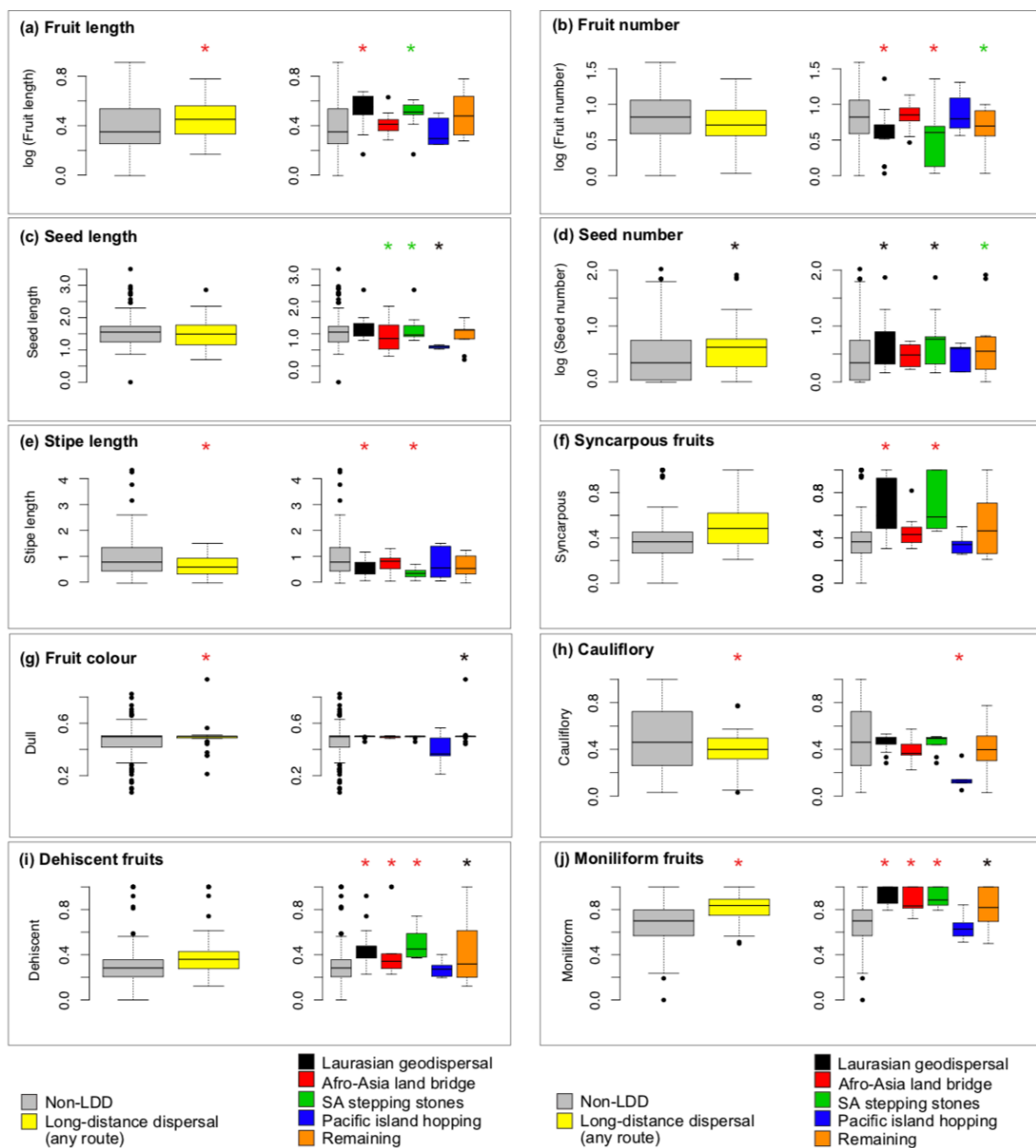
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587 **Figure 3: Annonaceae biogeographical inference.** The biogeographical reconstruction
 588 under the DEC+J model (dispersal = 0.0008, extinction = 0, jump-dispersal = 0.0166) on the
 589 Annonaceae maximum clade credibility (MCC) tree visualizes the 24 long-distance dispersal
 590 events (dashed branches). The pie-charts indicate the most probable ancestral area(s) (Africa,
 591 Asia, Pacific, North America, South America, Madagascar). The long-distance dispersal
 592 routes (Laurasian geodispersal, Afro-Asian land bridge, South America-Africa stepping
 593 stones and Pacific island hopping) are shown for the respective time periods. The subfamilies
 594 are indicated at the tips.



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 598

599 **Figure 4: Frugivory-related traits associated with intercontinental long-distance**
600 **dispersal in Annonaceae.** The average value of a trait (continuous traits, a-e) or the
601 probability of the presence of a trait (binary traits, f-j) is indicated for long-distance dispersal
602 events and dispersal routes as compared to Annonaceae lineages not involved in long-distance
603 dispersal, based on ancestral state reconstructions and biogeographical inference from the
604 DEC+J model. The traits associated with dispersal routes (represented by colours other than
605 grey) significantly deviating from Annonaceae not involved in long-distance dispersal (grey)
606 are inferred with linear models (continuous, a-e) or generalized linear models (binary traits, f-
607 j). The red asterisk indicates effects significantly supported by both the DEC and DEC+J
608 models, a green asterisk if only supported by DEC+J and a black asterisk if only supported by
609 DEC. Box-and-whiskers indicate average, 95% distribution and outliers of trait values. LDD
610 = long-distance dispersal.



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613

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830

831

832 **Biosketch:** Renske E. Onstein studies the broad-scale evolution, ecology and biogeography of
833 angiosperms in relation to their functional traits – ranging from the role of fruits and
834 frugivores in tropical rain forest radiations, to sclerophyllous leaf traits in Mediterranean-type
835 ecosystems.

836

837 **Data availability:** Sequences are available from GenBank (Appendix S1). The maximum
838 clade credibility (MCC) tree is available from Dryad XXX. The functional traits for
839 Annonaceae species measured in this study are available from the Supporting Information
840 (Appendix S1) and Dryad XXX.

841

842 **Author contributions:** R.E.O. conceived the ideas with input from W.D.K and H.S.; R.E.O.,
843 L.W.C., H.S. and T.L.P.C. collected the data; R.E.O. analysed the data; R.E.O. led the writing
844 and all authors discussed the results and commented on the manuscript.

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