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Selfing in epiphytic bromeliads compensates for the limited pollination services provided by nectarivorous bats in a neotropical montane forest

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27 **Abstract**

28 Plants with specialized pollination systems frequently exhibit adaptations for self-pollination, and this
29 contradictory situation has been explained in terms of the reproductive assurance function of selfing. In
30 the Neotropics, several plant lineages rely on specialized vertebrate pollinators for sexual reproduction,
31 including the highly diverse Bromeliaceae family, which also displays a propensity for selfing. Thus far,
32 the scarce evidence on the role of selfing in bromeliads and in other neotropical plant groups is
33 inconclusive. To provide insights into the evolution and persistence of self-fertilization in the breeding
34 systems of Bromeliaceae, we studied four sympatric epiphytic species from the genus *Werauhia*
35 (Tillandsioideae) in Costa Rica. We documented their floral biology, pollination ecology, and breeding
36 systems. We estimated the contribution of selfing by comparing the reproductive success between
37 emasculated flowers requiring pollinator visits and unmanipulated flowers capable of selfing and exposed
38 to open pollination across two flowering seasons. The studied species displayed specialized pollination by
39 nectar-feeding bats as well as a high selfing ability (autofertility index values > 0.53), which was attained
40 by a delayed selfing mechanism. Fruit set from natural cross-pollination was low ($<26\%$ in both years)
41 and suggested limited pollinator visitation. In line with this, we found a very low bat visitation to flowers
42 using video-camera recording, from 0 to 0.24 visits per plant per night. On the contrary, the contribution
43 of selfing was comparatively significant since 54-80% of the fruit set from unmanipulated flowers can be
44 attributed to autonomous self-pollination. We concluded that inadequate cross-pollination services
45 diminished the reproductive success of the studied *Werauhia*, which was compensated for by a delayed
46 selfing mechanism. The low negative effects of inbreeding on seed set and germination likely reinforce
47 the persistence of selfing in this bromeliad group. These results suggest that selfing in bat-pollinated
48 bromeliads may have evolved as a response to pollinator limitation.

49

50 **Keywords:** breeding systems, Bromeliaceae, chiropterophily, Costa Rica, pollinator limitation,
51 reproductive assurance

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56 **Introduction**

57 Selfing or the ability to self-fertilize in plants, is a relatively common reproductive strategy among
58 angiosperms (~20%) (Barrett, 2002), and in several species, the floral mechanisms that facilitate selfing
59 also co-exist with specialized pollination systems in a mixed mating system (Fenster & Martén-
60 Rodríguez, 2007). The maintenance of selfing as part of mixed mating systems in plants is largely
61 attributed to its benefits as a "reproductive assurance" mechanism in the face of unreliable cross-
62 pollination (Mallick, 2001; Herlihy & Eckert, 2002; Kalisz et al., 2004; Moeller & Geber, 2005; Moeller,
63 2006; Fenster & Martén-Rodríguez, 2007; Zhi-Qiang & Quing-Jun, 2008; Martén-Rodríguez & Fenster,
64 2010; Busch & Delph, 2012; Jones et al., 2013).

65

66 The ability to self-fertilize requires the loss of self-incompatibility mechanisms and the existence
67 of floral biology adaptations to facilitate the autonomous deposition of self-pollen onto the stigma. These
68 mechanisms include the absence of intra-floral herkogamy (Webb & Lloyd, 1986) and dichogamy (Bertin
69 & Newman, 1993). The establishment of self-fertilization is also contingent on the absence or reduced
70 inbreeding depression effects (Charlesworth & Charlesworth, 1987; Eckert et al., 2006). In addition, for
71 selfing to provide reproductive assurance, the reproductive success of a species must be constrained by
72 pollen availability or pollinator services (i.e., pollen limitation) (Eckert et al., 2006), and it should not
73 incur in pollen and ovules discount (Knight et al., 2005).

74

75 The Bromeliaceae family is a very diverse group of monocotyledonous plants, almost entirely
76 restricted to the American continent (Benzing, 2000). They contribute significantly to the floristic
77 diversity of vascular epiphytic floras in the Neotropics (Cascante-Marín and Nivia-Ruíz, 2013).
78 Bromeliads possess specialized pollination systems that involve vertebrate pollinators (hummingbirds and
79 nectarivorous bats) and insects to a lesser degree, mainly bees (Benzing, 2000; Kessler & Krömer, 2000;
80 Aguilar-Rodríguez et al., 2019a; Kessler et al., 2020). Even though most bromeliads exhibit adaptations
81 for cross-pollination, nearly two-thirds of the species investigated for their reproductive systems are
82 capable of selfing. This is more frequent in the subfamilies Tillandsioideae and Pitcairnioideae (Cascante-
83 Marín & Núñez-Hidalgo, 2023). However, little attention has been paid to detailed studies of selfing
84 mechanisms and their adaptive value in neotropical plants as a whole. Previous works (Wendt et al.,
85 2002; Matallana et al., 2010) have proposed that the prevalence of selfing among bromeliads represents a
86 reproductive isolation strategy (*sensu* Levin, 1971) to minimize the negative effects of hybridization in
87 sympatry. Nevertheless, the evidence supporting either hypothesis ("reproductive assurance" or
88 "reproductive isolation") is inconclusive in this important group of monocots (Cascante-Marín & Núñez-
89 Hidalgo, 2023).

90

91 The mechanisms of selfing vary with regard to the precise moment of its occurrence, and they
92 define the role of selfing, which has evolutionary consequences for plant fitness (Lloyd, 1992; Lloyd &
93 Schoen, 1992; Brys & Jacquemyn, 2011). Selfing may occur either before anthesis (prior selfing), during
94 anthesis when the flower is exposed to cross-pollination (competing selfing), or at the end of its life
95 (delayed selfing) (Schoen & Lloyd, 1992). Selfing that occurs late in the flower's life ("delayed selfing"),
96 when the possibility of cross-pollination has passed, is likely to result in reproductive assurance (Fenster
97 & Martén-Rodríguez, 2007; Goodwillie & Weber, 2018). Delayed selfing does not interfere with pollen

98 pick-up by pollinators or with the stigma's receipt of crossed pollen, hence decreasing pollen and ovule
99 discounting, respectively (Lloyd, 1992; Herlihy & Eckert, 2002).

100

101 This study seeks to further our understanding of the sexual reproductive systems of neotropical
102 plants, particularly the evolution and maintenance of selfing in the Bromeliaceae family. Using
103 information from floral biology, pollination ecology, and breeding systems of species from the genus
104 *Werauhia* J. R. Grant in the subfamily Tillandsioideae, we intend to provide insights into the ecological
105 causes for the persistence and predominance of self-fertilization in this plant group. *Werauhia* is proposed
106 as a monophyletic group (Barfuss et al., 2005) and is represented by one hundred recognized species
107 (Gouda & Butcher, 2016 and cont. updated) of epiphytic life-form and distributed mainly on the
108 mountains of southern Central America (Costa Rica and Panama) (Grant, 1995; Morales, 2003). Previous
109 studies in *Werauhia* indicate the presence of specialized pollination systems involving nocturnal
110 nectarivorous bats (Aguilar-Rodríguez et al., 2019a) and hummingbirds (Lasso & Ackerman, 2004), as
111 well as high selfing ability in *W. gladioliflora* (Cascante-Marín et al., 2005; Tschapka & Von Helversen,
112 2007), *W. nutans* and *W. noctiflorens* (Aguilar-Rodríguez et al., 2019b), and *W. sintenisii* (Lasso &
113 Ackerman, 2004).

114

115 We studied four *Werauhia* species that coexist simpatrically in a Costa Rican montane forest and
116 characterized their floral biology (herkogamy, dichogamy, anthesis, and senescence behavior of flowers),
117 pollination system (identified the main pollinators and their visitation rates), and the components of their
118 reproductive systems (i.e., self-compatibility, selfing capacity, and presence of agamospermy). We also
119 evaluated the presence of inbreeding depression in self-fertilized progeny and estimated the contribution
120 of selfing to reproductive success in natural conditions during two flowering episodes. We predict that
121 our study species will exhibit high self-compatibility and selfing capacity, and if selfing acts as a

122 safeguard against unpredictable cross-pollination (i.e., reproductive assurance), then it should occur at the
123 end of the flower life (“delayed selfing”) (sensu Goodwillie & Weber, 2018).

124

125 **Materials and methods**

126

127 **Study site**

128

129 This study was conducted at Cerros de (Hills of) La Carpintera Protective Zone in Costa Rica, between
130 2018 and 2021. The area comprises a small mountain formation in the eastern region of the Central
131 Valley of the country (9°52'–9°54" N; 83°57'–84°00' W; 1500–1850 m asl). The site comprises 2,396
132 hectares covered by patches of primary forest interspersed with late secondary forest, and pastures
133 (Sánchez et al., 2008). The rainfall regime is seasonal, with a well-defined dry season from December to
134 April. The site has a rich epiphytic flora and bromeliads are represented by 28 species from the genera
135 *Aechmea* (1 spp.), *Catopsis* (3), *Guzmania* (3), *Pitcairnia* (1), *Racinaea* (2), *Tillandsia* (11), *Vriesea* (1),
136 and *Werauhia* (6) (Sánchez et al., 2008).

137

138 **Study species**

139

140 We selected the more abundant *Werauhia* species at the study site: *W. ampla*, *W. nephrolepis*, *W.*
141 *pedicellata*, and *W. subsecunda* (Fig. 1). These are small to medium size and tank-forming bromeliads
142 that develop a single spiked or compound inflorescence per rosette. *Werauhia* species are distinguished
143 by having flowers with nocturnal anthesis, zygomorphic corollas with dull coloration (white or greenish),
144 basal appendages of petals with the dactyloid divided apex, and a cupular-shaped stigma without papillae
145 (Grant, 1995). The joint flowering period of the four species extends from November to August and

147 shows significant inter-specific temporal displacement (Cascante-Marín et al., 2017). Voucher specimens
148 are deposited in the Luis Fournier O. Herbarium (USJ) at the University of Costa Rica (*W. ampla* USJ-
149 100246, *W. nephrolepis* USJ-105232, *W. pedicellata* USJ-106525, and *W. subsecunda* USJ-111865).

150

151 **Floral biology**

152

153 We documented nine floral traits for each species: (i) number of flowers per inflorescence, (ii) floral
154 display (number of flowers open per day), (iii) color of the inflorescence bracts (peduncle, primary and
155 floral bracts), (iv) corolla color and shape (campanulate or bilabiate), (v) stigma and anthers position
156 relative to the corolla mouth, (vi) stigma-anthers separation or herkogamy, (vii) anthesis time and flower
157 longevity, (viii) time of anther dehiscence and stigma receptivity or dichogamy, and (ix) mechanism of
158 flower senescence. We tested stigma receptivity with a Peroxidase test (King, 1960; Kearns & Inouye,
159 1993), using the presence of bubbling (observed with a 20X hand magnifying glass) on the stigmatic
160 surface as an indicator of enzymatic activity.

161

162 We recorded the emission of floral volatile compounds through an organoleptic test (i.e., smelling
163 the open flower and noticing any fragrance). Floral nectar volume and sugar concentration were measured
164 in flowers from plants kept in a shadehouse at the study site. Before anthesis, flowers were isolated to
165 prevent nectar consumption by floral visitors. Using glass capillary tubes, the accumulated volume was
166 measured 2–4 hours after anthesis. A handheld refractometer (Bellingham & Standley Ltd., UK) was used
167 to estimate the sugar concentration in Brix degrees.

168

169 **Floral visitors and visitation frequency**

170

171 We recorded the flower visitors to each bromeliad species in the forest with six video camera traps
172 (Trophy cam, model 119476, Bushnell Corporation, Kansas, USA), during the flowering peaks of 2019,
173 2020, and 2021. The cameras were set to record 15-second-long videos when activated, followed by a
174 period of 30 seconds of inactivity, during the day and night. At each focal plant, the complete flowering
175 period of an inflorescence was monitored. Only in a few cases, it was interrupted due to battery depletion.
176 The video analysis included: (i) number of visits, (ii) visitor identity (e.g., bats, hummingbirds, others),
177 (iii) time and duration of each visit, and (iv) visitor behaviour (i.e., whether it contacted the anthers or
178 stigma). The visitation rate per night for the most frequent visitors was determined by dividing the total
179 number of recorded visits by the number of nights monitored each year.

180

181 To corroborate the chiropterophyllous transport of pollen, we captured bats to examine if they
182 were carrying pollen from the studied species. We placed six mist nets (9×2.5 and 3×2.5 m) once or twice
183 a week between January and February 2020, from 16:00 to 22:00 hours, in sites considered as "passage
184 zones" for bats (Wilson et al., 1996) and near flowering individuals of the studied species. This sampling
185 only included the flowering period of *W. ampla* and *W. subsecunda*. The captured bats were identified
186 following the taxonomic keys of York et al. (2019). Pollen was obtained from the top of the head and
187 snout (cheeks-nose) using transparent adhesive tape. The piece of tape with pollen was attached to a
188 microscope glass slide and a sampling area of 4.6 cm² was visually scanned under a light microscope in
189 the laboratory. We used a reference pollen collection from the study site to identify the pollen grains
190 carried by the bats.

191

192 **Controlled pollination treatments and breeding systems evaluation**

193

194 We conducted controlled pollinations on 73 plants (17 *W. ampla*, 15 *W. nephrolepis*, 16 *W. pedicellata*,
195 and 25 *W. subsecunda*) kept in a shade house at the study site, from September 2018 to July 2019. We
196 performed four pollination treatments: (i) manual self-pollination, (ii) manual cross-pollination, (iii)
197 pollinator exclusion (autonomous selfing), and (iv) emasculation (test of agamospermy). The
198 agamospermy test included stigma removal to avoid unnoticed contamination, this treatment did not
199 affect further floral anthesis. Hand pollinations were conducted 1–2 h after anthesis and flowers from all
200 treatments were bagged until their senescence. All treatments were performed on each plant and
201 randomly assigned to flowers in the same inflorescence. Fruit development was monitored in a monthly
202 basis and the proportion of developed fruit in each treatment was calculated before fruit dehiscence.

203
204 The components of the reproductive systems were estimated using the parameters described by
205 (Cascante-Marín & Núñez Hidalgo, 2023): (1) the *Self-compatibility index*: $SCI = P_a / P_x$ (Lloyd &
206 Schoen, 1992), the *Auto-fertility index*: $AFI = P_s / P_x$ (Lloyd & Schoen, 1992), and the *Agamospermy*
207 *index*: $AGI = P_{ag} / P_x$ (Riveros et al., 1996). For all indices: P_a = proportion of fruits after hand self-
208 pollination, P_{ag} = proportion of fruits after flower emasculation, P_s = proportion of fruits from flowers
209 excluded from visitors, and P_x = proportion of fruits after hand cross-pollination.

211 **Reproductive success and inbreeding depression**

212
213 We estimated the reproductive success per pollination treatment as the mean number of seeds per fruit in
214 a sample of 8 to 52 fruits per treatment and species. Potential effects of inbreeding depression at the
215 population level were tested by comparing seed production and seed germination capacity between
216 manually self- and cross-pollination treatments. We conducted a germination test using seeds from 8–46
217 fruits per treatment (8–12 plants per species). Seeds were mixed and a sample of 480 seeds per treatment

221 was distributed among 12 replicates of 40 seeds placed on wet towel paper in glass Petri dishes under lab
222 conditions. As control, a similar number of seeds from open pollinated fruits were germinated. To avoid
223 fungal contamination, we applied a commercial fungicide (Vitabax 40 WP) at the beginning of the
224 experiment. The seeds were monitored and wetted (if necessary) twice a week and the number of
225 germinated seeds recorded for two months. We considered a seed germinated when the radicle emergence
226 from the seed coat was noticeable.

227

228 We performed an ANOVA test to detect significant differences in mean seed production between
229 treatments per species and, after a significant result, we conducted *post-hoc* pairwise comparisons
230 (Tukey's HSD test). Differences in mean cumulative percent of germinated seeds among treatments (self-
231 and cross-pollinated, and natural pollination) for each species were evaluated using a non-parametric
232 Kruskal-Wallis test (Zar, 2010). We used the Wilcoxon test for paired comparisons between treatments
233 when significant differences were detected and applied a Bonferroni's correction (Zar, 2010). Analyses
234 were carried out using the built-in statistical functions available in the R software platform (R Core Team,
235 2019).

236

237 The reduction in fitness of selfed progeny was estimated with the *Inbreeding depression index*
238 (Charlesworth & Charlesworth, 1987): $IDI = 1 - (W_s / W_o)$, where W_s = mean number of seeds per fruit
239 or percentage of germinated seeds from manual selfing and W_o = mean number of seeds per fruit or
240 proportion of germinated seeds from manual outcrossing. An *IDI*-value = 0 indicates the absence of
241 inbreeding depression, while an *IDI* value = 1 indicates strong inbreeding depression.

242

243 **Reproductive assurance**

244

245 To estimate the contribution of selfing to reproductive success, we compared the fruit set between
246 emasculated and intact flowers under open pollination conditions in two consecutive flowering seasons.
247 We emasculated 474 flowers from 13–42 plants per species in 2020 and 975 flowers from 31–53 plants
248 per species in 2021. As control group, a similar number of intact flowers were selected in the same plants.
249 Since plants from the studied species usually do not reproduce in consecutive years, the groups of
250 manipulated plants differed in both years. Using an aluminium ladder, we included plants on host-trees
251 within reach of six meters in height.

252
253 Emasculation was conducted in the afternoon (14–17 h) before floral anthesis, swollen flower
254 buds in pre-anthesis were carefully open with a pair of tweezers and the anthers removed. This
255 manipulation did not alter the floral anthesis. In the case of species with a high floral display per night,
256 usually > 1 flower (*W. nephrolepis* and *W. pedicellata*), all flowers in anthesis were emasculated to avoid
257 the possibility of geitonogamy. Fruits from emasculated flowers indicates a successful pollinator visit,
258 whereas fruits from intact flowers may include both autonomous self- and cross-pollination.

259
260 We estimated the probability of fruit set between treatments (emasculated versus control) with a
261 Generalized Linear Model (GLM) using a binomial distribution (link = "logit") and a dichotomous
262 response variable (success vs. failure). The model included as predictor variables: “treatment”, “year”,
263 and their interaction, with categories “emasculated” and "year 2020" as reference. The model was
264 estimated with the base package of the platform R (R Core Team, 2019). The Hosmer & Lemeshow test
265 (*ResourceSelection* package; Lele et al., 2019) evaluated the fit of the logistic model to the data. For those
266 significant variables, we estimated the 'odds ratio' between the reference and respective categories of each
267 variable and its 95% confidence limits.

268

269 The contribution of selfing to the reproductive success (i.e., fruit set) of each species per year was
270 calculated using the *Reproductive Assurance Index* (Schoen & Lloyd, 1992): $RAI = (P_i - P_e) / P_i$; where
271 P_i is the proportion of fruits from intact flowers and P_e is the proportion of fruits from emasculated
272 flowers. Selfing contributes to reproduction if the RAI-value is greater than zero; when multiplied by 100,
273 it indicates its relative contribution to the total fruit set. We also estimated the RAI using data on seed set
274 from the 2021 season. For this, we counted the number of seeds in a sample of 16–32 fruits per treatment
275 from each of the four studied species.

276

277 **Results**

278

279 **Floral biology**

280

281 Mean flower production per inflorescence varied from eight flowers in *W. subsecunda* to 55 flowers in *W.*
282 *pedicellata*. Depending on the species, one to several flowers open per night, with *W. ampla* and *W.*
283 *subsecunda* being less susceptible to geitonogamy, both species mostly open one flower per night (Table
284 1). All species released floral volatiles reminiscent of fermented fruits or garlic scents, whereas nectar
285 production varied in terms of volume (11.9–598.1 μ L) and concentration (8–18 °Brix) per flower (Table
286 1).

287

288 In all studied species, reproductive organs were exposed to pollinators, the stigma and anthers
289 projecting from or close to the corolla mouth (Fig. 1). Herkogamy was absent in *W. nephrolepis* and *W.*
290 *subsecunda*, but variable in *W. ampla* and *W. pedicellata*, with some plants developing flowers with
291 approach herkogamy (i.e., the stigma longer than the anthers) (Fig. 1C and 1K). Flowers of *W. ampla* and

292 *W. nephrolepis* were distinguished by the upper portion of the style curving downward and away from the
293 anthers (Fig. 1C). The four species showed incomplete protogyny. The stigma receptivity occurred early,
294 sometimes even at the bud stage preceding anthesis, but soon it overlapped with pollen presentation.
295 Temporal separation between female and male function varied within and between species by up to two
296 hours (Table 1).

297

298 Flowers exhibited late-afternoon anthesis (15:00–18:00 h), remaining fully open at night and for a
299 period from 8 h in *W. nephrolepis* up to 24 h in *W. ampla* (Table 1). Flower senescence followed a similar
300 pattern among the studied species, at the end of the flower's life, the corolla loses its turgor and collapses
301 (Fig. 1 and Supplemental Videos S1 to S3). In the absence of herkogamy, the constriction of the petals
302 brings the anthers with remaining pollen grains into contact with the stigma, which is still receptive and
303 has accumulated a viscous fluid in the cupular stigmatic lobes. In *W. ampla* and *W. nephrolepis*, nectar
304 dripping on the lower petal may remove pollen and deposit it on the stigma, increasing the likelihood of
305 autonomous self-pollination.

307 **Pollinators and floral visitors**

308

309 The video recording data comprised 454 nights and 1448 monitored flowers (Table S1). Bats visited the
310 studied *Werauhia* on 33 occasions, usually between 19:00–23:00 h and 01:30–03:30 h, and each visit to a
311 flower lasted around two seconds. The video images did not allow a precise identification of the bat
312 species, but they revealed contact between the bat's head and the flower's reproductive organs (Fig. 1 and
313 Supplemental Video S4 to S7). Overall, the visitation rate per night per plant was quite low (0.07 visits)
314 and varied among years and species from zero to 0.24 (Table S1). In a few events, the video cameras

315 were activated at night, but no activity was documented, which suggests the possibility of unrecorded
316 visits.

317

318 Sporadic visits by the hummingbird *Lampornis calolaemus* (Trochilidae) to flowers of *W. ampla*,
319 *W. nephrolepis*, and *W. pedicellata* were also video-recorded during the late afternoon at the beginning of
320 flower anthesis (16:50–17:20 h) and the following morning (6:00–8:00 h) when flowers were wilting. A
321 nocturnal and arboreal mouse from genus *Reithrodontomys* (Rodentia: Cricetidae) was occasionally
322 recorded visiting flowers of *W. ampla* and *W. nephrolepis*. Stingless bees (*Trigona* sp., Apidae) were seen
323 on flowers of *W. ampla* and *W. nephrolepis* collecting pollen from the anthers in the following day of
324 anthesis.

325

326 During the mist-netting sampling of eight nights and with an effort of 675 m²/h, we captured 46
327 bats from nine genera. Pollen from the studied *Werauhia* species was recovered from three (out of five)
328 captured individuals of the nectarivorous leaf-nosed bats *Hylonycteris underwoodi* and from the single
329 capture of *Glossophaga soricina* (Table S2). Pollen counts varied between 7 and 5250 grains per sampled
330 individual. Additional pollen recovered from the bats mainly belonged to the shrubby epiphytic
331 nightshades: *Merinthopodium neuranthum* and *Schultesianthus leucanthus* (Solanaceae) (Table S2).

332

333 **Breeding systems**

334

335 Hand self- and cross-pollinations resulted in high percentages (>75%) of fully developed fruits, except in
336 *W. pedicellata* (50% and 58.1%, respectively) (Table 2). Fruit set from autonomous selfing was higher for
337 *W. subsecunda* (76.7%) and *W. nephrolepis* (71.1%) and moderate in *W. ampla* (43.3%) and *W.*
338 *pedicellata* (31.1%). The breeding systems of the four *Werauhia* species are characterized by high values

339 of self-compatibility (SCI = 0.86–1.14), with relatively high values of self-fertility (AFI = 0.53–1.00),
340 which indicate a high ability to self-pollinate by autonomous means. The agamospermy index suggested a
341 very low degree of potential apomixis in *W. nephrolepis* and *W. pedicellata* (AGI = 0.11 and 0.06,
342 respectively) (Table 2).

343

344 **Reproductive success and inbreeding depression**

345

346 The average seed set per fruit did not significantly differ between manually self- and cross-
347 pollinated fruits for each species (Fig. 2), supporting the high self-compatibility condition previously
348 recorded using fruit-set data. Moreover, inbreeding depression effects were absent or low for seed
349 production, with IDI values ranging from -0.10 to 0.15. Comparing the number of seeds produced by
350 autonomous selfing versus controlled self-pollination revealed no statistically significant differences,
351 indicating the high efficacy of selfing at the level of seed production. (Fig. 2). Similar amounts of seeds
352 were developed in fruits from open and controlled cross-pollination (Fig. 2).

353

354 Seed germination was high (>80%) and did not differ statistically between self-, cross-, and open
355 pollinated seeds, except for *W. pedicellata*, which selfed seeds had a lower germination rate (Fig. 3). The
356 studied species experienced null to low negative effects of inbreeding on their germination capacity,
357 except for *W. pedicellata* (IDI value = 0.34). In all species, seedlings remained alive by the end of the
358 experiment after two month of sowing.

359

360 **Reproductive assurance**

361

362 In all species and in both studied years, emasculated flowers developed fewer fruits compared to intact
363 flowers (Figure 4). The GLM results indicated a significant effect of "treatment", but neither "year" nor
364 their interaction did, except for *W. pedicellata* whose response was not consistent across years (Table 3,
365 Figure 4). The odds ratios indicated that intact flowers capable of autonomous selfing had 3.4 times (in
366 *W. ampla*) to nearly 12 times (in *W. subsecunda*) more chances of producing fruits than emasculated
367 flowers that require pollinator visits (Table 3).

368

369 The estimation of reproductive assurance indicated a contribution of autonomous selfing to fruit
370 set from moderate to high (54–80%), except for *W. pedicellata* in 2020, whose fruit set was affected by
371 herbivory (Figure 4). For seed production in 2021, the contribution of selfing to the number of seeds per
372 capsule was low ($RAI \leq 0.14$), except for *W. subsecunda* ($RAI = 0.48$) (Fig. 3).

373

374 **Discussion**

375

376 The Bromeliaceae family exhibits a tendency toward selfing, but evidence of its potential adaptive value
377 is lacking. In this study, we combined data from floral biology, pollination ecology, and breeding systems
378 to demonstrate that selfing contributes significantly to the reproductive success of bromeliads. The
379 studied *Werauhia* species from the Tillandsioideae subfamily showed a specialized pollination system
380 that promotes out-crossing but experienced low visitation by nectar-feeding bats. The reduced events of
381 cross-pollination were compensated by autonomous selfing that occurs at the end of the flower's life and
382 secures the plant's reproductive success.

383

384 **Specialized pollination and mechanism of selfing**

385

386 The studied *Werauhia* species conform to the traditional bat-pollination syndrome with nocturnal
387 anthesis behavior of flowers with dull coloration, emission of floral scents as chemical attractants, and
388 diluted nectar in high volume as reward (sensu Faegri & van der Pijl, 1979). The nocturnal video
389 recording of bats visiting the flowers and the captured bats carrying pollen grains from the studied species
390 confirmed this specialized pollination system.

391

392 Despite demonstrating unambiguous floral adaptations for cross-pollination, the breeding systems
393 of the studied *Werauhia* were highly self-compatible and able to self-fertilize autonomously. The
394 combination of incomplete protogyny and a lack of or variable herkogamy (in *W. ampla* and *W.*
395 *pedicellata*) is likely what facilitates autonomous deposition of self-pollen on the stigma of the studied
396 species. However, selfing did not appear to occur either before (prior) or during anthesis (competing)
397 (sensu Lloyd & Schoen, 1992). When flowers were fully open, direct contact between stigma and anthers
398 was prevented by the stigma's distinctive cup-shaped lobes, which served to conceal the receptive area
399 within (Brown & Gilmartin, 1989; Barfuss et al., 2016). Also, the ventral torsion of the style near the
400 stigma in *W. ampla* and *W. nephrolepis* may also reduce the chances of stigma-anther contact during
401 anthesis. This feature of the style and stigma is found in other species of *Werauhia*, and the degree of
402 torsion varies (Utley, 1983), but its potential significance to pollination has not been discussed
403 previously.

404

405 Rather, we found that autonomous selfing in the studied *Werauhias* occurred at the end of the
406 flower's life. The pattern of flower senescence by which the corolla closes and forces the anthers with
407 exposed pollen into contact with the still-receptive stigma corresponds to the mechanism of "corolla
408 closure" described by Goodwillie and Weber (2018). The stigmatic exudate that visibly accumulates in
409 the stigma lobes of the studied species probably helps the pollen grains stick when the corolla closes.

410 Following this evidence, self-pollination in the studied *Werauhia* would represent a mechanism of
411 “delayed selfing” (*sensu* Lloyd & Schoen, 1992), and it suggests that reproductive assurance rather than
412 reproductive isolation is its primary benefit, as the latter would most likely select for earlier or preemptive
413 selfing to prevent bats from depositing heterospecific pollen onto the stigmas (*sensu* Randle et al., 2016).
414

415 Within the Bromeliaceae family, chiroterophily is present in subfamily Pitcairnioideae
416 (*Pitcairnia*) but is better represented in Tillandsioideae, mainly in *Pseudoalcantarea* and *Vriesea*, and
417 *Werauhia* is thought to be the genus with the greatest specialization in bat pollination (reviewed by
418 Aguilar-Rodríguez et al., 2019a). Fenster and Martén-Rodríguez (2007) suggested that specialized
419 pollination is frequently associated with floral mechanisms to self-pollinate; however, several examples
420 indicate that for bat pollination such association is weak. In a group of bat-pollinated gesneriads, Martén-
421 Rodríguez & Fenster (2010) found they were unable to self-pollinate autonomously, while hummingbird-
422 pollinated species exhibit high potential for autonomous selfing. Additional examples of neotropical
423 chiropterophilous plants evidence the presence of self-incompatibility mechanisms or the inability to self-
424 pollinate autonomously (e.g. Sazima & Sazima, 1978; Gibbs et al., 1999; Gribel & Gibbs, 2002; Sazima
425 et al., 2003) suggesting that specialization in pollination and floral traits that promote selfing are not
426 necessarily associated in an evolutionary context (Fenster & Rodríguez 2007). Thus, the high frequency
427 of *Werauhia* species and bromeliads, in general, with specialized pollination systems and high selfing
428 ability might be a particularity of this plant lineage.

429

430 **Pollinators and pollinator limitation**

431

432 Two nectar-feeding bat species from the subfamily Glossophaginae (*Glossophaga soricina* and
433 *Hylonycteris underwoodi*) represent the most probable pollinators of the studied species. Our census was

434 limited in scope (two months), but according to a more extensive survey of the bat community (Durán,
435 2013), a third nectarivorous species (*G. commissarisi*) is present in our study site. Based on the frequency
436 of captures, our data suggest that *Hylonycteris underwoodi* is likely the most important pollinator of the
437 studied epiphytic bromeliads. This is a small nectarivorous bat distributed from Mexico to Panama in
438 primary and older secondary forests and from sea level to 2640 m asl (Wilson & Mittermeier, 2019).

439

440 Plants with specialized pollination systems are prone to pollen limitation due to unpredictable
441 visitation by their pollinators (Knight et al., 2005; Martén-Rodríguez & Fenster, 2010). In
442 chiropterophilous plants, bats are considered “good” pollinators because they carry large amounts of
443 pollen from different paternal genotypes and can disperse it over long distances (Fleming et al., 2009). In
444 spite of this, our evidence from camera traps suggests a low pollinator availability of nectar-feeding bats,
445 with visitation ranging from none to 0.24 visits per night per plant. Data from other bat-pollinated
446 bromeliads suggest varying but usually higher visitation rates; for instance, Aguilar *et al.* (2019b) found
447 no visitation to *Werauhia nutans* but up to 4.2 visits per flower per night in *Pseudalcantarea viridiflora*.
448 While in *W. gladioliflora*, Tschapka & von Helversen (2007) observed 1-44 visits per flower per night.
449 Bat visits to flowers can be quite fast (less than 0.5 seconds) and it is possible that camera traps have
450 underestimated the visitation rate. However, our flower emasculation experiment, which resulted in low
451 reproductive success (<26% fruit set), supports the idea of limited pollinator services in the studied
452 epiphytic bromeliads.

453

454 The low pollinator visitation recorded may arise from the interaction of several ecological factors
455 acting locally. A low diversity of pollinators has been associated with increased pollen limitation (Knight
456 et al., 2005). Species richness in nectar-feeding bat communities shows a decreasing pattern with respect
457 to elevation (Fleming et al., 2005), with fewer species in montane forests compared to lowland habitats.

458 The absence of *Anoura geoffroyi* (Phyllostomidae) at the study site is notable since it is a nectarivorous
459 species from montane forests and considered abundant throughout its distribution range (Ortega &
460 Alarcón-D., 2008). In Costa Rica, however, it is an uncommon and rarely captured species, although it is
461 apparently common in some localities (LaVal & Herrera-R., 2002; Wainwright, 2007). The lower
462 diversity (three species) of pollinating bats in our research site, located at around 1700 m asl, compared to
463 a Costa Rican lowland bat community with four nectarivorous species (Tschapka & von Helversen,
464 2007), presumably plays a role in the limited visitation we recorded.

465

466 Low floral visitation may also be indicative of a low population density of pollinators.

467 *Hylonycteris underwoodi* is a rare species that never occurs in dense populations and roosts in small
468 groups of one to four individuals (Wilson & Mittermeier, 2019). In a lowland bat community, this bat
469 species was unfrequently captured in mist nests and represented 4% of the captures (Tschapka & von
470 Helverson, 2007). Similarly, in a previous bat inventory at our study site and with a sampling effort
471 spanning a whole year (39 nights and 21 060 m²/h), Durán (2013) documented only five *H. underwoodi*
472 individuals from a total of 142 captured bats (3.5% of the captures). Overall, the evidence strongly
473 suggests that *H. underwoodi* has a low population density at our montane research site, which likely
474 accounts for the observed low visitation frequency to bromeliad flowers. According to Fleming et al.
475 (2005), nectarivorous bats density is probably low in most habitats; however, the aforementioned research
476 by Tschapka & von Helversen (2007) also revealed a higher abundance of bats that frequently visited the
477 flowers of *W. gladioliflora* in a lowland forest. This spatial variation in pollinator abundance may affect
478 the efficiency of selfing as a reproductive assurance mechanism.

479

480 Factors related to habitat fragmentation may, in turn, affect the density of resident bat pollinators
481 (Steffan-Dewenter & Tschardtke, 1999; Cunningham, 2000; Liu & Koptur, 2003; Knight et al., 2005) and

482 negatively impact pollination services. The studied montane forest is a medium-sized forest fragment (ca.
483 2.400 ha) loosely connected to major forested areas in the much larger Talamanca Mountain range. This
484 condition may limit long-distance migration, affect the stability of the local population of *H. underwoodi*,
485 or impede the establishment of other nectarivorous species such as *A. geoffroyi*. In addition, pollen grains
486 of non-bromeliad plants recovered from bats suggest that inter-specific competition among co-flowering
487 bat-pollinated plants may be a potential cause of decreased visitation. On the contrary, intra-specific
488 competition for pollinators among sympatric *Werauhia* is likely low, since the investigated species
489 exhibit a staggered flowering phenology in the study site (Cascante-Marín et al., 2017). Furthermore, this
490 phenological pattern may be an indicator that reproductive isolation is not the primary function of selfing
491 but rather its reproductive assurance function.

492

493 **Reproductive assurance**

494

495 The mechanism of autonomous delayed selfing of the studied *Werauhia* was key to their
496 reproductive success, representing 54 to 80 percent of the total fruit set. Recording the time of selfing in
497 bromeliad pollination studies is not a common practice (Núñez-Hidalgo & Cascante-Marín 2023), but the
498 few studies that have reported delayed selfing in bat-pollinated and highly autofertile bromeliads belong
499 to *Werauhia* species (Cascante-Marín et al., 2005; Aguilar-Rodríguez et al., 2019b). However, these
500 studies did not assess its contribution to reproductive success.

501

502 The establishment and persistence of selfing are counteracted by the negative effects of
503 inbreeding (Charlesworth & Charlesworth, 1987). Theoretically, the maintenance of selfing would occur
504 when the adequacy of the selfed progeny surpasses that of outcrossed origin by a factor of $W_s/W_o > 0.5$
505 (Herlihy & Eckert, 2002; Eckert et al., 2006). We found that inbreeding depression at early stages of the

506 progeny had low or null effects on the number of seeds (IDI-values ≤ 0.15) and germination capacity
507 (IDI-values ≤ 0.24) of selfed seeds. This likely contributes to the maintenance of selfing in the studied
508 *Werauhia* populations. However, life-time estimations of inbreeding depression would confirm or reject
509 the positive effects of selfing and its evolutionary stability (Delmas et al. 2014).

510

511 In floral emasculation experiments, reproductive assurance may be overestimated due to low
512 visitation caused by modifications to flower attractiveness (Eckert et al., 2004). In our case, anthers
513 removal may have caused a minor alteration to the flower's visual appearance, and we presume a non-
514 significant effect since it has been demonstrated that nectar-feeding bats depend more on olfactory and
515 acoustic cues when searching for nocturnal flowers (Gonzalez-Terrazas et al., 2016). Also, bats appear to
516 rely more on olfaction when flowers are situated against a complex background (Muchhala & Serrano,
517 2015), as is the case with epiphyte plants in the forest canopy.

518

519 Comparable data on manipulative experiments involving other bromeliads, as well as tropical
520 plants in general, are severely lacking (see Eckert et al. 2006, Busch & Delph 2012). Lasso & Ackerman
521 (2004) found that emasculated flowers of the hummingbird-pollinated *Werauhia sintenisii* from the island
522 of Puerto Rico experienced low pollinator visitation. The authors suggested the value of selfing in the
523 reproduction of this species. Studies from temperate zone plants are more prevalent in the literature (e.g.,
524 Eckert 2000, Kalisz et al. 2004, Moeller 2006, Brys & Jacquemyn 2011, Yang et al. 2018, Teixido &
525 Aizen 2019) and show that the effect of selfing on reproductive success exhibits temporal and spatial
526 variation. The few studies on tropical plants have found that the contribution of selfing to reproductive
527 assurance may vary among plants with different pollination systems in a group of gesneriads (Martén-
528 Rodríguez & Fenster, 2010). It was also found that the contribution of selfing to the reproduction of the

529 vine *Ipomoea hederacea* (Convolvulaceae) varied among reproductive seasons (Delgado-Dávila &
530 Martén-Rodríguez 2021).

531

532 Selfing capacity and the degree of self-compatibility in Bromeliaceae are positively associated,
533 with some of the variation explained by floral biology attributes such as anthers-stigma separation or
534 herkogamy (Cascante-Marín & Núñez-Hidalgo, 2023). We found that *W. ampla* and *W. pedicellata* were
535 highly self-compatible (SCI = 0.91 and 0.86, respectively) but exhibited lower selfing capacity (AFI =
536 0.53 and 0.54, respectively), which resulted in lower contribution to reproduction assurance. These
537 differences in selfing capacity can be explained by the observed variation in herkogamy in the studied
538 populations that may reduce the effectiveness of the selfing mechanism of corolla closure. Previous
539 studies have shown that autofertility is correlated with variations in herkogamy; furthermore, this floral
540 trait exhibits partitioning primarily between populations (Moeller, 2006). The variation of this floral trait
541 is poorly documented in tropical plants, and it has been suggested that it can evolve rapidly in response to
542 environmental changes affecting cross-pollination (Opedal, 2017; 2018).

543

544 Ecological factors may offset the beneficial effects of selfing, as shown by the contrasting
545 outcomes of the reproductive assurance estimation in *W. pedicellata*, despite its moderate selfing capacity
546 (AFI = 0.54). This unexpected result can be explained by herbivory caused by larvae of a butterfly
547 (Lepidoptera: Licaenidae) that consumed early-developing capsular fruits in several plants during the
548 2020 season. Herbivory of reproductive structures may alter the reproductive success of plants, as
549 documented in other bromeliad species (Cascante-Marín et al., 2007; Orozco-Ibarrola et al., 2015). This
550 particular situation likely accounts for the lower fruit set in open pollination recorded in the first
551 flowering season studied. This type of herbivory may result in complete loss of a plant's inflorescence, as
552 observed in plants kept in a greenhouse and field conditions.

553

554 **Concluding remarks**

555

556 This study provides novel evidence of the function of delayed selfing as a reproductive assurance
557 mechanism in the species-rich family Bromeliaceae, a plant lineage characterized by a tendency towards
558 self-fertilization. The alternative hypothesis of selfing as a mechanism of reproductive isolation is not
559 supported because of the non-overlapping flowering seasons previously reported for the investigated
560 *Werauhia* species in the study site (Cascante-Marín et al., 2017), which precludes heterospecific pollen
561 transfer. Moreover, it has been demonstrated that delayed selfing is an ineffective barrier against hybrid
562 fertilization (Brys et al., 2016). A comprehensive study of potential isolation mechanisms will confirm
563 this assumption.

564

565 We conclude that reproductive success in the studied *Werauhia* species is pollinator-limited due
566 to the low visitation rate of its main bat pollinator. The delayed-selfing mechanism is strengthened by the
567 lack of inbreeding depression and substantially contributes to reproductive success, compensating for the
568 limited cross-pollination services provided by nectar-feeding bats. This selfing mode may be common
569 among chiropterophilous bromeliads; however, the documented reproductive benefits may vary
570 depending on the ecological context of pollination. Some reports of delayed selfing in predominantly
571 ornithophilous bromeliad genera, such as *Tillandsia* (Orozco-Ibarrola et al., 2015) and *Pitcairnia* (Wendt
572 et al., 2002), warrant further investigation to test whether selfing as a mechanism of reproductive
573 assurance has also evolved in bromeliad lineages with other specialized pollination systems than
574 chiropterophily.

575

576 The high prevalence of selfing in Bromeliaceae suggests a potential ecological and evolutionary
577 advantage. Unveiling such benefits requires detailed studies combining floral biology, breeding systems,
578 and pollination in bromeliads and other tropical plants. Manipulative experiments that encompass
579 temporal and spatial variation in pollination conditions may help us understand the ecological factors that
580 shape the effects of selfing in tropical plants.

581

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591

592 **Authors contributions**

593 Both authors designed the conceptual framework of the study. S.N.H. collected most of the data
594 and led the analysis and interpretation of the data with support of A.C.M., while S.N.H. wrote the initial
595 drafts of the manuscript. A.C.M. contributed to the final version of the manuscript and gave the final
596 approval for publication.

597

598 **Data availability statement**

599 The data underlying this article are available in the article and in its online Supporting Information.

600 **Literature cited**

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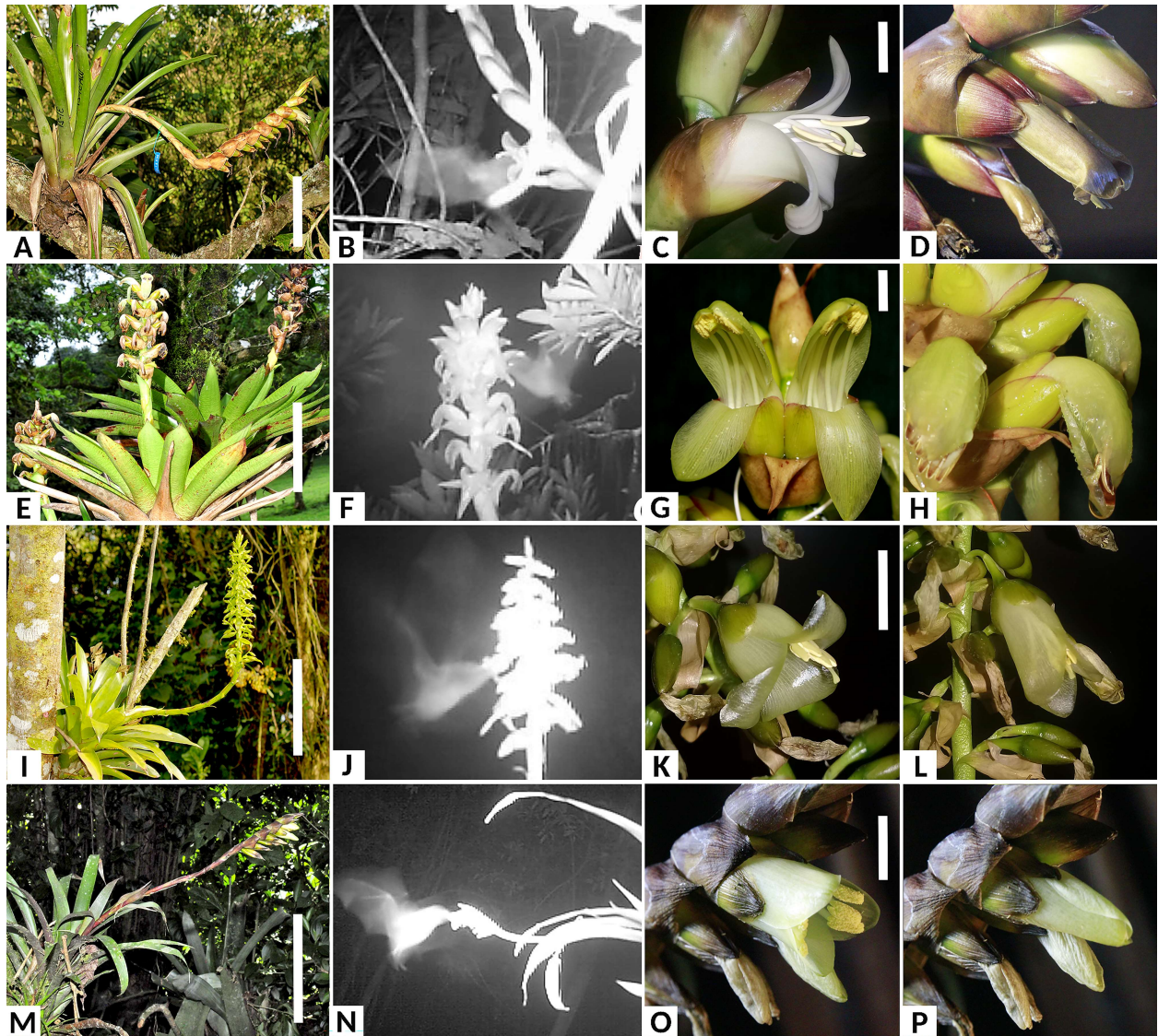
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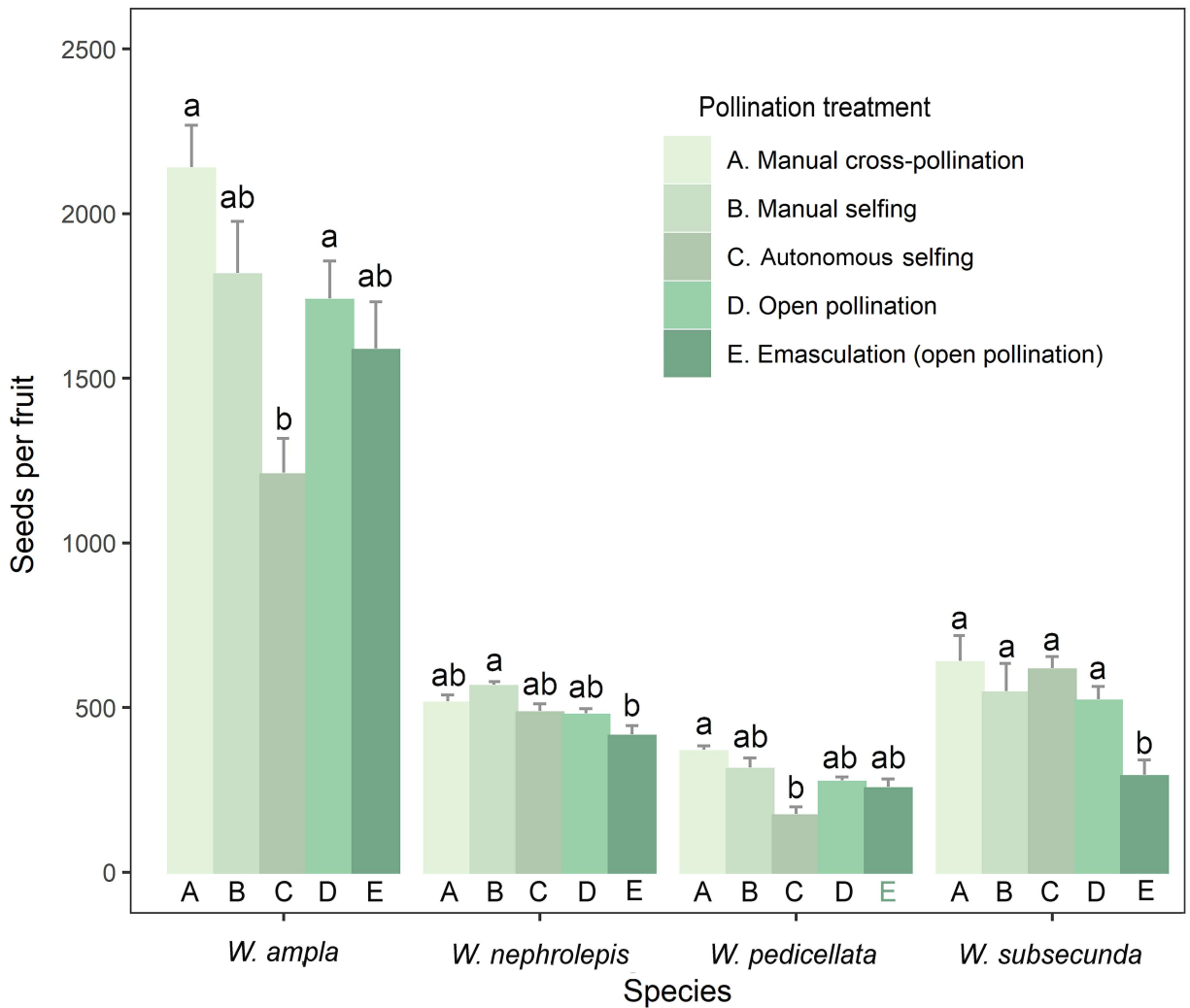
822 **Figure 1.** Studied species of *Werauhia* (Bromeliaceae: Tillandsioideae) in a montane forest, Cerros La
 823 Carpintera, Costa Rica. (A-D) *W. ampla*, (E-H), *W. nephrolepis*, (I-L) *W. pedicellata*, (M-P) *W.*

824 *subsecunda*. (B, F, J, N) Night-vision images of bats visiting inflorescences of the studied species and

825 recorded with video camera traps. (C, G, K, O) Flowers in anthesis. (D, H, L, P) Senescent flowers whose
 826 corollas have lost turgor. Scale bars = 10 cm (A, E, I, M) and 1.0 cm (C, G, K, O).

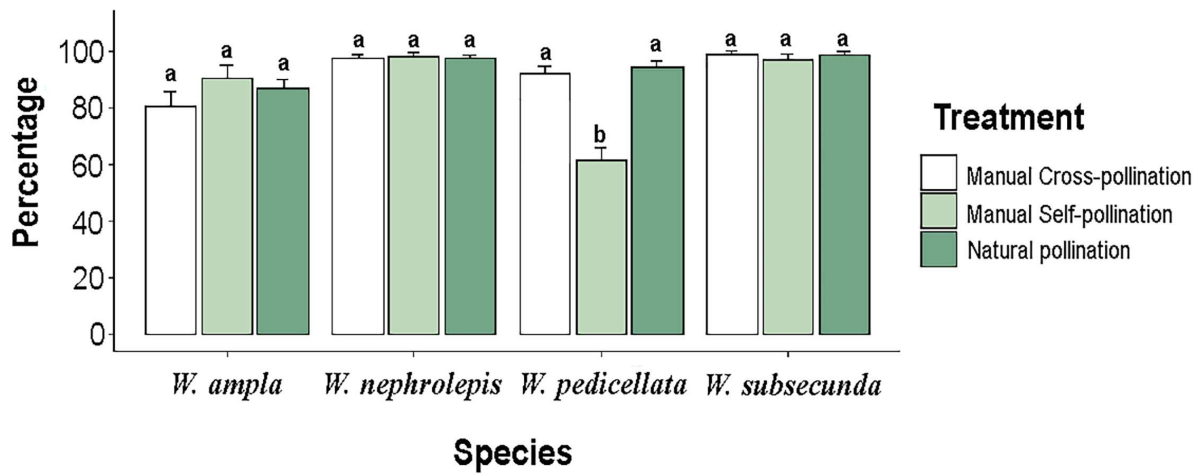
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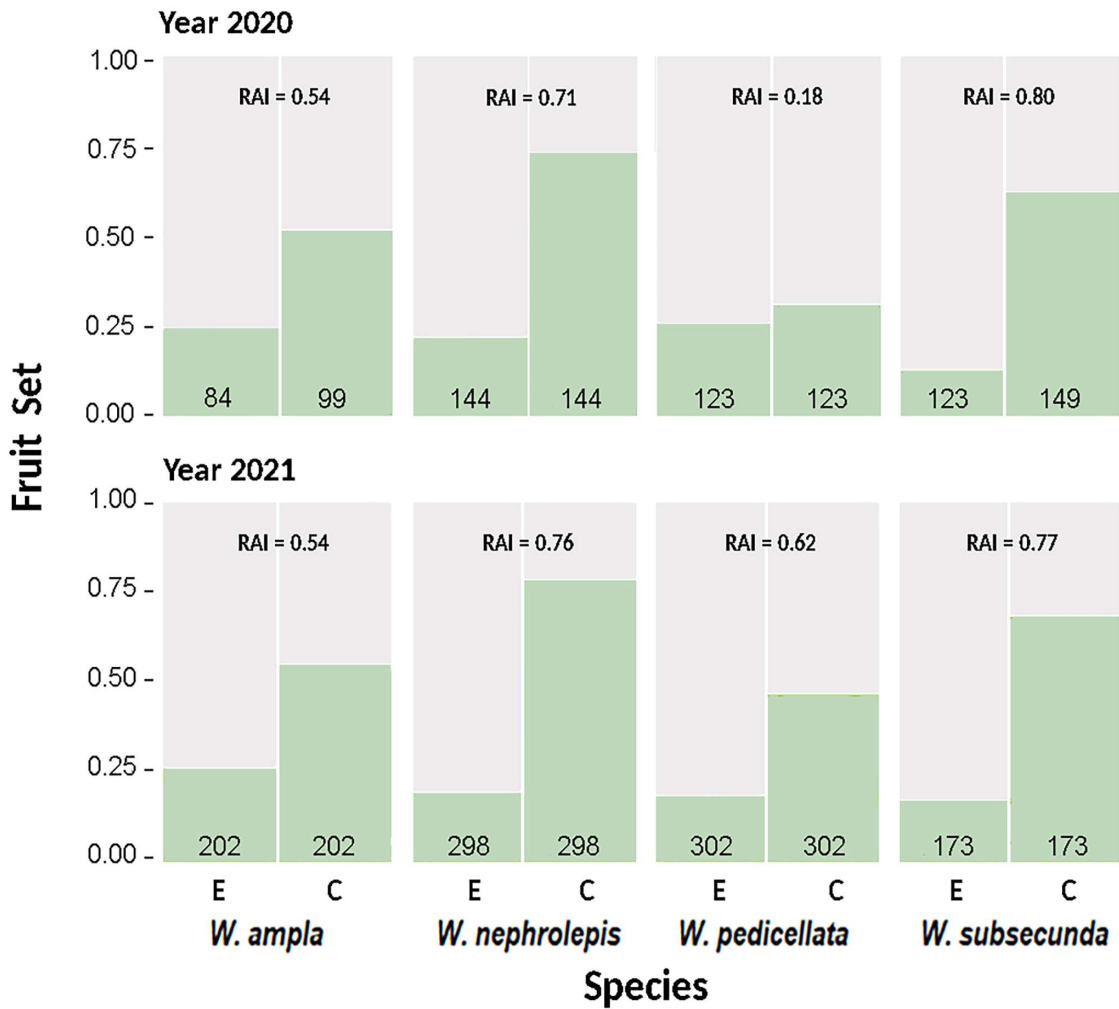
830 **Figure 2.** Seed set in four epiphytic *Werauhia* species (Bromeliaceae: Tillandsioideae) under different
 831 pollination treatments. Plants from a montane tropical forest, Cerros La Carpintera, Costa Rica. Bars are
 832 mean number of seeds per fruit and vertical lines are 1 SE. Different letters indicate significant
 833 differences between treatments per species after a Tukey test.

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Figure 3. Seed germination capacity of progeny sired from hand self- and cross-pollination and open pollination in four species of *Werauhia* (Bromeliaceae: Tillandsioideae) from a montane forest, Cerros La Carpintera, Costa Rica. Data are mean germination percentages from 12 replicates of 40 seeds per treatment after two-months of monitoring. Vertical lines = 1 SE. Different letters indicate significant differences between treatments per species after a Wilcoxon's test. The estimated values of the Inbreeding Depression Index were for *W. ampla* = -0.13, *W. nephrolepis* = 0.00, *W. pedicellata* = 0.34, and *W. subsecunda* = 0.02.



856 **Figure 4.** Fruit set from emasculated (E) and intact (C) flowers under open pollination conditions of four
 857 *Werauhia* (Bromeliaceae: Tillandsioideae) species in a montane forest, Cerros La Carpintera, Costa Rica.
 858 Bars represent the proportions of developed fruits per treatment in two consecutive reproductive seasons
 859 (2020 and 2021). The value of the *Reproductive Assurance Index* (RAI) is indicated for each species and
 860 year. The sample size (number of flowers) per treatment is indicated at the bottom of each column.

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TABLE 1. Floral biology traits of four sympatric *Werauhia* species (Bromeliaceae: Tillandsioideae) from a montane forest, Cerros La Carpintera, Costa Rica.

| Floral trait | <i>W. ampla</i> | <i>W. nephrolepis</i> | <i>W. pedicellata</i> | <i>W. subsecunda</i> |
|---|---|--|--|--|
| Flowers per inflorescence - mean±SD (range, sample size) | 13.3 ± 3.6 (7–24, 45) | 26.7 ± 6.1 (9–42, 42) | 54.7 ± 29.6 (20–145, 42) | 8 ± 1.8 (3–13, 50) |
| Floral display (open flowers per da) - mean±SD (range, sample size) | 1 (rarely 2) (37) | 6.5 ± 2.5 (2–14, 37) | 5.4 ± 2.7 (2–12, 32) | 1 (rarely 2 or 3) (62) |
| Color of peduncle, primary, and floral bracts at anthesis | Green to brown | Greenish | Green with reddish stripes | Green |
| Anthesis time | Late afternoon 15–17:30 h (n=72) | Late afternoon 16–17:30 h (n= 71) | Late afternoon 16–18:00 h (n= 91) | Late afternoon 15:30–17:00 h (n= 56) |
| Flower longevity | 24 h (n= 58) | 6–8 h (n= 55) | 16–19 h (n= 31) | 15–17 h (n= 32) |
| Corolla shape | Campanulate | Bilabiate | Campanulate | Campanulate |
| Corolla color | White-green and suffused with purple toward the petals apex | White-greenish | White-translucent | White-greenish |
| Herkogamy type, (anters-stigma separation, sampled flowers) | Absent or approach type, stigma curved (2–5 mm, n= 58) | Absent, stigma curved (n= 55) | Absent or approach type (1.5–2 mm , n= 31) | Mostly absent (n= 32) |
| Dichogamy type (temporal separation, sampled flowers) | Protogyny, incomplete (5–135 min, 58) | Protogyny, incomplete (10–60 min, 55) | Protogyny, incomplete (20–75 min, 31) | Protogyny, incomplete (5–70 min, 32) |
| Emission of floral scents (organoleptic test) | Slightly perceptible, fermented fruits | Perceptible, garlic and fermented fruits | Perceptible, garlic | Perceptible, fermented fruits |

| | | | | |
|---|---|--|---|--|
| Nectar volume (μ l) per flower - mean \pm SD, [range], (sample size) | 598.1 \pm 217.2 [184.2–952.9] (33 fl / 4 ind) | 327.7 \pm 199 [30–574.8] (30 fl / 6 ind) | 11.9 \pm 10.0 [1–50.5] (33 fl / 11 ind) | 35.3 \pm 29 [5–82.5] (20 fl / 5 ind) |
| Nectar concentration ($^{\circ}$ Brix) - mode, range (sample size) | 17, 12–18 (33 fl. / 4 ind.) | 12, 8–14 (30 fl. / 6 ind.) | 12, 3–13 (33 fl. / 11 ind.) | 12, 8–13 (20 fl. / 5 ind.) |

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TABLE 2. Results of controlled pollination treatments and values of indexes that describe the breeding systems of four epiphytic *Werauhia* species (Bromeliaceae: Tillandsioideae) in a montane forest, Cerros La Carpintera, Costa Rica. Data are fruit percentages (%) and in parenthesis the number of developed fruits / manipulated flowers.

| Experimental variable | <i>W. ampla</i> | <i>W. nephrolepis</i> | <i>W. pedicellata</i> | <i>W. subsecunda</i> |
|--------------------------------|-----------------|-----------------------|-----------------------|----------------------|
| Number of plants (N) | 17 | 15 | 16 | 25 |
| Manual self-pollination | 75.0% (12/16) | 100% (36/36) | 50% (15/30) | 87.5% (14/16) |
| Manual cross-pollination | 82.4 % (14/17) | 94.3 % (33/35) | 58.1 % (18/31) | 76.5 % (13/17) |
| Autonomous self-pollination | 43.3% (13/30) | 71.1% (32/45) | 31.1% (14/45) | 76.7% (23/30) |
| Agamospermy | 0 (0/19) | 10.5% (4/38) | 3.4% (1/29) | 0 (0/17) |
| Self-Compatibility Index (SCI) | 0.91 | 1.06 | 0.86 | 1.14 |
| Auto-Fertility Index (AFI) | 0.53 | 0.75 | 0.54 | 1.00 |
| Agamospermy Index (AGI) | 0.00 | 0.11 | 0.06 | 0.00 |

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TABLE 3. Parameter estimates for the Generalized Lineal Models on the production of fruits between emasculated and unmanipulated flowers under open-pollination conditions in four *Werauhia* species (Bromeliaceae: Tillandsioideae) in a montane forest, Cerros La Carpintera, Costa Rica.

| Parameters by species | d.f. | Estimate | S.E. | Wald Chi-Square | P-value | Odds ratio | Confidence interval (95%) |
|------------------------------|------|----------|------|-----------------|---------|------------|---------------------------|
| <i>W. ampla</i> | | | | | | | |
| Intercept | 1 | -1.16 | 0.26 | -4.54 | <0.001 | | |
| Treatment (control) | 1 | 1.22 | 0.33 | 3.76 | <0.001 | 3.40 | 1.80–6.44 |
| Year (2021) | 1 | 0.10 | 0.30 | 0.34 | 0.732 | 1.11 | - |
| Treatment × year | 1 | 0.03 | 0.39 | 0.09 | 0.929 | 1.03 | - |
| Error | 583 | | | | | | |
| <i>W. nephrolepis</i> | | | | | | | |
| Intercept | 1 | -1.29 | 0.20 | -6.38 | <0.001 | | |
| Treatment | 1 | 2.32 | 0.28 | 8.37 | <0.001 | 10.17 | 5.9–17.51 |
| Year | 1 | -0.17 | 0.25 | -0.68 | 0.498 | 0.84 | - |
| Treatment × year | 1 | 0.48 | 0.35 | 1.38 | 0.164 | 1.62 | - |
| Error | 880 | | | | | | |
| <i>W. pedicellata</i> | | | | | | | |
| Intercept | 1 | -1.09 | 0.21 | -5.24 | <0.001 | | |
| Treatment | 1 | 0.28 | 0.29 | 0.99 | 0.321 | 1.33 | - |
| Year | 1 | -0.44 | 0.26 | -1.70 | 0.088 | 0.65 | - |
| Treatment × year | 1 | 1.12 | 0.34 | 3.28 | 0.001 | 3.07 | 1.57– 6.01 |
| Error | 846 | | | | | | |
| <i>W. subsecunda</i> | | | | | | | |
| Intercept | 1 | -1.97 | 0.28 | -7.16 | <0.001 | | |
| Treatment | 1 | 2.48 | 0.32 | 7.68 | <0.001 | 11.96 | 6.34–22.53 |
| Year | 1 | 0.37 | 0.34 | 1.09 | 0.278 | 1.45 | - |
| Treatment × year | 1 | -0.09 | 0.42 | -0.21 | 0.831 | 0.92 | - |

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875 **List of Supporting information**

876

877 **APPENDIX.** Raw data from experiments.

878

879 **TABLE S1.** Visitation data of nectarivorous bats to flowers of four epiphytic bromeliads from genus
880 *Werauhia* (Bromeliaceae: Tillandsioideae) in a montane forest, Cerros La Carpintera, Costa Rica. Data
881 from six video-camera traps from the flowering periods of 2019, 2020, and 2021.

882

883 **TABLE S2.** Number of pollen grains per plant species recovered from the six nectarivorous bats captured
884 in a montane forest, Cerros La Carpintera, Costa Rica. Data from a sampling effort of 675 m²/h during
885 eighth nights from January to February 2020.

886

887 **VIDEO S1.** Time-lapse video of a flower senescence of *Werauhia ampla* (Bromeliaceae). Frame rate: 30
888 fps. Duration: 15 seconds. Author: Alfredo Cascante-Marín.

889

890 **VIDEO S2.** Time-lapse video of a flower senescence of *Werauhia subsecunda* (Bromeliaceae). Frame
891 rate: 30 fps. Duration: 27 seconds.

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893 **VIDEO S3.** Time-lapse video of a flower senescence of *Werauhia nephrolepis* (Bromeliaceae). Frame
894 rate: 30 fps. Duration: 16 seconds.

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896 **VIDEO S4.** Slow motion video (10X) of a bat visiting a nocturnal flower of *Werauhia ampla*
897 (Bromeliaceae). Duration: 22 seconds.

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899 **VIDEO S5.** Slow motion video (10X) of a bat visiting a nocturnal flower of *Werauhia nephrolepis*
900 (Bromeliaceae). Duration: 7 seconds.

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902 **VIDEO S6.** Slow motion video (10X) of a bat visiting a nocturnal flower of *Werauhia pedicellata*
903 (Bromeliaceae).

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905 **VIDEO S7.** Slow motion video (10X) of a bat visiting a nocturnal flower of *Werauhia subsecunda*
906 (Bromeliaceae). Duration: 10 seconds.

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