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1 **Title: The morphological allometry of four closely related and coexisting insect species**
2 **reveals adaptation to the mean and variability of the resource size.**

3

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5

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12

1 **1** Declaration of Authorship: PFP and SV conceived and designed the experiment. FD and
2 PFP performed the field work, PFP and MCBV measured the insects. EF and MCBV
3 analyzed the data. EF, SV and MCBV wrote the manuscript.

13 **Abstract**

14 The size of organisms may result from various, sometimes antagonistic forces operating on
15 distinct traits, within an evolutionary framework that may also be constraining. Morphological
16 allometry, referring to the way trait size scales with body size, has been shown to reflect
17 ecological adaptation to the mean size of the resource exploited. We examined the allometric
18 relationships between rostrum and body size among four insect (*Curculio* spp.) specialists of
19 oak acorns. In all four species, weevil females drill a hole with their rostrum prior depositing
20 one or a few eggs inside the seed. The four weevil species, that coexist on the same individual
21 trees, displayed partitioned egg-laying periods in the year, thereby encountering acorns of
22 different size and maturation stage. We found marked differences in the allometric slope
23 among females: species laying eggs late in the season had a steeper slope, leading to
24 increasingly longer rostrum relative to body length, along with the mean size of the growing
25 acorns. Females of the smallest species had the longest oviposition period and also had the
26 steepest slope, which provided them with the most variable rostrum length, thereby matching
27 the variable size of the resource through time. Our work highlights the need to consider not
28 only the average size but also the degree of variability in resource size to understand the
29 adaptive value of allometric relationships.

30 **Key-Words:** rostrum size; scaling relationship; oak weevil; *Curculio* spp.; egg-laying;
31 variability in resource size

32 **Introduction**

33 The ways the size of traits covary with body size is a major issue in evolutionary ecology, and
34 scaling relationships have been shown to result from various, sometimes opposite, selective
35 forces operating on various traits, while being embedded in a conservative phylogenetic
36 framework. Allometric relationships can be measured during growth (ontogenetic allometry),
37 among individuals of the same population and status (static allometry), or between
38 populations or species (evolutionary allometry) (Pélabon et al. 2014). Quantifying the impact
39 of biomechanical, developmental or genetic constraints on trait expression (allometric
40 constraints) and that of functional optimisation (allometric adaptation) would help to
41 understand the diversity of traits and reveal their evolutionary potential (Brakefield and
42 Roskam 2006; Voje and Hansen 2013; Pélabon et al. 2014; Harrison 2015; Poe et al. 2021).

43 The evolution of morphological allometry has been extensively studied in sexual selection
44 and has major impact on primary and secondary sexual traits (Green 1992; Baker and
45 Wilkinson 2001; Mezey and Houle 2005; Kodric-Brown et al. 2006; Eberhard 2009; Eberhard
46 et al. 2009; Houle et al. 2011; Voje et al. 2013; Lemaître et al. 2014; Pélabon et al.
47 2014; Hansen 2015). The traits of an organism may be subject to various selection
48 pressures, which may lead to changes in their scaling relationship with body size and in the
49 overall body shape. Such evolutionary changes have been shown to promote ecological
50 adaptation to biophysical environmental features (e.g., shell shape characterizing distinct
51 ecotypes in a snail, Hollander et al. 2006), or to the size of the exploited resource (Boag 1984;
52 Toju and Sota 2006; Skandalis et al. 2017; Pigot et al. 2020). There is a paradox between the
53 evolutionary potential of scaling relationships revealed in artificial selection experiments, and
54 the very conserved size allometries observed on a broad taxonomic scale (Bolstad et al. 2015),
55 suggesting that allometric constraints are responsible for preventing rapid divergent
56 adaptation (Rombaut et al. 2022).

57 Most studies examining the scaling relationships between the traits involved in resource use,
58 and discussing their adaptive value, have considered the average size and not the variability in
59 the size of the items used. We explored the relationship between allometric size relationships
60 in weevils (*Curculio* spp.) and the mean and variability of the resource used. Weevils provide
61 a biological system well suited to study evolutionary aspects of morphological allometric
62 relationships: they are phytophagous insects specialist of plant host species, mainly nut seeds,
63 which females use for oviposition. In a number of species, females cling to the nut to bore a
64 hole with their rostrum before depositing one or a few eggs inside with their extended
65 ovipositor (Donaldson 1992; Bonal et al. 2011; Zhang et al. 2021). Rostrum is an oral
66 appendage notably elongated in Curculionid species (Davis 2017) and its size differs greatly
67 among *Curculio* species, resulting from an ecological adaptation to the average size of mature
68 seeds exploited (Hughes and Vogler 2004a). Previous studies suggest highly conserved
69 scaling relationships between rostrum and body size among *Curculio* species (Bonal et al.
70 2011; Toju and Sota 2006; but see Donaldson 1992), with species or populations differing
71 from each other in mean body size and/or rostrum to body size ratio. In this study, we
72 examined the allometric relationships between rostrum and body length in four oak weevil
73 species that are phylogenetically close to each other, coexist on the same trees, and are all
74 specialised in exploiting oak acorns in which females lay their eggs and the larvae fully
75 develop (Coutin 1992; Hughes and Vogler 2004b; Venner et al. 2011).

76 We first determined the size of oak acorns at the time each of the four species oviposit, then
77 we tested which of the two opposite predictions the observed patterns most accurately fitted:
78 (i) conserved scaling relationships among species and between males and females within
79 species, provided that these are phylogenetically close to each other (Hughes and Vogler
80 2004b; Péliesson et al. 2011). Differences in rostrum size would then possibly occur as a
81 consequence of mean differences between the weevil species in either body size or rostrum-
82 to-body size ratios. This prediction would be in line with previous results found on weevil
83 species (Toju and Sota 2006; Bonal et al. 2011). (ii) As these four species have partitioned

84 oviposition period over the year (Pélisson et al. 2013), the oak acorns that females
85 encounter should have distinct size characteristics (mean and variance), which could support
86 distinct rostrum size and possibly distinct scaling relationship between rostrum and body size.
87 Rostrum size would be expected to increase, on average, as females lay eggs late in the season
88 (i.e., into big nuts), and this should be accompanied by increased hypermetry in the rostrum vs
89 body size relationship. In the species with the longest oviposition period, females are likely to
90 encounter acorns of more variable size throughout the season; rostrum size should therefore
91 be more variable between females, which would be achieved through either increased
92 variability in body size or stronger hypermetry.

93

94 **Materials and Methods**

95 **1 Insect Sampling**

96 The weevil communities under study are composed of four closely related species (*Curculio*
97 *elephas*, *C. glandium*, *C. pellitus*, *C. venosus*) infesting and competing for acorns of the same
98 oak trees. Adults of three species (*C. pellitus*, *C. venosus* and *C. glandium*) emerge in April,
99 in a rather synchronous way, which is two to three months before the earliest female was
100 found ready to oviposit (Venner et al. 2011, Pélisson et al. 2012). Adults of the remaining
101 species (*C. elephas*) emerge far later in the season, i.e., between the end of July and the
102 beginning of August, and females are sexually receptive and ready to lay eggs into the acorns
103 from their first day of emergence. Adults of all four species die at the end of the breeding
104 season.

105 For all species, adults of both sexes were sampled over five years (2004 to 2009 except 2008)
106 on four ~150-200 year-old sessile oak trees located in a fragmented, agricultural landscape
107 near Lyon (France) (*Quercus petraea*: N 45°45'; E 5° 16'). Each year we sampled insects on
108 oak trees on a weekly basis from early April to mid-October, i.e., from the time when the first

109 adult weevils emerge above ground until no more adult weevil could be trapped on two
110 consecutive sampling sessions.

111 The weevils were live-trapped by consistently beating each of several branches with a wooden
112 stick within one hour after sunrise (Schauff 1986). Insect weevils that had fallen from the
113 branches were collected on a textile sheet on the ground under the canopy, brought back to the
114 laboratory in a cooling box, and their sex and species were identified on the basis of
115 morphological criteria (Hoffmann 1954; Venner et al. 2011; Péliesson et al. 2013). Body and
116 rostrum length were measured on the live-trapped insects to the nearest 0.1 mm using a
117 binocular microscope (Zeiss stemi-C; Zeiss, Illkirch, France; magnification x16); body length
118 was measured from the head - excluding the rostrum - to the end of the elytra covering the
119 end of the abdomen, and rostrum was sized after being gently flattened.

120 **2 Determination of the egg-laying period**

121 To detect the onset of the breeding period for each species, two females of each species were
122 randomly selected, whenever available, at each sampling session in 2009. These females were
123 dissected under a binocular microscope and their total oocytes were counted (mature and
124 immature). We also checked the presence of spermatozoa in the spermathecae of females
125 using DAPI staining (DiAminido Phenyl Indol). For each spermatheca, we used a 20µl
126 staining solution (15µl of Vectashield without DAPI blended with 5µl of Vectashield with
127 DAPI) to fix the coverslip on the glass slide. Following staining, each sample was stored two
128 days at 4°C in the dark before checking the presence of sperm with a fluorescence microscope
129 (Imager Z1 AxioCam). The onset of the breeding period was set for each species as the day
130 when the first female had chorionated eggs and sperm in her spermatheca. The end of the
131 breeding season was the day when the last female was sampled on the trees. After discarding
132 females trapped outside their breeding period, we calculated the laying period for each species
133 as the time interval when 90% of the females were trapped (5-95 percentiles).

134 **3 Acorn sampling**

135 As the acorns used for oviposition by female oak weevils grow and mature throughout the
136 season, their size was likely to differ depending on the species and its oviposition period. In
137 2009, we collected oak acorns from the branches of the trees weekly from mid-June, i.e. from
138 fruit initiation until the 2 October, when no more fruit could be found on the branches and the
139 very last female weevils were caught. At each sampling session, 15 healthy (i.e.,
140 unparasitized) acorns were randomly selected and brought back to the laboratory. The acorns
141 were removed from their cups whenever feasible, measured with an electronic caliper to the
142 nearest 0.1 mm for their maximum length (L1), and their maximum diameter taken along two
143 perpendicular axes (L2 and L3).

144 We estimated their volume considering acorns as ellipsoids, computing the formulae :

145
$$volume = \frac{4}{3} \times \pi \times \frac{L_1}{2} \times \frac{L_2}{2} \times \frac{L_3}{2}$$

146 We used the cubic root of the estimated acorn volume for subsequent analyses, since it was in
147 the same dimension unit as body or rostrum length of insects, for comparative purposes. At
148 the first two sampling sessions, the acorns were too immature to be removed from their cup
149 prior to being sized, yet these dates were outside the egg-laying period for any of the weevil
150 species, thereby allowing unbiased analyses of the fruit size.

151 **Statistical treatment**

152 We used a non-parametric Kruskal-Wallis test to detect interspecific differences in the egg-
153 laying period, using the Julian date of capture of fertile females of each species as the variable
154 of interest. We also used Bartlett's test on the same variable to see if these periods could vary
155 in duration between species.

156 We set up a general model to test whether the allometric relationships between rostrum and
157 body length are likely to differ between the four weevil species, between males and females
158 within the same species, or whether the inter-sexual differences depend on the species

159 considered. To this end, we fitted linear relationships between log-transformed rostrum and
160 body lengths, testing the effect of sex, species and their interaction using ANCOVA (Pélabon
161 et al. 2014; Kilmer and Rodríguez 2017). We then tested for interspecific differences in the
162 scaling relationship between rostrum and body length in males and females separately, in
163 order to detect possible sex-specific differences. We estimated the allometric coefficients
164 between rostrum and body length from the most complete model and calculated their 95%
165 confidence intervals.

166 We also examined a possible non-linear allometric relationship between rostrum and body
167 length in females. For each species, we selected the best-fitted model out of five models,
168 based on AIC values: Neutral, Segmented Neutral (single step model), Linear (already fitted
169 in the previous section), Quadratic, and Segmented (with a single breakpoint estimated,
170 separating the two best-fitted linear regression models).

171 We examined whether the variability in weevil size could be linked to that of acorn size: we
172 computed for each weevil sex and species the coefficient of variation of rostrum length, body
173 length and the rostrum to body length ratio, which we plotted against the coefficient of
174 variation of the acorn size at the time acorns were used by each species. 95% confidence
175 intervals were computed for these values by means of bootstrapping , a resampling method
176 robust to the actual non-normal distribution of the statistic (Hinkley 1988).

177 All analyses were performed with the R free software environment, using the 'boot' and
178 'segmented' packages in addition to the basic functionalities (R core team 2018).

179

180 **Results**

181 **1 Seasonal distribution of oviposition, size of acorns at oviposition and size of**
182 **females of the four oak weevil species.**

183 The females of all four species laid their eggs into oak acorns at very different times of the
184 year (Fig. 1a; Table S1; Kruskal-Wallis test: Chi-square = 332.4, df = 3, $P < 2.2 \times 10^{-16}$) and
185 the laying period was very variable (Bartlett test: Chi-square = 103.72, df = 3, $P < 2.2 \times 10^{-16}$).

186 Females of all four species exhibited distinct size characteristics (Table 1; ANOVA test of the
187 effect of species on body length variation: $F_{3,569} = 310.9$, $P < 0.0001$; $R^2 = 0.62$), and due to the temporal sharing of the resource, laid eggs into oak acorns of very
188 different sizes (Fig. 1b; Table S2; Kruskal-Wallis test: Chi-square = 194.3, df = 3, $P < 2.2 \times 10^{-16}$;
189 16; Bartlett test: Chi-square = 150.2, df = 3, $P < 2.2 \times 10^{-16}$). *C. glandium* and *C. elephas* are the
190 smallest and largest species, respectively (Fig. 1c). All four species were similarly ranked for
191 both rostrum length and average acorn size at oviposition. Considering the ratio of rostrum to
192 body length, however, the females of *C. glandium* almost reached the maximum values of *C.*
193 *elephas* (Fig. 1d), while *C. venosus* and *C. pellitus* (the two early species that oviposit in small
194 fruits) had much lower ratio values.

196 *C. glandium* was the species where females had the most variable rostrum to body length ratio
197 (Table 1; Bartlett's test: Chi-square = 186, df = 3, $P < 0.0001$), and the highest coefficient of
198 variation of rostrum length while experiencing the greatest variation in acorn size (Fig. 1e,
199 dots). Their body size was not especially variable, compared to other female species (Fig. 1e,
200 triangles). Males *C. glandium* did not show any particular variability, neither in their rostrum
201 nor their body size (Fig. 1f).

202

203 **2 Allometric relationships between rostrum and body length as a function of**
204 **species and sex**

205 The linear relationship between log-transformed rostrum and body length differed
206 significantly between the four species and the two sexes, indicating different allometry rules
207 in this group (Table 2a). Considering females and males separately, the slope of the regression
208 statistically differed among females but not among males (Table 2b, 2c).

209 ***Sex differences***

210 In two of the four species, i.e. *C. elephas* and *C. glandium*, females showed a significantly
211 greater allometric slope than males (C.g.: 0.606 ± 0.067 (estimated difference \pm s.e.), $t=9.07$,
212 $P < 0.0001$; C.e.: 0.278 ± 0.107 , $t=2.61$, $P=0.0091$), while no significant difference was found
213 between the two sexes for *C. venosus* and *C. pellitus* (C.v.: 0.008 ± 0.090 , $t=0.09$, $P=0.93$;
214 C.p.: 0.204 ± 0.207 , $t=0.99$, $P=0.32$). Overall, within each of the four species, females had a
215 longer rostrum than males at the same body size (Fig. 2 A-D) and had a greater rostrum to
216 body length ratio compared to conspecific males (ANCOVA: Species:Sex interaction, $F_{obs} =$
217 1226.5 , $df = 3$ and 943 , $P < 2.2e-16$). The largest differences between the two sexes were
218 found for the species *C. glandium* and *C. elephas* (Table S3, Figs. 2C and 2D).

219 ***Interspecific differences among females***

220 Only *C. glandium* females showed marked hypermetry between rostrum and body length
221 (Table 3, Fig. 2), while *C. elephas* and *C. pellitus* females did not deviate statistically from the
222 isometric relationship and while *C. venosus* females showed hypometry. All species showed a
223 monotonic linear static allometry between rostrum and body length (see Table S4, Fig. 3),
224 with the exception of *C. glandium* females which fitted a segmented linear model with a
225 single breakpoint dividing the sample into 45 and 173 females further assigned to the 'small-
226 bodied' and 'large-bodied' groups, respectively (Fig. 3 and S1; $R^2 = 0.79$).

227 In *C. glandium*, small-bodied females experienced greater increase in their rostrum length for
228 a given variation in body size than did large-bodied females ('small-bodied' group, slope =
229 1.968 [$1.53-2.41$; 95% CI]; 'large-bodied' group, slope = 1.236 [$1.07-1.40$; 95% CI];

230 ANOVA, interaction body length x group size on rostrum size: $F_{obs} = 9.54$, $df = 1$ and 214 , P
231 $= 0.0023$). Large *C. glandium* females, being consistently smaller than *C. elephas* females,
232 had on average a greater slope value than *C. elephas* females (ANOVA, interaction body
233 length x species: $F_{obs} = 5.56$, $df = 1$ and 261 , $P = 0.02$). The size of *C. glandium* females at
234 the time of their capture did not vary during the egg laying period (see fig S2).

235 **Discussion**

236 Allometry rules between traits of organisms are usually highly conserved between related
237 species, which is acknowledged to be the result of constraints or stabilizing selection (Pélabon
238 et al. 2014; Bolstad et al. 2015; Uyeda et al. 2017; Marcy et al. 2020). Examining the scaling
239 relationships between rostrum and body size in four species of phytophagous insects of the
240 genus *Curculio*, we found strong divergences between species despite being phylogenetically
241 closely related, coexisting and dependent on the same resource. Our results suggest that
242 rostrum size may evolve, as well as its relationship with body size, towards an optimized use
243 of the acorns whose mean size and variance differ according to their period of exploitation by
244 the insect species.

245 The weevils of the genus *Curculio* are mostly host plant specialists and constitute an
246 ecologically diverse group, and it has been shown that the rostrum size of females belonging
247 to this genus has evolved with the size of the mature seeds of the host plant (Hughes and
248 Vogler 2004a). At the intraspecific level, female rostrum size has been shown to coincide
249 with the average thickness of plant tissue that females must drill through before oviposition,
250 consistent with the high adaptive value of this female trait associated with oviposition
251 behavior (Toju and Sota 2006; Toju 2008). Here, we found marked differences in mean
252 rostrum size, paralleling the seasonal partitioning of oviposition that encompasses the period
253 of acorn growth and maturation (Pélisson et al. 2013). Species that oviposited earlier in the
254 season, on small and immature fruits, had smaller rostrums than those that oviposited later, on
255 larger fruits. Our results suggest that the seasonal partitioning of the oviposition period, which

256 leads to strong differences in fruit size at oviposition, promoted a divergent evolution of
257 rostrum size among coexisting species, amplified by distinct scaling relationships between
258 this trait and body size.

259 In this study, the consistent hypometry found in males contrasted with the diversity of the
260 scaling relationships observed in females of the four weevil species, suggesting that the
261 rostrum size of females may be under divergent selection. Rostrum length in females may
262 ultimately be determined by the morphological attributes of the seeds - and/or their variability
263 - at the time these are exploited. The early-laying species *C. venosus* showed hypometric
264 relationships: large females had a relatively smaller rostrum than small females, a conserved
265 trait that may be optimal for laying eggs into small, immature fruits. Females of the later
266 species, *C. elephas*, did not differ statistically in their slope from *C. pellitus*, but had the
267 longest rostrum size for a given body size. In *C. elephas* females, the largest of the four oak
268 weevil species, rostrum is proportional to body size, allowing them to excavate a deep hole to
269 reach the base of large, mature acorns where they lay their eggs (Merville et al. 2014).

270 Of the four weevil species, *C. glandium* females had the steepest allometric slope of rostrum
271 versus body size, higher by 32 to 66% than females of the other three *Curculio* species.
272 Furthermore, *C. glandium* females are the only ones for which the non-linear scaling
273 relationship best fitted the observations: females above a threshold body size experienced
274 shallower, yet still hypermetric slope. Such hypermetry gives females exaggerated variability
275 in rostrum size relative to body size, and the ability to infest acorns of different sizes and
276 developmental stages. Of the four weevil species, *C. glandium* females exhibited the greatest
277 variability in rostrum size, but not body size, suggesting stabilizing selection on body size
278 and/or stronger selectional direction on trait than on body size (Blanckenhorn 2000,
279 Bonduriansky and Day 2003).

280 Our results differ from those described by Bonal et al (2011) who found that *C. glandium* and
281 *C. elephas* females had the same relationship between rostrum and body length (slope and
282 intercept), suggesting that the longer rostrum of *C. elephas* was merely due to their greater

283 body size. Their study may differ from ours in three ways: (i) data were not log transformed,
284 (ii) the insect weevil populations were trapped on different oak species, with *Q. ilex* and *Q.*
285 *humilis* producing smaller mature fruits than those produced by *Q. petraea* studied here, and
286 (iii) rostrum size is adjusted to elytron length, not body length as in our study. Still, their
287 results seem consistent with a possible non-linear scaling relationship in *C. glandium* females
288 (Fig. 1 of Bonal et al. 2011), similar to our results, although the non-linearity of the
289 relationship was not tested in their study.

290 All four weevil species are specialists of oak acorns, a resource whose availability is highly
291 fluctuating from year to year (Schermer et al. 2019, 2020). In years of low fruit abundance,
292 early species should have an advantage because they exploit the resource first: their small
293 rostrum allows them to lay eggs optimally in these small, immature fruits. In years of high
294 fruit abundance, late weevil species would benefit most as they lay eggs in safe, large, mature
295 acorns with their large rostrum. A series of low and high fruiting years would then alternately
296 favor early and late ovipositing females. In contrast to the other species, *C. glandium* females
297 have a prolonged oviposition period, and also show the most variable rostrum length, afforded
298 by a strong hypermetry with their body size. The heterogeneity of rostrum length among
299 individuals may result from a bet-hedging strategy, induced by maternal effects: the variable
300 number of eggs laid per acorn should lead to variable larval mass and adult size, and females
301 would then have offspring with highly variable rostrum size. In this sense, the hypermetry
302 found in *C. glandium* females would favor risk spreading, allowing the same genotype to
303 efficiently exploit the resource earlier or later in the season, depending on the quantity of
304 acorns available, which varies from year to year. Future investigations are needed to assess
305 whether females in this species do select acorns assortatively with their own size and/or
306 whether egg-laying efficiency varies according to the degree of assortment between the
307 acorns and their own size.

308 Our study reveals diverse scaling relationships between rostrum and body size in females of
309 four phylogenetically related species: this suggests divergent evolution of the allometric rules

310 underlying morphological traits in this group of insects. Scaling relationships between traits
311 and body size in insects have been experimentally shown to evolve in response to direct
312 selection (Bolstad et al. 2015). From a broader perspective, the responsiveness of allometric
313 relationships can have a decisive impact on the assembly of species within communities.
314 Body size is often considered sufficient to summarize the ecological niche of competing
315 species, given that many traits covary with body size, and mainly differentiate species in the
316 way they interact with each other and with the environment (Ritchie 2009; Almaraz and Oro
317 2011; Mancini et al. 2014; Ofstad et al. 2016). Our work, however, **calls for** going beyond
318 body size by reconsidering species of similar size but with distinct allometric relationships
319 that could generate cryptic niche partitioning, thereby stabilizing their coexistence. In that
320 sense, the variability of the size of the resource shared by the species may have a major
321 impact on divergent evolution of morphological allometry, ecological niche partitioning and
322 species assemblage.

323

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327

328 |

329 | **Declarations**

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331 Conflict of Interest: The authors declare that they have no conflict of interest.

332 Ethical approval : All applicable institutional and/or national guidelines for the care and use
333 of animals were followed.

334 Consent to participate: All patients included in this study gave written informed consent to
335 participate in this research.

336 Consent for publication All patients provided written informed consent to publish the data
337 contained within this article.

338 Availability of data and materials: The datasets used and/or analysed during the current study
339 are available from the corresponding author on reasonable request.

340 Code availability: Not applicable

341 **References**

- 342 Almaraz P, Oro D (2011) Size-mediated non-trophic interactions and stochastic predation drive
343 assembly and dynamics in a seabird community. *Ecology* 92:1948–1958.
344 <https://doi.org/10.1890/11-0181.1>
- 345 Baker RH, Wilkinson GS (2001) Phylogenetic analysis of sexual dimorphism and eye-span allometry
346 in stalk-eyed flies (Diopsidae). *Evolution* 55:1373–1385. [https://doi.org/10.1111/j.0014-](https://doi.org/10.1111/j.0014-3820.2001.tb00659.x)
347 [3820.2001.tb00659.x](https://doi.org/10.1111/j.0014-3820.2001.tb00659.x)
- 348 Blanckenhorn WU (2000) The evolution of body size: what keeps organisms small? *Q Rev Biol.*
349 75:385–407. doi: 10.1086/393620.
- 350 Boag PT (1984) Growth and allometry of external morphology in Darwin’s finches (*Geospiza*) on Isla
351 Daphne Major, Galapagos. *J Zool* 204:413–441. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-7998.1984.tb02380.x)
352 [7998.1984.tb02380.x](https://doi.org/10.1111/j.1469-7998.1984.tb02380.x)
- 353 Bolstad GH, Cassara JA, Márquez E, et al (2015) Complex constraints on allometry revealed by
354 artificial selection on the wing of *Drosophila melanogaster*. *Proc Natl Acad Sci USA*
355 112:13284–13289. <https://doi.org/10.1073/pnas.1505357112>
- 356 Bonal R, Espelta JM, Vogler AP (2011) Complex selection on life-history traits and the maintenance
357 of variation in exaggerated rostrum length in acorn weevils. *Oecologia* 167:1053–1061.
358 <https://doi.org/10.1007/s00442-011-2036-7>
- 359 Bonduriansky R, Day T (2003) The evolution of static allometry in sexually selected traits. *Evolution*
360 57(11): 2450–8. doi: 10.1111/j.0014-3820.2003.tb01490.x
- 361 Brakefield PM, Roskam JC (2006) Exploring Evolutionary Constraints Is a Task for an Integrative
362 Evolutionary Biology. *Am Nat* 168:4–13. <https://doi.org/10.1086/509049>
- 363 Coutin R (1992) Original characteristics of the evolving cycles of some European weevil species:
364 *Curculio elephas* Gyll., *C. nucum* L., *C. glandium* Marsh., *C. venosus* Grav. and *C. villosus* F.
365 In: *Memoires de la Societe Royale Belge d’ Entomologie (Belgium)*. *Memoires van de*
366 *Koninklijk Belgische Vereniging voor Entomologie*. v. 35(1).

- 367 Davis SR (2017) The Weevil Rostrum (Coleoptera: Curculionoidea): Internal Structure and
368 Evolutionary Trends. *Bull Am Mus Nat Hist* 416:1–76. [https://doi.org/10.1206/0003-0090-](https://doi.org/10.1206/0003-0090-416.1.1)
369 416.1.1
- 370 Donaldson JS (1992) Adaptation for oviposition into concealed cycad ovules in the cycad weevils
371 *Antliarhinus zamiae* and *A. signatus* (Coleoptera: Curculionoidea). *Biol J Linn Soc* 47:23–35.
372 <https://doi.org/10.1111/j.1095-8312.1992.tb00653.x>
- 373 Eberhard W, Rodriguez RL, Polihronakis M (2009) Pitfalls in understanding the functional
374 significance of genital allometry. *J Evol Biol* 22:435–445. [https://doi.org/10.1111/j.1420-](https://doi.org/10.1111/j.1420-9101.2008.01654.x)
375 9101.2008.01654.x
- 376 Eberhard WG (2009) Static allometry and animal genitalia. *Evolution* 63:48–66.
377 <https://doi.org/10.1111/j.1558-5646.2008.00528.x>
- 378 Green AJ (1992) Positive allometry is likely with mate choice, competitive display and other
379 functions. *Anim Behav* 43:170–172. [https://doi.org/10.1016/S0003-3472\(05\)80086-7](https://doi.org/10.1016/S0003-3472(05)80086-7)
- 380 Hansen TF (2015) Evolutionary Constraints
- 381 Harrison JF (2015) Evolvability and nonevolvability of allometric slopes. *Proc Natl Acad Sci USA*
382 112:13426–13427. <https://doi.org/10.1073/pnas.1517621112>
- 383 Hinkley DV (1988) Bootstrap methods. *J R Stat Soc Series B*, 50:321-337.
384 <https://doi.org/10.1111/j.2517-6161.1988.tb01731.x>
- 385 Hoffmann A (1954) Faune de France. Coleopteres Curculionides, (Deuxième partie). P. Lechevalier,
386 Paris, France
- 387 Hollander J, Adams DC, Johannesson K (2006) Evolution of adaptation through allometric shifts in a
388 marine snail. *Evolution* 60:2490–2497. <https://doi.org/10.1111/j.0014-3820.2006.tb01884.x>
- 389 Houle D, Pélabon C, Wagner GP, Hansen TF (2011) Measurement and Meaning in Biology. *Q Rev*
390 *Biol* 86:3–34. <https://doi.org/10.1086/658408>
- 391 Hughes J, Vogler AP (2004a) Ecomorphological Adaptation of Acorn Weevils to Their Oviposition

392 Site. *Evolution* 58:1971–1983. <https://doi.org/10.1111/j.0014-3820.2004.tb00483.x>

393 Hughes J, Vogler AP (2004b) The phylogeny of acorn weevils (genus *Curculio*) from mitochondrial
394 and nuclear DNA sequences: the problem of incomplete data. *Molecular Phylogenetics and*
395 *Evolution* 32:601–615. <https://doi.org/10.1016/j.ympev.2004.02.007>

396 Kilmer JT, Rodríguez RL (2017) Ordinary least squares regression is indicated for studies of
397 allometry. *J Evol Biol* 30:4–12. <https://doi.org/10.1111/jeb.12986>

398 Kodric-Brown A, Sibly RM, Brown JH (2006) The allometry of ornaments and weapons. *Proc Natl*
399 *Acad Sci USA* 103:8733–8738. <https://doi.org/10.1073/pnas.0602994103>

400 Lemaître J-F, Vanpé C, Plard F, Gaillard J-M (2014) The allometry between secondary sexual traits
401 and body size is nonlinear among cervids. *Biol Lett* 10:20130869

402 Mancini P, Hobson K, Bugoni L (2014) Role of body size in shaping the trophic structure of tropical
403 seabird communities. *Mar Ecol Prog Ser* 497:243–257. <https://doi.org/10.3354/meps10589>

404 Marcy AE, Guillerme T, Sherratt E, et al (2020) Australian Rodents Reveal Conserved Cranial
405 Evolutionary Allometry across 10 Million Years of Murid Evolution. *Am Nat* 196:755–768.
406 <https://doi.org/10.1086/711398>

407 Merville A, Vallier A, Venner S, et al (2014) Determining the instar of a weevil larva (Coleoptera:
408 Curculionidae) using a parsimonious method. *Eur J Entomol* 111:567–573.
409 <https://doi.org/10.14411/eje.2014.056>

410 Mezey JG, Houle D (2005) The dimensionality of genetic variation for wing shape in *Drosophila*
411 *melanogaster*. *Evolution* 59:1027–1038. <https://doi.org/10.1111/j.0014-3820.2005.tb01041.x>

412 Ofstad EG, Herfindal I, Solberg EJ, Sæther B-E (2016) Home ranges, habitat and body mass: simple
413 correlates of home range size in ungulates. *Proc R Soc B* 283:20161234.
414 <https://doi.org/10.1098/rspb.2016.1234>

415 Pélabon C, Firmat C, Bolstad GH, et al (2014) Evolution of morphological allometry. *Ann NY Acad*
416 *Sci* 1320:58–75. <https://doi.org/10.1111/nyas.12470>

- 417 Pélisson P-F, Bel-Venner M-C, Rey B, et al (2012) Contrasted breeding strategies in four sympatric
418 sibling insect species: When a proovigenic and capital breeder copes with a stochastic
419 environment. *Funct Ecol* 26:198–206. <https://doi.org/10.2307/41428802>
- 420 Pélisson P-F, Bernstein C, François D, et al (2013) Dispersal and dormancy strategies among insect
421 species competing for a pulsed resource. *Ecol Entomol* 38:470–477.
422 <https://doi.org/10.1111/een.12038>
- 423 Pélisson PF, Henri H, Bel-Venner MC, et al (2011) Identification at the larval stage of four *Curculio*
424 species coexisting on oak trees using PCR-RFLP. *Entomol Exp Appl* 138:77–82.
425 <https://doi.org/10.1111/j.1570-7458.2010.01077.x>
- 426 Pigot AL, Sheard C, Miller ET, et al (2020) Macroevolutionary convergence connects morphological
427 form to ecological function in birds. *Nat Ecol Evol* 4:230–239.
428 <https://doi.org/10.1038/s41559-019-1070-4>
- 429 Poe S, Donald LAH, Anderson C (2021) What constrains adaptive radiation? Documentation and
430 explanation of under-evolved morphologies in *Anolis* lizards. *Proc R Soc B* 288:20210340.
431 <https://doi.org/10.1098/rspb.2021.0340>
- 432 R Core Team (2018). R: A language and environment for statistical computing. R Foundation
433 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- 434 Ritchie ME (2009) Scale, Heterogeneity, and the Structure and Diversity of Ecological Communities.
435 Princeton University Press
- 436 Rombaut LMK, Capp EJR, Cooney CR, et al (2022) Allometric conservatism in the evolution of bird
437 beaks. *Evol Lett* 6:83–91. <https://doi.org/10.1002/evl3.267>
- 438 Schauff ME (1986) Collecting and preserving insects and mites: Tools and techniques, Systematic
439 Entomology Laboratory
- 440 Schermer É, Bel-Venner M, Fouchet D, et al (2019) Pollen limitation as a main driver of fruiting
441 dynamics in oak populations. *Ecol Lett* 22:98–107. <https://doi.org/10.1111/ele.13171>

- 442 Schermer É, Bel-Venner M, Gaillard J, et al (2020) Flower phenology as a disruptor of the fruiting
443 dynamics in temperate oak species. *New Phytol* 225:1181–1192.
444 <https://doi.org/10.1111/nph.16224>
- 445 Skandalis DA, Segre PS, Bahlman JW, et al (2017) The biomechanical origin of extreme wing
446 allometry in hummingbirds. *Nat Commun* 8:1047. <https://doi.org/10.1038/s41467-017-01223->
447 [x](https://doi.org/10.1038/s41467-017-01223-x)
- 448 Toju H (2008) Fine-Scale Local Adaptation of Weevil Mouthpart Length and *Camellia* Pericarp
449 Thickness: Altitudinal Gradient of a Putative Arms Race. *Evolution* 62:1086–1102.
450 <https://doi.org/10.1111/j.1558-5646.2008.00341.x>
- 451 Toju H, Sota T (2006) Adaptive divergence of scaling relationships mediates the arms race between a
452 weevil and its host plant. *Biol Lett* 2:539–542. <https://doi.org/10.1098/rsbl.2006.0514>
- 453 Uyeda JC, Pennell MW, Miller ET, et al (2017) The Evolution of Energetic Scaling across the
454 Vertebrate Tree of Life. *Am Nat* 190:185–199. <https://doi.org/10.1086/692326>
- 455 Venner S, Pélisson P-F, Bel-Venner M-C, et al (2011) Coexistence of Insect Species Competing for a
456 Pulsed Resource: Toward a Unified Theory of Biodiversity in Fluctuating Environments.
457 *PLoS ONE* 6:e18039. <https://doi.org/10.1371/journal.pone.0018039>
- 458 Voje KL, Hansen TF (2013) Evolution of Static Allometries: Adaptive Change in Allometric Slopes
459 of Eye Span in Stalk-Eyed Flies. *Evolution* 67:453–467. <https://doi.org/10.1111/j.1558->
460 [5646.2012.01777.x](https://doi.org/10.1111/j.1558-5646.2012.01777.x)
- 461 Voje KL, Hansen TF, Egset CK, et al (2013) Allometric constraints and the evolution of allometry.
462 *Evolution* 68:866–885. <https://doi.org/10.1111/evo.12312>
- 463 Zhang G, Guo W, Wang X, et al (2021) Structural comparison of the rostra of two species of weevils
464 coexisting on *Ailanthus altissima*: the response to ecological demands of egg deposition. *Ecol*
465 *Evol* 21:101. <https://doi.org/10.1186/s12862-021-01824-7>

466 **Tables**467 **Table 1** - Size characteristics of females from the four oak weevil species.

	Rostrum size (mm)			Body size (mm)		Rostrum to Body size ratio	
	n	Mean	Sd	Mean	Sd	Mean	Sd
<i>C. venosus</i>	221	3.73	0.24	6.83	0.42	0.546	0.020
<i>C. pellitus</i>	42	4.38	0.30	7.31	0.35	0.599	0.028
<i>C. glandium</i>	218	5.57	0.76	6.00	0.52	0.925	0.070
<i>C. elephas</i>	92	7.84	0.74	7.90	0.69	0.993	0.037

468

469

470 **Table 2** - Analysis of variance of models testing the effects of sex, species and their interaction in the
 471 relationships between log-transformed rostrum length and body length a) Full model ($R^2 = 0.97$), b)
 472 females only ($R^2 = 0.968$) and c) males only ($R^2 = 0.729$).

a) Full Model sex*species

	Df	Sum of squares	Mean square	F value	P
Body length (log)	1	15.649	15.65	5953.6	< 2.2e-16
Species	3	36.07	12.02	4574.4	< 2.2e-16
Sex	1	30.60	30.60	11642.8	< 2.2e-16
Body length (log) : Species	3	1.42	0.47	179.5	< 2.2e-16
Body length (log) : Sex	1	0.016	0.016	6.1	0.01375
Species : Sex	3	8.60	2.87	1090.6	< 2.2e-16
Body length (log) : Species : Sex	3	0.083	0.028	10.5	8.4e-07
Residuals	935	2.46	0.003		

b) Females only

Body length (log)	1	4.77	4.77	1829.4	< 2.2e-16
Species	3	40.28	13.43	5148.6	< 2.2e-16
Body length (log) : Species	3	0.22	0.07	28.4	< 2.2e-16
Residuals	565	1.47	0.0026		

c) Males only

Body length (log)	1	1.48	1.48	556.3	< 2.2e-16
Species	3	1.23	0.41	153.6	< 2.2e-16
Body length (log) : Species	3	0.0049	0.0016	0.6	0.6066
Residuals	370	0.98	0.0027		

473

474

475 **Table 3** - Estimated parameters of the allometric relationship between rostrum and body length (log-
 476 transformed values) of males and females of the four oak weevil species. Letters in uppercase indicate
 477 statistically distinct slope values.

	Sex	Sample size	Intercept	Slope (s.e)	Slope CI 95 %
<i>C. venosus</i>	Male	119	-0.533	0.869 (0.071) ^a	[0.728;1.009]
<i>C. pellitus</i>	Male	43	-0.460	0.883 (0.130) ^a	[0.628;1.138]
<i>C. glandium</i>	Male	167	-0.303	0.830 (0.054) ^a	[0.723;0.937]
<i>C. elephas</i>	Male	49	-0.199	0.725 (0.088) ^a	[0.550;0.901]
<i>C. venosus</i>	Female	221	-0.338	0.861 (0.055) ^{a,b}	[0.752;0.970]
<i>C. pellitus</i>	Female	42	-0.687	1.087 (0.161) ^{a,b}	[0.772;1.403]
<i>C. glandium</i>	Female	218	-0.861	1.436 (0.040)^c	[1.358;1.514]
<i>C. elephas</i>	Female	92	-0.015	1.003 (0.060) ^b	[0.886;1.121]

478

479 **Figure Legends**

480 **Fig 1** a) Seasonal time partitioning of egg-laying and b) size characteristics of the oak acorns at the
481 time of egg-laying by females belonging to each of the four oak weevil species (*C. venosus* (V), *C.*
482 *pellitus* (P), *C. glandium* (G) and *C. elephas* (E)); c) Body length of females of the four weevil
483 species; d) Species and sex differences in the rostrum to body length ratio; e) variability in the size of
484 females as a function of the variability in the size of acorns at the time of egg-laying per species; f)
485 idem for males. Coefficients of variation of the rostrum (red dots) and body (black triangles) size are
486 shown together with their 95% confidence intervals

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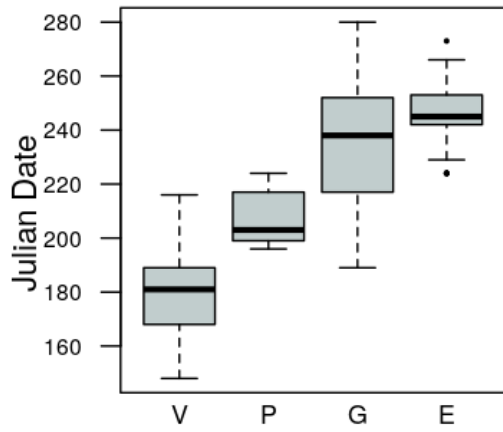
488 **Fig 2** Allometric relationship between rostrum and body length in females (cross) and males (open
489 circles) of the four oak weevil species: (A) *C. elephas*, (B) *C. glandium*, (C) *C. pellitus* and (D) *C.*
490 *venosus*. Data are log-transformed. Linear regression models are shown (full lines) with their
491 confidence interval (95%, grey shade). Dotted dark lines in the panels A-D have a slope of 1 and pass
492 through the mean points for males and females, for illustrative purposes

493

494 **Fig 3** Allometric relationship between rostrum and body length in males (dotted lines) and
495 females (full lines) of all four species. Only the model is shown (Linear regression estimates
496 together with their CI 95%) for clarity purpose

497

a)



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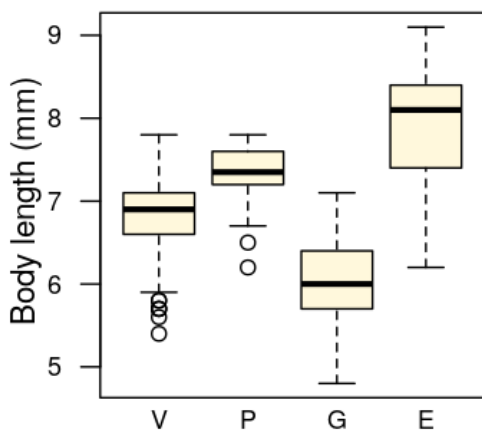
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c)



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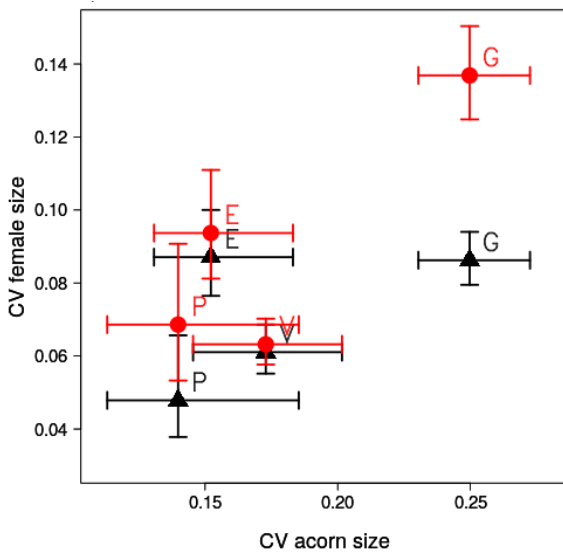
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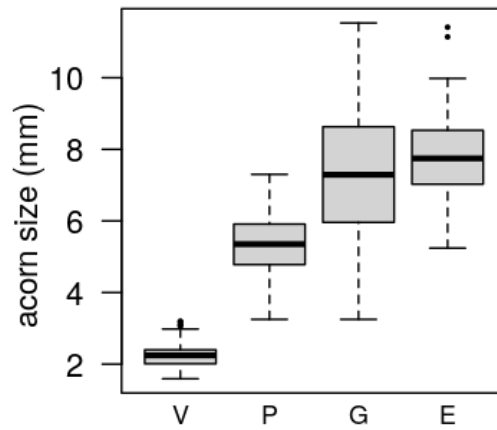
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e)

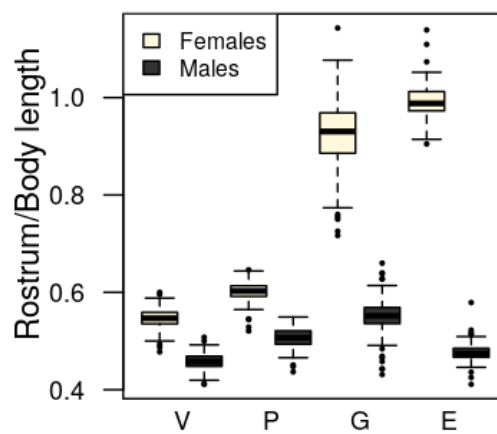


510 **Fig 1**

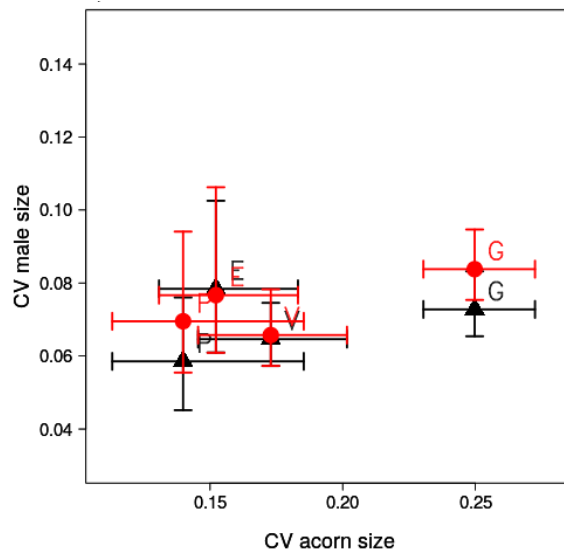
b)

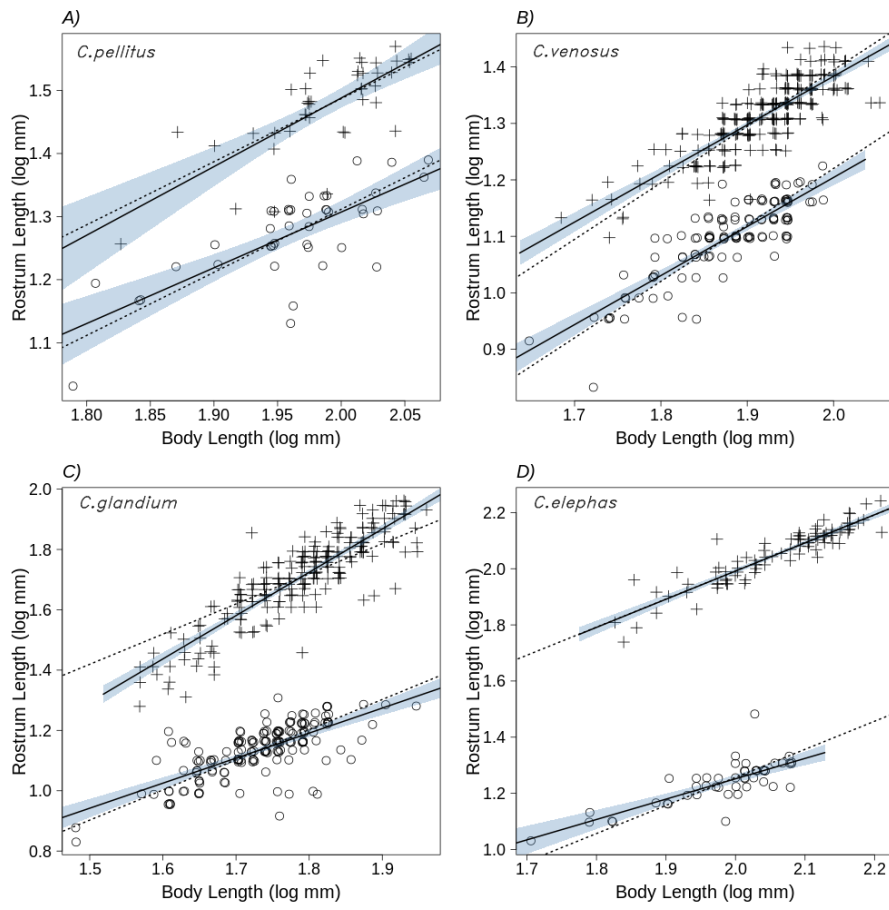


d)



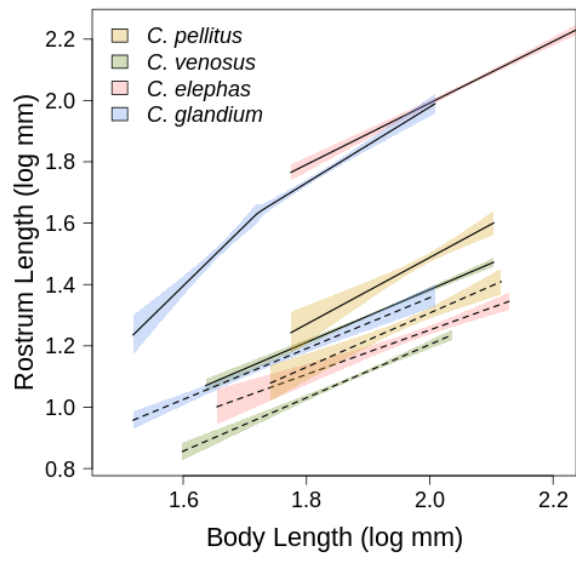
f)





511 **Fig 2**

512



513 Fig 3