

1 **Escalation and constraints of antagonistic armaments in water striders**

2

3 Antonin Jean Johan Crumière<sup>1,2</sup>, David Armisen<sup>1</sup>, Aïdamalia Vargas-Lowman<sup>1</sup>, Martha  
4 Kubarakos<sup>1,3</sup>, Felipe Ferraz Figueiredo Moreira<sup>3</sup> and Abderrahman Khila<sup>1\*</sup>

5

6 \* : To whom correspondence should be addressed: [abderrahman.khila@ens-lyon.fr](mailto:abderrahman.khila@ens-lyon.fr)

7 Phone: +33625671544

8

9 **Author affiliations:**

10 <sup>1</sup> : Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Université Claude Bernard  
11 Lyon 1, CNRS UMR 5242, Ecole Normale Supérieure de Lyon, 46, allée d'Italie, 69364 Lyon  
12 Cedex 07, France

13

14 <sup>2</sup> Current address: Section for Ecology and Evolution, Department of Biology, University of  
15 Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

16

17 <sup>3</sup> Current address : Department of Pharmaceutical Sciences, University of Toronto, Toronto,  
18 Ontario M5S 3M2, Canada

19

20 <sup>4</sup>: Laboratório de Biodiversidade Entomológica, Instituto Oswaldo Cruz, Fundação Oswaldo  
21 Cruz, Rio de Janeiro 21040-360, Brazil

22

23 **Abstract:**

24 Sexual conflict may result in the escalating coevolution of sexually antagonistic traits. However,  
25 our understanding of the evolutionary dynamics of antagonistic traits and their role in association  
26 with sex-specific escalation remains limited. Here we study sexually antagonistic coevolution in  
27 a genus of water striders called *Rhagovelia*. We identified a set of male grasping traits and  
28 female anti-grasping traits used during pre-mating struggles and show that natural variation of  
29 these traits is associated with variation in mating performance in the direction expected for  
30 antagonistic co-evolution. Phylogenetic mapping detected signals of escalation of these sexually  
31 antagonistic traits suggesting an ongoing arms race. Moreover, their escalation appears to be  
32 constrained by a trade-off with dispersal through flight in both sexes. Altogether our results  
33 highlight how sexual interactions may have shaped sex-specific antagonistic traits and how  
34 constraints imposed by natural selection may have influenced their evolution.

35

36

37 **Keywords:**

38 Sexual conflict, antagonistic armaments, coevolution, water striders, sexual dimorphism

39

40 **Introduction:**

41 The evolutionary interests of males and females in reproductive interactions often diverge,  
42 leading to the coevolution of sexually antagonistic traits that are favoured in one sex at a fitness  
43 cost to the other (1). The evolution of these dimorphic traits can, in turn, cause antagonistic  
44 selection and episodes of escalation of armaments between the sexes (2, 3). Conflict resolution,  
45 through the evolution of sex biased gene expression and sexual dimorphism, is expected to drive  
46 divergence of the sexes sometimes resulting in striking cases of sexual dimorphism (4, 5). In the  
47 specific case of sexual conflict over mating rate, the evolution of antagonistic armaments, such  
48 as grasping traits in males, can be matched by the evolution of counter-adaptations as anti-  
49 grasping traits in females (6, 7). These sex-specific armaments can lead to episodes of escalation  
50 and ultimately arms race (3, 8).

51

52 Antagonistic co-evolution of the sexes has attracted much attention and many studies focused on  
53 sex chromosome evolution (3, 9), sex biased gene expression (10-12), and on the dynamics of  
54 morphological co-evolution of sexually antagonistic traits (2, 13, 14). However, our

55 understanding of the function of sexually antagonistic traits during interactions between the  
56 sexes, their impact on the outcome of sexual interactions, and the factors that escalate or  
57 constrain the expression of these traits remain unclear. Waters striders have been widely used as  
58 a model to study sexual dimorphism and antagonistic co-evolution (7, 8, 15). Sexual dimorphism  
59 is particularly dramatic in the tropical genus *Rhagovelia* (Insecta, Gerromorpha, Heteroptera)  
60 (16-18), where both sexes often bear morphological traits reminiscent to those found in species  
61 with strong sexual conflict (1, 16-18). However, the process of sexual selection driving sexual  
62 dimorphism in *Rhagovelia* is unknown (1, 19). Here, through morphological and behavioral  
63 quantifications, we identified a large set of potentially sexually antagonistic armaments in the  
64 *Rhagovelia* genus and tested their role during sexual interactions in a representative species  
65 called *Rhagovelia antilleana* (16-18). We then tested how variation in sexually antagonistic traits  
66 affects male and female success during sexual interactions. We show that this variation is  
67 associated with trade offs with flight capability and egg storage. Finally, we used phylogenetic  
68 reconstruction of male and female secondary sexual traits to understand how antagonistic  
69 coevolution and diversification may have shaped the divergence of the sexes in this lineage and  
70 assess the extent of evolutionary arms race.

71

72

## 73 **Results**

### 74 **Sexual dimorphism in *Rhagovelia***

75 Sexual dimorphism in the water strider genus *Rhagovelia* ranges from subtle to spectacular  
76 differences in morphology between the sexes (Figure 1A-D'). *Rhagovelia* populations contain  
77 both winged and wingless morphs (17, 18). In both male morphs, the foreleg tarsi are equipped  
78 with prominent, hook-like, combs that are absent in the females (Figure 1 E and E'). The rearlegs  
79 of males possess several rows of spikes of different sizes along the trochanter, femur, and tibia  
80 (Figure 1F, F' - G, G'). In addition, the femurs of the rearlegs are very thick compared to those  
81 of the females (Figure 1F, F'). Staining for actin fibres revealed that the width of the femur is  
82 entirely occupied by muscles (Figure S1), suggesting that thicker femurs have the potential to  
83 apply stronger grip force (Figure 2D-F). In females, winged morphs possess a prominent spike-  
84 like projection of the pronotum (Figure 1 H') that is not found in wingless females (Figure 1C')  
85 or winged males (Figure 1H). Wingless females have a narrow-shaped abdomen (Figure 2B) that  
86 can be even more pronounced in some species such as *Rhagovelia obesa* (Figure 2C). We have  
87 never observed winged females with narrow abdomen or wingless females with pronotum  
88 projection indicating that these two traits are mutually exclusive (Figure 2A-B). This observation  
89 suggests that female morph-specific strategies to reduce mating frequency have evolved in  
90 *Rhagovelia* females.

91

92 **Mating behaviour in *Rhagovelia antilleana***

93 Pre-mating interactions in *R. antilleana* include vigorous struggles between males and females  
94 characteristic of sexual conflict over mating rate (1, 8, 20). Behavioural quantification revealed  
95 that males attack females preferably on the side by grasping female's mid- and rearlegs (328 out  
96 of 461 attacks, Table 1). We did not detect a significant male preference for winged or wingless  
97 morphs (Figure S2A; n=36 winged females and n=36 wingless females; Wilcoxon test: p=0.94  
98 n.s.), however we observed that wingless females were mainly attacked from the side (63.2%)  
99 and the back (29.6%), while winged females were more likely to be attacked from the side  
100 (78.5%) possibly due to the presence of the pronotum projection (Table 1). When mounted,  
101 males use the sex combs to clasp the pronotum of the female and tighten the grip on her legs  
102 using their modified rearlegs (Supplementary video 1). Females shake their body vigorously and  
103 perform repeated somersaults, which frequently result in rejecting the male (Supplementary  
104 video 2, Table 1). The struggles are vigorous and their duration is typically 0.68 +/- 0.99 seconds  
105 in average, but can vary between fractions of a second (<0.15 seconds) to several seconds (9.75  
106 seconds) (Figure S2B). These pre-mating struggles are indicative of sexual conflict over mating  
107 rate and the sex-specific modifications we observed might be the result of antagonistic  
108 interaction between the sexes in this species.

109

110 **Effect of natural variation in male and female antagonistic traits on mating rate**

111 Artificially generated variation in sexually antagonistic traits is known to results in variation in  
112 mating success (20). Here, we wanted to test whether natural variation in male persistence and  
113 female resistance traits is also associated with variation in mating success. In *R. antilleana*, only  
114 winged females have the pronotum projection (Figure 2A) and only wingless females have  
115 narrow abdomen (Figure 2B-C), whereas in males there is considerable natural variation in the  
116 degree of elaboration of the rear-legs (Figure 2D-F). To determine the extent to which this  
117 variation affects individual performance during pre-mating struggles, we used a tournament  
118 design (Figure S3) (21) composed of 10 replicates with 8 males and 8 females each for a total of  
119 80 males and 80 females. This experiment separated males and females based on the outcome of  
120 premating struggles into successful, intermediate, and unsuccessful individuals (Figure S3,  
121 Figure 3). Successful males are those who copulate after a premating struggle and successful  
122 females are those who reject males after pre-mating struggle (Figure S3). We found that  
123 successful males have significantly more rear-leg tibia spikes and thicker rear-leg femurs  
124 compared to unsuccessful males (respectively Wilcoxon test:  $p = 0.045^*$ ; Student t-test:  $p =$   
125  $0.02^*$ ; Figure 3A and B), however body size, number of spikes on rear-leg femur, and the length  
126 of rear-leg tibia and femur did not significantly differ between the two groups (Figure 3 C-F).

127 This result highlights the importance of the rear-legs for increasing male mating frequency and  
128 therefore male fitness (1). In females, our data indicated that individuals with a pronotum  
129 projection were significantly more efficient in rejecting males than females with narrow  
130 abdomen (Cochran-Mantel-Haenszel chi-squared test: 23,45; df = 2; p-val: 8.089e-06\*\*\*;  
131 Figure 3G). To further test the validity of this conclusion, we conducted an experiment where the  
132 performance of 30 wingless females (narrow abdomen and absence of pronotum projection) was  
133 compared to that of 18 winged females (wide abdomen) from which we amputated the pronotum  
134 projection. We found that winged females with amputated pronotum projection were less  
135 efficient at rejecting males, which resulted in higher mating frequently (Figure 3G-H). This  
136 result indicates that in the absence of the pronotum projection, narrow abdomen increases  
137 female's ability to resist harassing males. Therefore, alternative morph-specific strategies  
138 evolved in the females possibly under selection by male harassment. Altogether, these  
139 experiments demonstrate that natural variation in sexually antagonistic traits is associated with  
140 variation in the ability of both sexes to control mating rate.

141

#### 142 **Armament escalation is constrained by flight capability in *Rhagovelia***

143 Because natural variation in antagonistic traits can result is variation in the performance of males  
144 and females during pre-mating struggles, we investigated which factor might influence this



145 variation. Many studies described the presence of trade-offs between flight capability and certain  
146 life history traits such as fertility in a number of insects (22-24). We therefore tested whether  
147 wing polymorphism in *Rhagovelia antilleana* affects variation in male's and female's sexually  
148 antagonistic traits. The pronotum projection, a sexually antagonistic trait that increases the ability  
149 of females to resist male harassment, only develops in winged female morphs (Figure 1H'). The  
150 development of the wings is accompanied by the development of flight muscles, which fill most  
151 of the space in the thorax (Figure 2A') (25-27). These winged females position their developing  
152 eggs mostly in the abdomen (Figure 2A'). In wingless females of both *Rhagovelia antilleana* and  
153 *Rhagovelia obesa*, we found that the absence of flight muscles provides a space in the thorax that  
154 is occupied by the developing eggs (Figure 2B', C'). Furthermore, we found that wingless  
155 females contained a significantly higher number of developing eggs than winged females,  
156 consistent with the known trade off between flight and fertility (respectively 4.15 mean  $\pm$  2.96  
157 SD; n=20 and 1.35 mean  $\pm$  2.35  $\pm$  SD; n=20; Student t-test;p-val 0.002094\*\*; Figure 2A'-C').  
158 These data suggest that the presence of wings favours the development of the pronotum  
159 projection and constrains the narrowing of the abdomen (Figure S4). These constraints may have  
160 driven the evolution of alternative sex-specific and morph-specific strategies to escape fitness  
161 costs due to frequent mating in females. In males, we observed that winged morphs typically  
162 have the thinnest rear-leg femurs (Figure 2D) and were under-represented in the successful group

163 of our tournament set-up, contrary to wingless morphs that have the largest rear-leg femurs  
164 (Cochran-Mantel-Haenszel chi-squared test: 10,957; degree of freedom: 2; p-val: 0,004177\*\*;  
165 Figure 3I). These observations highlight a trade-off between dispersal and mating success also in  
166 males. Altogether, these results suggest that some life history traits such as fertility and dispersal  
167 constrain the escalation of sexually antagonistic armaments in both sexes (28, 29).

168

### 169 **Signs of evolutionary arms race between the sexes in the *Rhagovelia* genus**

170 Coevolution of the sexes due to antagonistic interactions could lead to escalation and arms race  
171 that deeply shape the evolutionary trajectory of lineages in nature (2, 6, 8, 13, 14). We therefore  
172 tested the evolution of armament and presence of arms race in the *Rhagovelia* genus by  
173 analysing phylogenetic patterns of correlation of male and female phenotypic complexity in  
174 terms of secondary sexually antagonistic traits (2, 13, 14). We generated a matrix of these traits  
175 in both males and females in a total of thirteen species including nine *Rhagovelia* and four  
176 closely related outgroups (Table S1 and S2). We mapped both male and female traits on a  
177 phylogeny to determine the patterns of phenotypic complexity between the sexes (Figure 4,  
178 Figure S5, Figure S6). Our reconstruction showed a strong phylogenetic signal where males of  
179 the *Rhagovelia* genus evolved an increasing number of secondary sexual traits (Figure 4). This  
180 pattern indicates substantial escalation of conflict in males (2, 6, 13, 14). In females, we detected

181 the evolution of anti-grasping traits in a clade where males are the most armed (Figure 4),  
182 suggesting female counter-adaptation to male escalation (2, 6, 13, 14). Altogether, these patterns  
183 of armament of the two sexes suggest an on-going arms race in the *Rhagovelia* genus.

184

## 185 **Discussion**

186 We have shown that sexual conflict over mating rate generates sexual dimorphism via the  
187 development of secondary sexual traits in the *Rhagovelia* genus. Males have evolved a set of  
188 persistence grasping traits aimed at increasing mating, whereas females evolved a set of anti-  
189 grasping traits aimed at decreasing mating. Natural variation in these antagonistic traits directly  
190 impacts mating frequency and therefore influences the fitness of both sexes. Among male  
191 grasping traits are the sex combs known in other insects (30-34). An additional male set of  
192 grasping traits consists of elaborations in the rearlegs, which are used as clasps that neutralize  
193 female's legs (Supplementary video 1). Our analysis revealed that higher number of spines on  
194 the tibia and larger femur are the two main structures associated with male mating success.  
195 Females on the other hand, possess morph-specific modifications consisting of a narrow  
196 abdomen in wingless morphs and a pronotum projection in winged morphs. Both of these traits  
197 increase the chances for the female to reject harassing males. The projection forms a barrier and  
198 increases the distance between the abdomen of the male and the female, likely making it easier

199 for the female to resist. Our experiments also showed that females with narrow abdomen are  
200 better at rejecting males than females to which we removed the pronotum projection and that  
201 have a wide abdomen. Because males use their rearlegs as clamps on the legs of the female, it is  
202 possible that the narrowing of the abdomen allows the female to slide out of this grip. Altogether  
203 our observations indicate a role of these sex-specific phenotypes in male persistence and female  
204 resistance (2, 6).

205  
206 Our results also indicated that dispersal, manifested by the presence of wings (16, 35, 36),  
207 constrains the degree of expression of sexually antagonistic traits in both sexes. This constraint  
208 reduces elaboration that in turn reduces the ability of the sexes to control mating frequency.  
209 Therefore, there is a tradeoff between dispersal and the expression of sexually antagonistic traits  
210 in *Rhagovelia*. Interestingly in the case of winged females, wing development allows the  
211 extension of the pronotum, which is an efficient female resistance trait. This suggests that the  
212 development of this structure might be contingent on the activation of the wing development  
213 program, although this hypothesis remains to be experimentally tested. How the decision to  
214 develop into winged or wingless morphs and whether sexual conflict affects this decision in  
215 *Rhagovelia* is unknown. *Rhagovelia* is a tropical genus and populations occur in large groups  
216 that can reach hundreds of individuals, suggesting that male harassment is frequent (37). It is

217 possible that under these conditions, winged morphs are favored to allow for dispersal and  
218 occupation of niches with lower population densities. However, winged morphs may not be  
219 favored in other contexts because of the trade-offs we observed between the presence of wings,  
220 the number of eggs produced by females, and the low success in pre-mating interactions we  
221 observed for males. It is possible that trade-offs, between mating frequency, dispersal, and  
222 fertility have shaped phenotypic evolution in this group of insects.

223

224 Finally, antagonistic sexual interactions and constraints have driven evolutionary escalation in  
225 the *Rhagovelia* genus (2, 6). Our analysis has shown that, across this genus, males have evolved  
226 an arsenal of traits across different species while females only evolved two morph-specific  
227 armaments (i.e. narrow abdomen and pronotum projection) in only in two species from a single  
228 clade in our sample. These are *Rhagovelia antilleana* and *Rhagovelia obesa* both belonging to  
229 the *collaris* complex (18). The presence of modified females in this group, with the pronotum  
230 projection in winged females and narrow abdomen in wingless females (18), suggests that sexual  
231 antagonism had strongly impacted the evolution of the genus. In addition to morphological  
232 modifications, the females have evolved complex behavior among their resistance arsenal  
233 through shaking and vigorous somersaults. It would be interesting for future studies to

234 investigate the difference in female resistance behavior across species and assess to which extant

235 females rely on behavior relative to morphological modifications.

236

## 237 **Conclusion**

238 Our approach has linked behavioral quantifications, morphological observations, mating

239 performance assessment and phylogenetic analysis for a better understanding of morphological

240 evolution and diversification. The results we obtained through this study highlight how sexual

241 interactions and natural selection can enhance and constraint antagonistic coevolution between

242 males and females and participated to phenotypic diversification of closely related species.

243 **Methods**

244 Insect sampling and culture

245 Species were collected during fieldwork in the locations indicated in Table S1. Lab populations  
246 were established for *Rhagovelia antilleana*. They were kept in water tanks at 25°C, 50%  
247 humidity, 14 hours of daylight and fed daily on crickets. Styrofoam floaters were provided for  
248 adult female egg laying. Adults and nymphs were raised in independent tanks to decrease nymph  
249 cannibalism.

250

251 Imaging

252 Picture acquisition and observation of secondary sexual traits were performed using a SteREO  
253 Discovery V12 (Zeiss), a Stereomicroscope M205 FA (Leica) and using Scanning Electron  
254 Microscopy at Centre Technologique des Microstructures (UCBL).

255

256 Muscle staining

257 Male rear-leg femurs were dissected and opened using forceps and scalpel to remove the cuticle.  
258 Femurs were fixed with Formaldehyde 4% in PTW 1% during 20 minutes. Femurs were then  
259 washed in 5 successive 10-minute baths of PTW 1%. The muscles were marked with 1/1000

260 Phalloidine 488 (Invitrogen) for 1 hour followed by 5 successive 10-minute baths in PTW 1%.

261 The femur were mounted in 50% glycerol + Dapi.

262

263 Analyse of male behaviour during pre-mating struggles

264 We observed 36 non-virgin couples composed of one male and one female (18 wingless and 18

265 winged females) using the Phantom Miro M310 Digital High Speed Camera and PCC Software

266 (Vision research, Ametek) for video acquisition. We recorded 223 interactions between males

267 and the wingless females and 238 between males and the winged females. We then analysed how

268 males attack the female and classify the different types of attack into “anteriorly” if males attack

269 from the front and block the female T1-legs (fore-legs), “posteriorly” if males attack from the

270 back and block the female T3-legs (rear-legs), and from the side if the males block female T1-

271 and T2-legs (mid-legs), T1- and T3-legs or T2- and T3 legs.

272

273 Determining male preference for female morphs

274 To determine male preference over types of female morphs we have put 2 non-virgin males with

275 2 non-virgin females (one winged and one wingless) into a common plastic container with water

276 and recorded the number of interactions with males for each female morphs during 20 minutes.

277 We repeated this procedure 18 times for a total of 36 winged and 36 wingless females.



278

279 Recording duration of interactions related to female morphs

280 To determine if the pronotum projection in females influence the duration of interactions, we  
281 recorded the time of the interactions for 36 non-virgin couples composed of one male and one  
282 female (18 wingless and 18 winged females) during 20 minutes each using a stopwatch with  
283 centisecond option. Interactions shorter than 0,15 seconds were too fast to take not and were not  
284 take into account in the analysis of data. A total of 604 and 489 interactions were recorded for  
285 respectively winged and wingless females.

286

287 Quantification of trait functions

288 Non-virgin males and females were isolated into two different buckets, one for each sex, during  
289 5 days to increase the motivation to mate. We set-up 10 tournaments between 8 males and 8  
290 females (80 males and 80 females in total) to split them in three different categories: successful,  
291 intermediate and unsuccessful, depending the ability in copulating for males and the ability in  
292 rejecting male for females. Both males and females were introduced at the same time in a  
293 common bucket of water. During the first round, for each mating we removed the couple and  
294 interrupted the mating by separating the male and the female in order to preserve the motivation  
295 to mate for the second round. The male was classified as successful and the female as

296 unsuccessful. Inversely, the remaining males that did not succeed to mate were classified as  
297 unsuccessful in mating and remaining females as successful in rejecting. The first round goes on  
298 until we obtained 4 successful males and 4 unsuccessful females (Figure S3). The different  
299 rounds of this experiment occurred for few minutes to several hours depending on the activity of  
300 the bugs. For the second round, we used two different buckets. In the first bucket, we introduced,  
301 at the same time, the 4 successful males with the 4 successful females, and in the second bucket  
302 we introduced, at the same time, the 4 unsuccessful males with the 4 unsuccessful females. At  
303 each mating, we removed the couple, as in the first round. The second round goes on until we  
304 obtained 2 successful males and 2 unsuccessful females per buckets. At the end, we were able to  
305 obtain the following rank for both males and females: successful for males that have mated twice  
306 and females that have never mated, intermediate for individuals that mated once, and  
307 unsuccessful for males that have never mated and for females that mated twice (Figure S3).  
308 Then, using SteREO Discovery V12 (Zeiss) with ZEN 2011 software (Zeiss) we took pictures of  
309 each individual to quantify the individual phenotypes. We measured the length and the width of  
310 the rear-leg femur, the length of the rear-leg tibia and we counted the number of spikes on the  
311 rear-leg femur and the number of spikes on the rear-leg tibia. We also recorded the presence of a  
312 pronotum projection, narrow abdomen and body length in females. To further test the role of the  
313 pronotum projection, we manually removed the pronotum projection from 18 winged females

314 using forceps and kept these females in lab condition during one week to allow them to recover.  
315 Then we set-up 6 new tournaments to compared the ability of these 18 winged females in  
316 rejecting males against 30 wingless females. All data are available in the Dryad Digital  
317 Repository: *Will be provided before publication*.

318

### 319 Statistical analysis

320 Cochran-Mantel-Haenszel chi-squared tests (Figure 3 G-I) were performed on data of the  
321 tournament using only the replicates where both morph types were present in order to compare  
322 performance (i.e. winged vs. wingless; 9 replicates (72 females) for pronotum projection vs.  
323 narrow abdomen; 6 replicates (48 females) for amputated pronotum vs. narrow abdomen; 8  
324 replicates (64 males) for winged vs. wingless morphs). Proportional odds logistic regression  
325 (MASS package) (38) were performed on data of the tournament using all individuals in order to  
326 analyse phenotypes related to mating performance. Shapiro tests, and Student t-tests and  
327 Wilcoxon tests for analysis of male preference, duration of interactions, comparison of trait  
328 values for successful and unsuccessful males, depending whether variables followed normal  
329 distributions, were performed using RStudio version 1.0.153. R Script used in this study is  
330 available in the Dryad Digital Repository: *Will be provided before publication (or upon request)*.

331

332 Phylogenetic reconstruction

333 Sequences were retrieved from in house transcriptome databases and from (39) for the following  
334 markers: *12S RNA*; *16S RNA*; *18S RNA*; *28S RNA*; *Cytochrome Oxydase subunit I (COI)*;  
335 *Cytochrome Oxydase subunit II (COII)*; *Cytochrome Oxydase subunit III (COIII)*; *Cytochrome b*  
336 (*cyt b*); *NADH-ubiquinone oxidoreductase chain 1 (ND1)*; *Ultrabithorax (Ubx)*; *Sex combs*  
337 *reduced (Scr)*; *Gamma interferon inducible thiol reductase (gilt)*; *Antennapedia (Antp)*; *Distal-*  
338 *less (dll)* ; *Doublesex (dsx)*. All these markers were submitted to GenBank and their accession  
339 numbers can be found in the Dryad Digital Repository: *Will be provided before publication (or*  
340 *upon request)*. Phylogenetic reconstruction (Figure S5) was performed using MrBayes version  
341 3.2.6 and PhyML version 3.0 in Geneious 7.1.9 as described in (39). Concatenation of sequence  
342 alignments and phylogenetic tree in Newick format are also available in the Dryad Digital  
343 Repository: *Will be provided before publication (or upon request)*.

344

345 Assessment of armament escalation

346 We created a matrix of presence/absence of secondary sexual traits for both males and females  
347 (Table S2). Based on our observation, we found eight traits in males; the sex combs in the  
348 forelegs, the different rows of spines (up to 5 rows) and the shape of the different segments of  
349 rear-legs. In females, these traits include the pronotum projection and the narrow abdomen

350 shape. Then, we mapped the sexually antagonistic traits individually on our phylogeny to  
351 reconstruct the ancestral state of each of them (Figure S6). Finally, we performed a  
352 reconstruction using the combination of all traits, in males and females separately, to determine  
353 the pattern of phylogenetic complexity in this sample. Reconstruction of ancestral state was  
354 performed using Mesquite version 3.2 (40) and mapping of phenotypic complexity was  
355 performed using contMap (package phytools, (41)) in RStudio version 1.0.153.

356

#### 357 Observation and imaging of egg position and egg counting

358 We used CT Scan to observe egg position without disrupting internal morphology of our  
359 samples. We fixed females of *R. antilleana* (n= 5 winged; n=5 wingless) and *R. obesa* (n=5  
360 wingless) during 2 days using Bouin solution (MM France). Then, samples were washed twice  
361 with PBS 1X and were emerged 4 days in 0,3% phosphotungstic acid + 70% ethanol + 1%  
362 Tween20 solution to improve the contrast during microtomography acquisition (42). Specimens  
363 were scanned using Phoenix Nanotom S (General Electrics) using the following parameters: 30  
364 kV tensions, 200  $\mu$ A intensity, 2400 images with time exposure of 2000 ms and a 2.5  $\mu$ M voxel  
365 size. Three-dimensional images were reconstructed with the software attached to the machine  
366 (data rec) and then visualized with VGStudioMax. Longitudinal pictures were acquired using  
367 this same software to assess the presence of wing muscles and the position of eggs. Further

368 egg counting was performed after dissection of 20 winged and 20 wingless *R. antilleana*  
369 females.

370

### 371 Two-dimensional morphometric analyses of body shape

372 Pictures of adult females of *R. antilleana* (n= 10 winged; n=10 wingless) and *R. obesa* (n=9  
373 wingless) were acquired using SteREO Discovery V12 (Zeiss). The body outline of each  
374 individual was extracted using Adobe Illustrator CC 2017 (Adobe). The analysis of outlines of  
375 the different conditions and Principal Component Analysis were performed using the package  
376 Momocs (43) in Rstudio version 1.0.153.

377

### 378 **Declarations**

#### 379 Ethics approval and consent to participate

380 Specimens from Brazil were collected under SISBIO permit # 43105-1. Specimens from other  
381 locations did not request any official authorizations.

382

#### 383 Consent for publication

384 Not applicable.

385

386 Availability of data and material

387 The data collected and resources used to perform analysis will be available on Dryad Digital  
388 Depository.

389

390 Competing interests

391 Abderrahman Khila is an Associate Editor at BMC Evolutionary Biology.

392

393 Funding

394 This work was supported by ERC-CoG # 616346, CNPq-PVE # 400751/2014-3 and by  
395 CEBA:ANR-10-LABX-25-01 to A. Khila.

396

397 Authors' contributions

398 Conception and Design of experiment: A.J.J.C. and A.K.

399 Acquisition of data: A.J.J.C., D.A., A.V.L., M.K., F.F.F.M. and A.K.

400 Analysis and Interpretation: A.J.J.C. and A.K.

401 Drafting manuscript: A.J.J.C. and A.K.

402 Revising manuscript: A.J.J.C., D.A., A.V.L., M.K., F.F.F.M. and A.K.

403

404 Acknowledgements

405 We thank A. Herrel, N. Nadeau, J. Abbott and L. Rowe for helpful discussions ; A. Le Bouquin,  
406 S. Viala, C. Finet, F. Bonneton, A. Decaras, R. Arbore, W. Toubiana for comments on the  
407 manuscript ; S. Viala for help with scanning electron microscopy and Centre Technologiques des  
408 Microstructure at Universite Claude Bernard Lyon 1 for access to SEM microscope, M. Bouchet  
409 for help with CT Scan, L. Souquet for help with shape analysis, and A. Le Bouquin and P.  
410 Joncour for help with statistics.

411

412 **References**

- 413 1. Arnqvist G, Rowe L. Sexual conflict. Princeton, N.J.: Princeton University Press; 2005.  
414 xii, 330 p. p.
- 415 2. Arnqvist G, Rowe L. Antagonistic coevolution between the sexes in a group of  
416 insects. *Nature*. 2002;415(6873):787-9.
- 417 3. Pennell TM, Morrow EH. Two sexes, one genome: the evolutionary dynamics of  
418 intralocus sexual conflict. *Ecology and evolution*. 2013;3(6):1819-34.
- 419 4. Bonduriansky R, Chenoweth SF. Intralocus sexual conflict. *Trends in ecology &*  
420 *evolution*. 2009;24(5):280-8.



- 421 5. Rowe L, Day T. Detecting sexual conflict and sexually antagonistic coevolution.  
422 Philosophical transactions of the Royal Society of London Series B, Biological sciences.  
423 2006;361(1466):277-85.
- 424 6. Arnqvist G, Rowe L. Correlated evolution of male and female morphologies in water  
425 striders. *Evolution; international journal of organic evolution*. 2002;56(5):936-47.
- 426 7. Weigensberg I, Fairbairn DJ. The sexual arms race and phenotypic correlates of  
427 mating success in the waterstrider, *Aquarius remigis* (Hemiptera: Gerridae). *Journal of*  
428 *Insect Behavior*. 1996;9(2):307-19.
- 429 8. Arnqvist G, Rowe L. Sexual Conflict and Arms Races between the Sexes - a  
430 Morphological Adaptation for Control of Mating in a Female Insect. *Proceedings of the*  
431 *Royal Society B-Biological Sciences*. 1995;261(1360):123-7.
- 432 9. Dean R, Perry JC, Pizzari T, Mank JE, Wigby S. Experimental evolution of a novel  
433 sexually antagonistic allele. *PLoS genetics*. 2012;8(8):e1002917.
- 434 10. Mank JE. The transcriptional architecture of phenotypic dimorphism. *Nature ecology*  
435 *& evolution*. 2017;1(1):6.
- 436 11. Mank JE, Wedell N, Hosken DJ. Polyandry and sex-specific gene expression.  
437 *Philosophical transactions of the Royal Society of London Series B, Biological sciences*.  
438 2013;368(1613):20120047.

- 439 12. Snook RR, Bacigalupe LD, Moore AJ. The quantitative genetics and coevolution of  
440 male and female reproductive traits. *Evolution; international journal of organic evolution*.  
441 2010;64(7):1926-34.
- 442 13. Bergsten J, Miller KB. Phylogeny of Diving Beetles Reveals a Coevolutionary Arms  
443 Race between the Sexes. *Plos One*. 2007;2(6).
- 444 14. Kuntner M, Coddington JA, Schneider JM. Intersexual arms race? Genital coevolution  
445 in nephilid spiders (Araneae, Nephilidae). *Evolution; international journal of organic*  
446 *evolution*. 2009;63(6):1451-63.
- 447 15. Arnqvist G. SPATIAL VARIATION IN SELECTIVE REGIMES: SEXUAL SELECTION IN  
448 THE WATER STRIDER, GERRIS ODONTOGASTER. *Evolution; international journal of*  
449 *organic evolution*. 1992;46(4):914-29.
- 450 16. Andersen NM. The semiaquatic bugs (Hemiptera: Gerromorpha). Klampenborg,  
451 Denmark.: Scandinavian Science Press LTD.; 1982.
- 452 17. Moreira FFF, Ribeiro JRI. Two new Rhagovelia (Heteroptera: Veliidae) and new  
453 records for twelve species in southeastern Brazil. *Aquatic Insects*. 2009;31(1):45-61.
- 454 18. Polhemus DA. Systematics of the genus Rhagovelia Mayr (Heteroptera: Veliidae) in  
455 the Western Hemisphere (exclusive of the angustipes complex). Lanham , Md.:  
456 Entomological Society of America; 1997. ii, 386 p. p.

- 457 19. Andersson MB. Sexual selection. Princeton, N.J.: Princeton University Press; 1994.  
458 xx, 599 p. p.
- 459 20. Khila A, Abouheif E, Rowe L. Function, developmental genetics, and fitness  
460 consequences of a sexually antagonistic trait. *Science (New York, N Y )*.  
461 2012;336(6081):585-9.
- 462 21. Ivy TM, Sakaluk SK. Sequential mate choice in decorated crickets: females use a fixed  
463 internal threshold in pre- and postcopulatory choice. *Animal Behaviour*. 2007;74:1065-72.
- 464 22. Karlsson B, Johansson A. Seasonal polyphenism and developmental trade-offs  
465 between flight ability and egg laying in a pierid butterfly. *Proceedings Biological sciences*.  
466 2008;275(1647):2131-6.
- 467 23. Simmons LW, Emlen DJ. Evolutionary trade-off between weapons and testes.  
468 *Proceedings of the National Academy of Sciences of the United States of America*.  
469 2006;103(44):16346-51.
- 470 24. Langelotto GA, Denno RF, Ott JR. A trade - off between flight capability and  
471 reproduction in males of a wing - dimorphic insect. *Ecology*. 2000;81(3):865-75.
- 472 25. Braendle C, Davis GK, Brisson JA, Stern DL. Wing dimorphism in aphids. *Heredity*.  
473 2006;97(3):192-9.

- 474 26. Kawada K. Forms and morphs of aphids. In: Minks AKH, P., editor. Aphids, Their  
475 Biology, Natural Enemies and Control. Vol 2A. Amsterdam: Elsevier; 1987. p. 255-66.
- 476 27. Miyazaki M. Forms and morphs of aphids. In: Minks AKH, P., editor. Aphids, Their  
477 Biology, Natural Enemies and Control. Vol 2A. Elsevier: Amsterdam ed1987. p. 163-95.
- 478 28. Denno RF, Olmstead KL, Mccloud ES. Reproductive Cost of Flight Capability - a  
479 Comparison of Life-History Traits in Wing Dimorphic Planthoppers. Ecological Entomology.  
480 1989;14(1):31-44.
- 481 29. Moran NA. The Evolutionary Maintenance of Alternative Phenotypes. American  
482 Naturalist. 1992;139(5):971-89.
- 483 30. Chesebro J, Hrycaj S, Mahfooz N, Popadic A. Diverging functions of Scr between  
484 embryonic and post-embryonic development in a hemimetabolous insect, *Oncopeltus*  
485 *fasciatus*. Developmental biology. 2009;329(1):142-51.
- 486 31. Markow TA, Bustoz D, Pitnick S. Sexual selection and a secondary sexual character in  
487 two *Drosophila* species. Animal Behaviour. 1996;52:759-66.
- 488 32. Polak M, Starmer WT, Wolf LL. Sexual selection for size and symmetry in a  
489 diversifying secondary sexual character in *Drosophila bipectinata* *duda* (Diptera :  
490 *Drosophilidae*). Evolution. 2004;58(3):597-607.

- 491 33. Spieth HT. Mating Behavior within the Genus *Drosophila* (Diptera). Bulletin of the  
492 American Museum of Natural History. 1952;99(7):401-74.
- 493 34. Tanaka K, Barmina O, Sanders LE, Arbeitman MN, Kopp A. Evolution of Sex-Specific  
494 Traits through Changes in HOX-Dependent doublesex Expression. Plos Biology. 2011;9(8).
- 495 35. Fairbairn DJ, King E. Why do Californian striders fly? Journal of evolutionary biology.  
496 2009;22(1):36-49.
- 497 36. Spence JR. The Habitat Templet and Life-History Strategies of Pond Skaters  
498 (Heteroptera, Gerridae) - Reproductive Potential, Phenology, and Wing Dimorphism.  
499 Canadian Journal of Zoology-*Revue Canadienne De Zoologie*. 1989;67(10):2432-47.
- 500 37. Ditrich T, Papacek M. Effect of population density on the development of *Mesovelia*  
501 *furcata* (Mesoveliidae), *Microvelia reticulata* and *Velia caprai* (Veliidae) (Heteroptera:  
502 Gerromorpha). European Journal of Entomology. 2010;107(4):579-87.
- 503 38. Venables WN, Ripley BD. Modern applied statistics with S. 4th ed. New York:  
504 Springer; 2002. xi, 495 p. p.
- 505 39. Crumiere AJJ, Santos ME, Semon M, Armisen D, Moreira FFF, Khila A. Diversity in  
506 Morphology and Locomotory Behavior Is Associated with Niche Expansion in the Semi-  
507 aquatic Bugs. *Current biology : CB*. 2016;26(24):3336-42.

508 40. Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis.

509 Version 3.40. <http://mesquiteproject.org> 2018 [

510 41. Revell LJ. phytools: an R package for phylogenetic comparative biology (and other  
511 things). *Methods in Ecology and Evolution*. 2012;3(2):217-23.

512 42. Metscher BD. MicroCT for comparative morphology: simple staining methods allow  
513 high-contrast 3D imaging of diverse non-mineralized animal tissues. *BMC physiology*.  
514 2009;9:11.

515 43. Bonhomme V, Picq S, Gaucherel C, Claude J. Momocs: Outline Analysis Using R.  
516 *Journal of Statistical Software*. 2014;56(13):1-24.

517

518

519

520

521

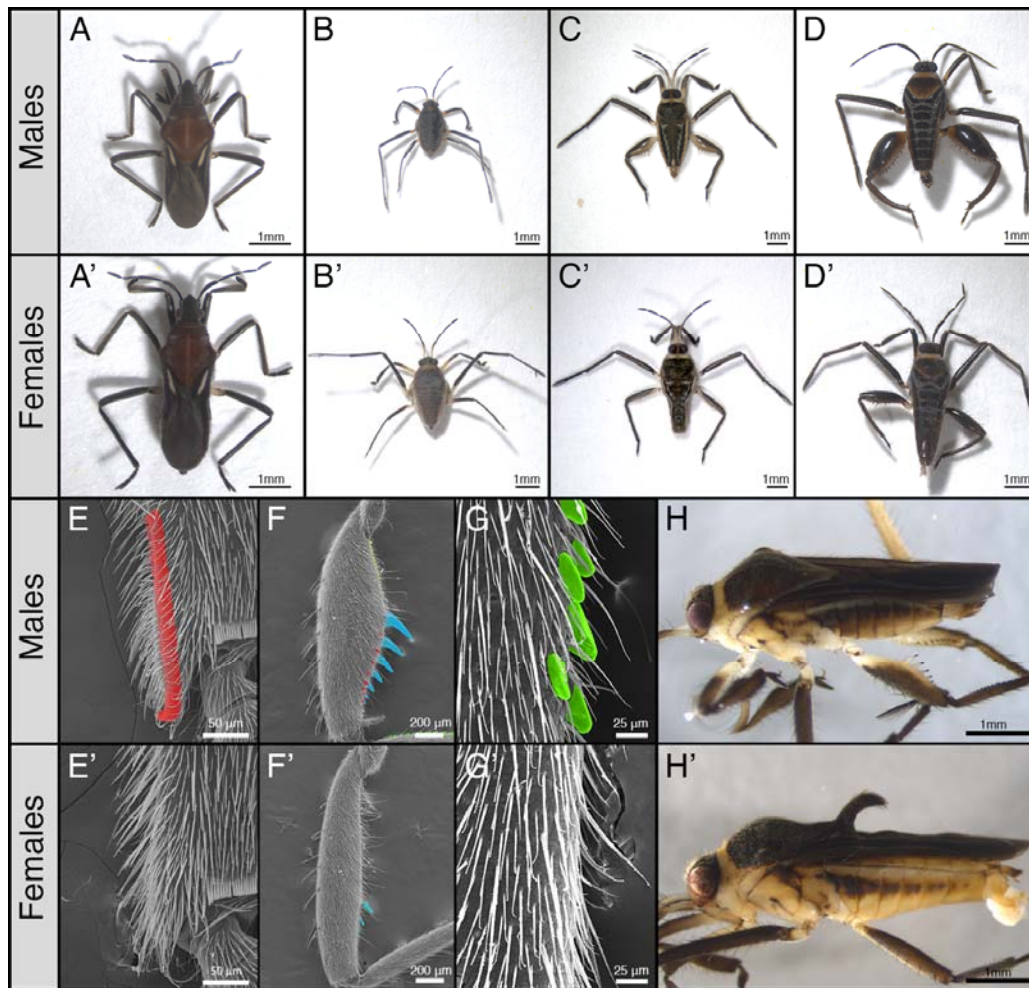
522

523

524

525

526  
527  
528  
529  
530  
531  
532  
533  
534  
535



536 **Figure 1: Sexual dimorphism and secondary sexual traits in the genus *Rhagovelia*.**

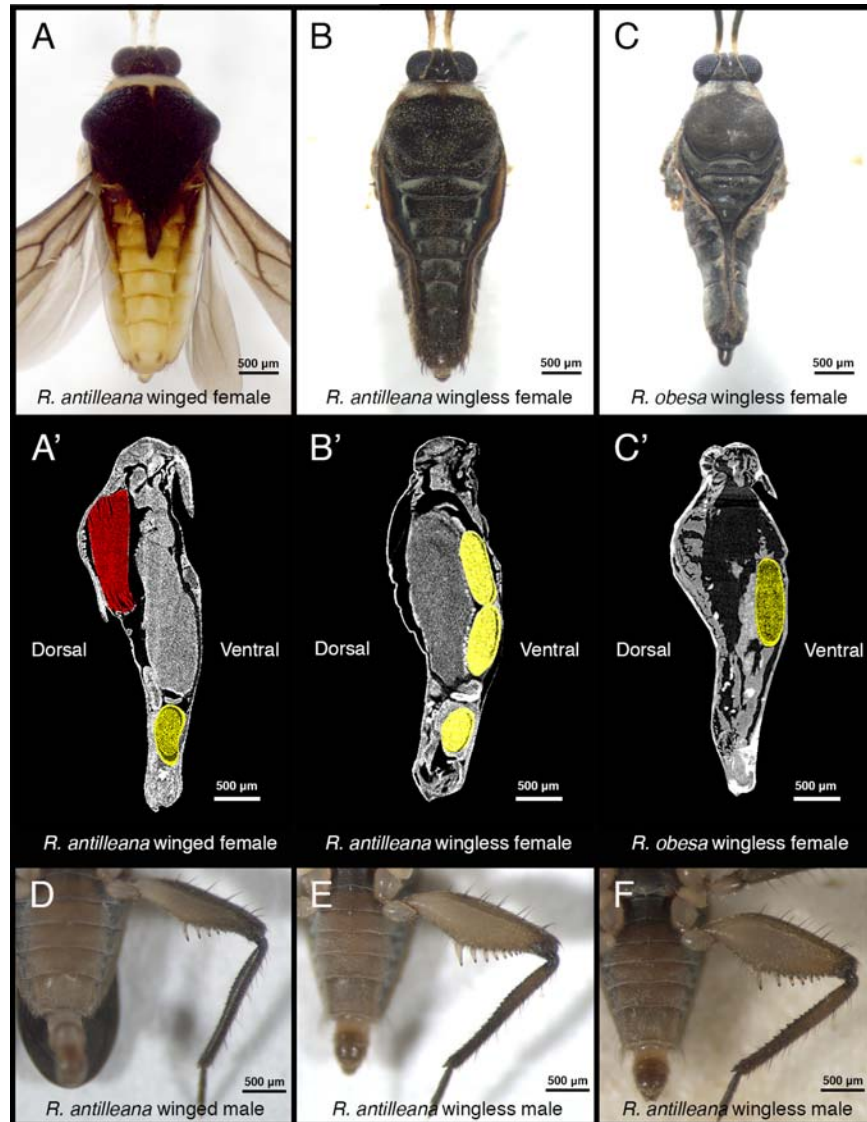
537 The outgroup *Oiovelia cunucunumana* (A, A') does not show strong dimorphism. In the genus  
538 *Rhagovelia*, sexual dimorphism can be subtle as in *Rhagovelia plumbea* (B, B') or more extreme  
539 as in *Rhagovelia antilleana* (C, C') or *Rhagovelia* sp.2 (D, D'). This dimorphism affects  
540 secondary sexual traits such as the sex combs (in red) present in male fore-legs (E) and absent in  
541 females (E'); the presence of spikes (in blue, yellow and red) and the larger femur of the rear-leg  
542 femur in males (F) compared to females that only have some small femur spikes (F'); and the  
543 presence of spikes (in green) along the rear-leg tibia in males (G) that are also absent in females  
544 (G'). Females possess secondary sexual traits such as a narrow abdomen (C') and a pronotum  
545 projection (H') that are absent in males (C, H).

546

547

548

549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560

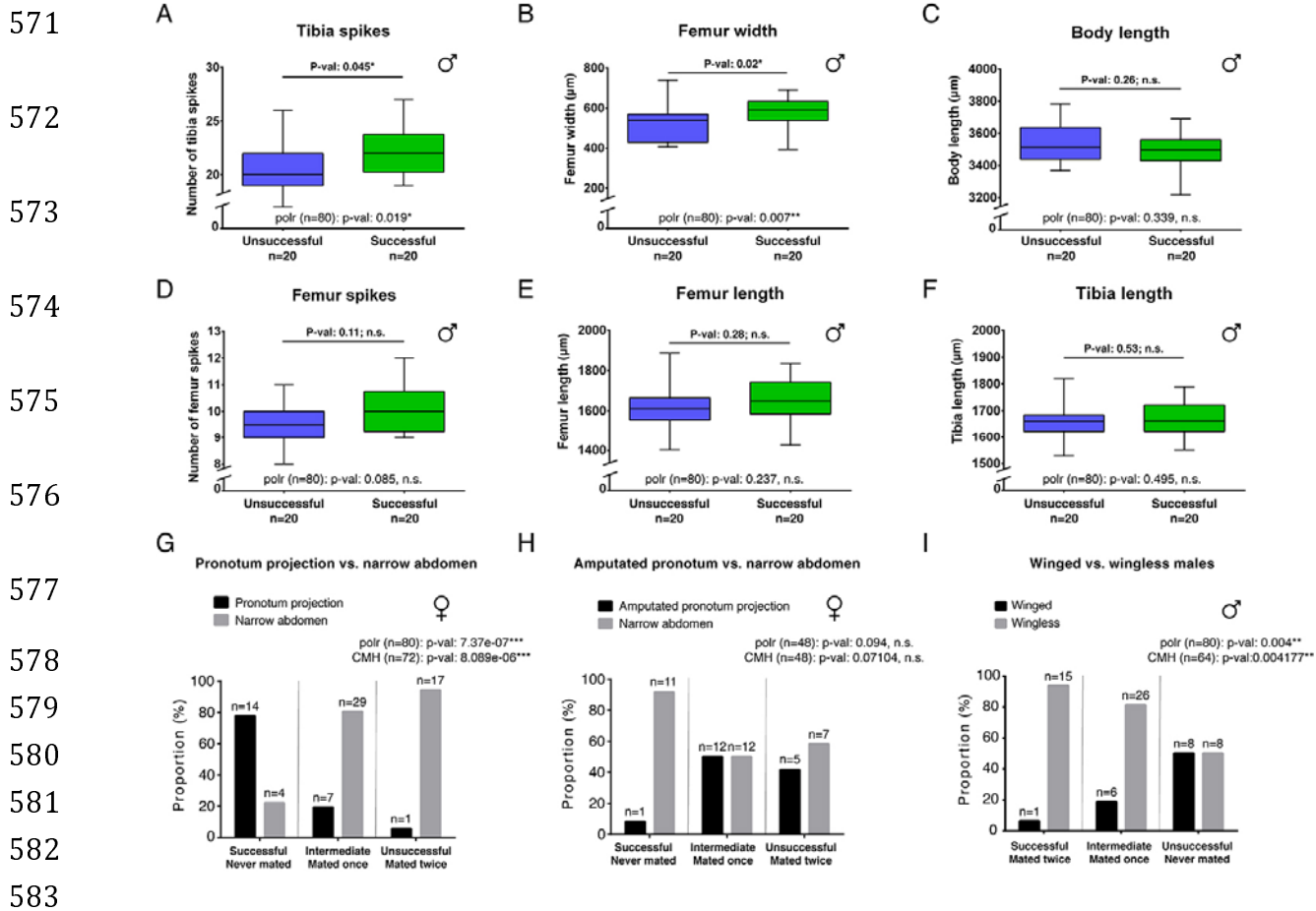


561 **Figure 2: Variation in secondary sexual traits in *Rhagovelia*.**

562 Winged *R. antilleana* females (A) have a large abdomen compared to wingless females (B). The  
563 presence of wing muscles (A', in red) in the thorax constrains the eggs to be located in the  
564 abdomen (in yellow) while in wingless females (B') the space in the thorax is free from wing  
565 muscles and can be occupied by eggs, thus allowing the development of a narrow abdomen. In  
566 species such as *R. obesa* (C), the narrow abdomen is even more pronounced and eggs are located  
567 in the thorax (C'), thus confirming our observation. There is also variability in the rear-leg in  
568 males, especially in the size of the femur. Winged males (D), have thin femurs while other males  
569 have intermediate (E) or large femurs (F).

570





**Figure 3: Morphological comparisons and effect on mating performance.**

Successful males have significantly higher number of tibia spikes (A) and larger femur (B) compared to unsuccessful males. There are no significant differences between the two classes of males for body length (C), number of femur spikes (D), femur length (E) and tibia length (F). Student t-tests were performed for all comparisons except for the number of femur spikes (Wilcoxon test). (G, H and I) show the distribution of the different morphotypes in the different classes of performance with the associated results of Cochran-Mantel-Haenszel Chi-square test. Significant p-values indicate association between the morphotype and the performance. Females with pronotum projection are more efficient than females with narrow abdomen in rejecting males (G). Females with amputated pronotum projection lost their ability to efficiently reject males (H). Wingless males are more efficient than winged males in mating (I). A *proportional odds logistic regression* analysis (polr) for the different phenotypic variables is indicated in all graphs. Significant p-values indicate association between the phenotypic trait measured and success in mating efficiency for males and male rejection for females. *n* indicates sample size.

599

600

601

602

603

604

605

606

607

608 **Figure 4: Comparison of male and female phenotypic complexity.**

609 The phylogeny of our samples shows a higher complexity of male phenotypes, in terms of  
610 number of secondary sexual traits, compared to outgroups (left phylogeny). This complexity is  
611 followed by higher complexity in females in two species *R. antilleana* and *R. obesa*, the ones  
612 that possess narrow abdomen and pronotum projection, suggesting an ongoing arms race.

613

614

615

616

617

618

619

620

621

622

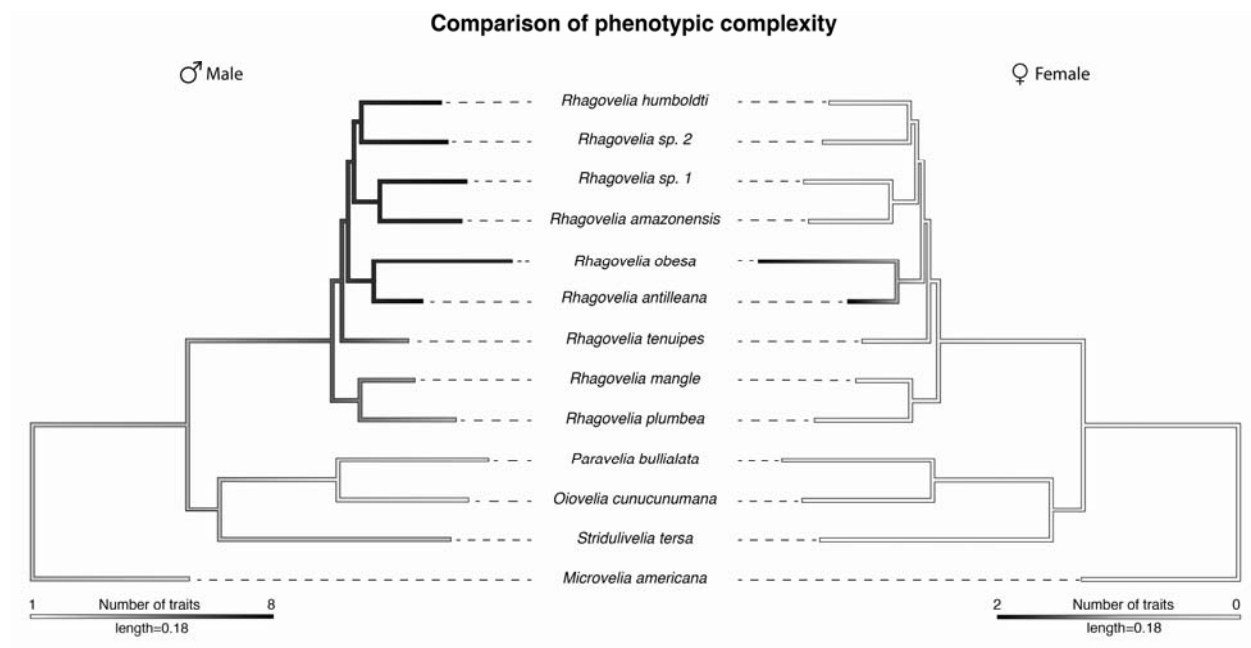
623

624

625

626

627



628

629 **Table 1: Quantification of preferential way of attack used by males on different**

630 **female morphs.**

	<b>Number of male attacks on wingless females</b>	<b>Number of male attacks on winged females</b>
<b>Anteriorly (L1+L1)</b>	16 (7.2 %)	13 (5.5 %)
<b>Posteriorly (L3+L3)</b>	66 (29.6 %)	38 (16 %)
<b>Side (L1+L2)</b>	41 (18.4 %)	52 (21.8 %)
<b>Side (L1+L3)</b>	7 (3.1 %)	14 (5.9 %)
<b>Side (L2+L3)</b>	93 (41.7 %)	121 (50.8 %)
<b>Total</b>	n=223 (100 %)	n=238 (100 %)
<b>Mating</b>	5 (2.2 %)	2 (0.8 %)

631 Males preferentially attack females on the side rather than anteriorly or posteriorly. This

632 tendency increases when males try to mate with a winged female. A total of 223 and 238

633 interactions with wingless and winged females respectively were observed during 18 trials.

634

635

636

637

638

639

640

641

642

643

644

645

646

647