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1 ***Curculio Curculis lupus: biology, behavior and morphology of***  
2 ***immatures of the cannibal weevil Anchylorhynchus eriospathae***  
3 ***G. G. Bondar, 1943***

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36 **Abstract**

37 Weevils are one of the largest groups of living organisms, with more than 60,000  
38 species feeding mostly on plants. With only one exception, their described larvae are typical  
39 plant-feeders, with mouthparts adapted to chewing plant material. Here we describe the  
40 second case of a weevil with early-instar larvae adapted to killing conspecifics. We have  
41 studied the life history of *Anchylorhynchus eriospathae* G. G. Bondar, 1943, a species whose  
42 immatures feed internally on palm flowers and fruits. We provide detailed descriptions of  
43 all immature stages, including the extremely modified first-instar larva. Unlike other  
44 weevils and later instars, this stage exhibits a flat body with very long ventropedal lobe  
45 setae, a large and prognathous head with a gula, and falciform mandibles, each with a  
46 serrate retinaculum, that are used to fight with and eventually kill other first-instar larvae.  
47 We also provide biological notes on all stages and the results of behavioral tests that  
48 showed that larval aggression occurs only among early life stages. Finally we show that  
49 adult size is highly dependent on timing of oviposition. This specialized killer first instar  
50 probably evolved independently from the one other case known in weevils (in *Revena*  
51 *rubiginosa*). Interestingly, both lineages inhabit the same hosts, raising the possibility that  
52 both intra- and inter-specific competition shaped those phenotypes. Given the scarcity of  
53 knowledge on early larval stages of concealed insect herbivores, it is possible that our  
54 findings represent an instance of a much broader phenomenon. Our observations also  
55 allowed us to conclude that *Anchylorhynchus eriospathae* and *A. hatschbachi* G. G. Bondar,  
56 1943 are actually the same species, which we synonymize here by considering the latter as  
57 a junior synonym (new synonymy).

58

59 **Introduction**

60 Insect herbivores feeding on limited and contained resources such as seeds often  
61 experience strong inter and intra- specific competition. Competition might be even stronger  
62 among endophytic larvae, since they have reduced mortality caused by parasitoids and  
63 pathogens (Hawkins *et al.*, 1997; Cornell & Hawkins, 1995). Much attention has been  
64 devoted to the processes and outcomes of intra-specific competition in seed-feeding insects.  
65 This is usually accomplished by analyzing survival curves in controlled experiments, from  
66 which one can infer the process of competition (Smith & Lessels, 1985). However, such  
67 studies usually miss the opportunity to observe the morphology and behavior mediating the  
68 competitive interaction, and different behavioral processes could result in the same  
69 outcome (Smith & Lessels, 1985). For example, a contest outcome could be mediated by  
70 surviving larvae incidentally killing their conspecifics (e. g. Mano & Toquenaga, 2011) or by  
71 attacking them (e. g. Guedes *et al.*, 2010; Alves-Costa & Knogge, 2005). The latter could  
72 further involve cannibalism, a common phenomenon in juvenile stages of phytophagous  
73 insects with poorly understood consequences (Richardson *et al.*, 2010).

74 A spectacular example of interference competition between seed-feeding larvae is that  
75 of *Revena rubiginosa* (C. H. Boheman, 1836) (Curculionidae, Baridinae). Adults of *Revena*  
76 *rubiginosa* lay their eggs inside developing fruits of the palm tree *Syagrus romanzoffiana*  
77 (Cham.) Glassman (Alves-Costa & Knogge, 2005; Guix & Ruiz, 1997). First-instar larvae have  
78 a specialized morphology, with falcate mandibles resembling those of carnivorous insects  
79 (Alves-Costa & Knogge, 2005). Even though they allegedly do not consume conspecific  
80 larvae, they actively use their mandibles to kill other individuals, and only one larva  
81 survives per fruit. Mandibles of later instar larvae have the typical form found in other

82 Curculionidae, with strong and stout triangular mandibles used for chewing vegetable  
83 matter.

84 *Revena rubiginosa* is the only known case among weevils of extreme larval adaptations  
85 to killing conspecifics. However, this might be very common. Weevils (superfamily  
86 Curculionoidea) figure prominently among the herbivores that feed on plant reproductive  
87 organs. Within this diverse group comprising over 60,000 species, there are numerous  
88 lineages that independently evolved this feeding habit (Oberprieler *et al.*, 2007). It is likely  
89 that larvae in those taxa experience processes of intra-specific competition similar to that of  
90 *Revena*. Since the larval morphology and behavior of the great majority of those species is  
91 unknown, we have little idea of how widespread such specializations are.

92 Weevils in the genus *Anchylorhynchus* C. J. Schoenherr feed internally on female flowers  
93 and developing fruits of palms in the genera *Syagrus* Mart., *Butia* Becc. (Becc.) and  
94 *Oenocarpus* Mart. (occasionally, also in the coconut *Cocos nucifera* L.) (de Medeiros &  
95 Núñez-Avellaneda, 2013; Valente & de Medeiros, 2013; Vaurie, 1954). Even though there  
96 are a few taxonomic revisions based on adults (Viana, 1975; Vaurie, 1954; Bondar, 1943;  
97 Voss, 1943) and several reports of larval feeding habits in terms of host plants (de Medeiros  
98 & Núñez-Avellaneda, 2013; Silberbauer-Gottsberger *et al.*, 2013; da Silva *et al.*, 2012;  
99 Bondar, 1943; Faust, 1894), their larvae were never described. Here we report a second  
100 case of a weevil showing morphological specializations to kill conspecifics, by studying the  
101 development, morphology and behavior of *Anchylorhynchus eriospathae* G. G. Bondar, 1943.

## 102 **Materials and methods**

### 103 **Collecting and rearing**

104 We studied beetles from a population of *Butia eriospatha* (Mart. Ex Drude) Becc. found  
105 in the main campus of the University of São Paulo (Cidade Universitária Armando Salles de  
106 Oliveira). This locality is not part of the native range of this palm widely used for  
107 landscaping (Lorenzi *et al.*, 2010), but the area known as “Praça do Relógio” in the main  
108 campus was reformulated in 1997 according to a landscape design to represent the six most  
109 important ecosystems in Brazil. One of those ecosystems is the *Araucaria* forest, and many  
110 individuals of *B. eriospatha* are planted as representatives of this ecosystem (Fig. 1). It is  
111 likely that larvae and pupae of *A. eriospathae* were introduced together with their host  
112 plants, which were transplanted as mature individuals (see results for information on pupal  
113 sites). There is a native species of palm associated with *Anchylorhynchus* in the same locality  
114 (*A. aegrotus* O. I. Fahraeus, 1843 in *Syagrus romanzoffiana*), but we chose to study *B.*  
115 *eriospatha* because individuals start flowering while they are still short (< 1.5 m high),  
116 facilitating *in situ* observations.

117 Between October 2011 and January 2012, we collected samples from inflorescences in  
118 different stages (from open female flowers to young fruits, Fig. 2). We marked those  
119 inflorescences and repeatedly visited them 2-3 times per week to collect new larvae by  
120 cutting a few rachillae in each visit. Larvae and pupae were also searched in the soil  
121 surrounding trees and in the decaying material that accumulates on the persistent bases of  
122 old leaves. In the laboratory, rachillae were cut into several pieces and placed over moist  
123 filter paper in closed petri dishes. Larvae were reared in the lab at room temperature and  
124 observed daily to record duration and number of instars.

## 125 **Morphology**

126 Live and fixed larvae were observed under a stereomicroscope and illustrated with the  
127 help of a *camera lucida* attached to a Leitz microscope Zeiss SM-Lux or a Wild

128 stereomicroscope M5A. To illustrate mouthparts, we made temporary preparations with  
129 glycerin in excavated slides. Black-and-white illustrations were inked in tracing paper and  
130 colored illustrations were prepared with color pencil. Illustrations were then scanned and  
131 processed in Adobe Photoshop CS3. Measurements were taken with an eyepiece  
132 micrometric scale attached to a stereomicroscope. Photos were taken using a camera  
133 attached to a stereomicroscope or an Automontage system (Leica M125 stereomicroscope  
134 coupled to digital camera Leica DFC).

135 After preliminary trials transplanting larvae to larger fruits resulted in larger adults, we  
136 decided to test the relationship between host plant fruit size and adult body size. We  
137 measured the length and width of the adult pronotum and the width of the ovaries from  
138 which they emerged for the first 50 adults to emerge starting on 13/VII/2012. Those  
139 individuals were collected as eggs between November and December 2011, from fruits still  
140 attached to their host plants. We used the geometric mean of pronotum length and width as  
141 a proxy for body size and correlated that with ovary size. Additionally, we fitted a linear  
142 regression using fruit size as an independent variable.

### 143 **Behavior**

144 We observed the egg-laying behavior in the field, and most larval behaviors in the lab.  
145 We opened infested fruits or flowers and placed another larva to observe their interaction.  
146 This was done repeatedly combining pairs of different instars. To test the specificity of the  
147 response of *A. eriospathae*, we also tested interacting first-instar larvae with larvae of the  
148 pineapple beetle *Urophorus humeralis* (J. C. Fabricius, 1798) (Coleoptera, Nitidulidae), a pest  
149 on ripe fruits. Larvae of *U. humeralis* never meet larvae of *A. eriospathae* in nature, but they  
150 gently bite each other and other larvae upon contact (D. C. Bená, personal observation).

151 **Results**

152 **Descriptions**

153 **Fourth (last) instar larva** (Figs. 3–5 and Fig. 9E)

154 **Diagnosis.** Length: 6.6–9.5 mm; prothorax width: 1.6–2.1 mm. Subcylindrical, C-  
155 shaped, weakly curved dorso-ventrally. Head with one pair of stemmata. Epipharyngeal  
156 lining reduced, anterior margin with only three pairs of spatulate setae and without setae  
157 and sensilla between labral rods.

158 **Description.** Head (Figs. 3B-C). Hypognathous; coronal suture about 1/3 of epicranial  
159 length; frontal suture well developed, with 2/3 of frons length; endocarena present. One  
160 pair of stemmata, located laterally to antennal base. Fronto-clypeal suture simply curved  
161 not sinuous. Cephalic capsule with four pairs of minute epicranial posterior setae (*pes*), *pes2*  
162 not aligned with other three; five pairs of dorsal epicranial setae (*des*), *des1* and *des3* and  
163 *des5* located alongside frontal suture, *des2* and *des4* located more laterally, *des2* slightly  
164 longer than other four; four pairs of frontal setae (*fs*), *fs1* and *fs3* shorter than *fs2* and *fs4*, *fs3*  
165 do not surpass anterior margin of frons; *fs4* surpass anterior margin of clypeus; lateral  
166 epicranial setae (*les*) absent; two pairs of ventral epicranial setae (*ves*), subequal. Clypeus  
167 (Fig. 4A) transverse, trapezoidal, posterior margin with 3 pairs of setae (*cls1-3*). Labrum  
168 (Fig. 4A) semicircular, anterior margin trilobate, with three pairs of setae (*lrms*).  
169 Epipharynx (Fig. 4C) trapezoidal, anterior margin with three pairs of spatulate setae,  
170 parallel to anterior margin: two pairs of anteromedial setae (*ams1-2*) and one pair of larger  
171 anterolateral setae (*als*); labral rods convergent backwards, without stem; setae and  
172 sensilla absent. Antennae (Fig. 4B) 1-segmented, with one elongate sensorial cone, bearing  
173 six minute sensilla, sensillum II longer and wider than others, III and IV similar sized, I and  
174 VI much smaller. Mandibles (Figs. 4J-K) cuneiform, symmetrical, with one large apical tooth



175 and one small median tooth; with two dorsal setae. Maxillae (Figs. 4D–E): cardo elongate-  
176 oval, glabrous; stipes widened distally. ventrally with four sensilla: two in the outer margin,  
177 one located anteriorly and other posteriorly, and two very approximate in the inner margin;  
178 mala with a dorsal row of three spatulate setae and a ventral row of six aligned spatulate  
179 setae; palpi 2-segmented, palpomere I with three sensilla, one setiform. Hypopharynx (Fig.  
180 4D). Ligula with triangular elevation. Labium (Fig. 4E): prementum sclerotized, with two  
181 pairs of setae, posterior pair about four times longer than anterior pair; mentum with three  
182 pair of setae, two lateral and one median; labial palpi 2-segmented, palpomere I as long as  
183 wide, palpomere II elongate, shorter and more slender than I. Gula absent.

184 Thorax (Figs. 5A, D, G). Pro-, meso- and metathorax transverse. Prothorax with 11 pairs  
185 of pronotal setae (*prns1–11*); ventropleural lobe with three pairs of setae (*vpls1–3*); pedal  
186 area with four pedal setae, 2 larger and two smaller (*pdas1–4*); mediosternal lobe unisetose  
187 (*msts1*). Meso- and metathorax: prodorsum with two setae (*prs*); postdorsum of meso- and  
188 metathorax with four pairs of postdorsal setae (*pds1–4*); dorsopleural lobe of meso- and  
189 metathorax with three pairs of setae (*dpls1–3*); ventropleural lobe of meso- and metathorax  
190 unisetose (*vpls*); pedal area with four pair of setae (*pdas1–4*), two large and two small;  
191 mediosternal lobe unisetose (*msts1*). Prothoracic spiracle (Fig. 4L) annular, biporous, with  
192 six oblique airtubes directed backwards.

193 Abdomen (Figs. 5B–C, E–F, H–I). 9-segmented; segments I–VII similar, with three dorsal,  
194 transverse plicae; segment VIII with two dorsal, transverse plicae; segment IX not plicate.  
195 Segments I–VII: postdorsal area with a transverse row of two pairs of postdorsal setae  
196 (*mds1–2*); mesodorsum with four pairs of mesodorsal setae (*mds1–4*); mediosternal area  
197 unisetose (*msts*); dorsopleural lobe with one pair of dorsopleural setae (*dpls1–2*);  
198 ventropleural lobe unisetose (*dpls1*); laterosternal lobe unisetose (*lsts*); mediosternal area

199 unisetose (*msts*). Segment VIII: predorsum with one pair of setae (*prs*) and postdorsum with  
200 two pairs of setae (*pds1-2*). Segment IX reduced, trapezoidal, with three pairs of setae  
201 located posteriorly; laterosternal and ventropleural lobes very reduced. Segment X very  
202 reduced, elliptical, ventral. Anal slit terminal, surrounded by four fleshy lobes. Abdominal  
203 spiracles I-VIII (Figs. 4M-N) annular; biporous; airtubes with six annuli, spiracles I-VIII  
204 turned backwards.

205 Alimentary canal (Fig. 4O) lacking mycetomes; posterior ventriculus (*pov*) two coiled;  
206 with 20 short, papilliform gastric caeca (*gcc*), axially aligned forming two compact lines on  
207 lower ventricular coil; Malpighian tubules (*Mgt*) arranged 3+3.

208 **Third instar larva** (Figs. 4H-I)

209 Length: 6.2–7.0 mm ; prothorax width: 1.5–1.6 mm.

210 **Second instar larva** (Figs. 4F-G and Fig. 9D)

211 Length: 3.0–5.1 mm ; prothorax width: 0.9–1.1mm.

212 **Remarks.** Second and third instar closely resemble fourth instar larvae, and share very  
213 similar chaetotaxy. Body milky white, with a few short and fine setae.

214 **First instar larva** (Figs. 6-7 and Figs. 9B-C)

215 **Diagnosis.** Length: 1.5 mm; largest prothorax width: 0.7 mm. Body strongly flattened.

216 Head prognathous, about one fourth of body length, gula present; one pair of stemmata;

217 mandibles falciform with serrate retinaculum. Ventropedal lobe setae of thorax and

218 abdomen very elongate, about as wide as prothorax width.

219 **Description.** Head (Figs. 7A–B). Prognathous. Epicranium deeply emarginate behind.  
220 Coronal suture absent; frontal suture about  $4/5$  as long as epicranial length, subdivided in  
221 basal third forming two elliptical areas; endocarina present, with  $1/2$  of frons length;  
222 fronto-clypeal suture present; *pes4* inserted laterally, not aligned with other *pes* (*pes3–4*);  
223 *des2* inserted very close to frontal suture; *fs1* located in elliptical area formed by division of  
224 frontal suture, *fs1*, *fs3* and *fs4* subequal, larger than *fs2*; *fs4* surpassing anterior margin of  
225 labrum; *ves2* slightly longer than *vs1*. Labrum (Fig. 7A) trapezoidal, with one pair of setae  
226 (*lrms*). Epipharynx (Fig. 7E) with two pairs of sensorial spots located medially; labral rods  
227 curved, divergent posteriorly. Antenna (Fig. 7F) 1-segmented, with elongate sensorial cone  
228 and five spatulate elongate sensilla: I, II and V thinner, III and IV larger and broader.  
229 Mandibles (Figs. 7G–H) falciform, elongate, symmetrical, with one apical (dorsal) and one  
230 subapical (ventral) teeth, and one posterior, triangular-shaped retinaculum (dorsal); inner  
231 ventral margins of dorsal tooth and retinaculum serrate. Maxillae (Figs. 7C–D) stipes with  
232 three long setae in ventral view (Fig. 7D); mala, in dorsal view (Fig. 7C), with spiniform  
233 projections located in basal half. Hypopharynx (Fig. 7C) with sinuous anterior margin.  
234 Labium (Fig. 7D): prementum with two pairs of setae, posterior pair slightly longer than  
235 anterior pair. Gula present (Fig. 7B), trapezoidal, transverse.

236 Thorax (Fig. 6). Pro- meso- and metathorax transverse, flattened.

237 Abdomen (Fig. 6). Segments I -IX flattened. Segment VIII with one transverse row of  
238 three setae. Segment IX reduced, trapezoidal, with one transverse row of six setae;  
239 laterosternal and ventropleural lobes ventralized and reduced. Segment X very reduced,  
240 circular, terminal.

241 **Remarks.** The first instar larva is very different from the fourth instar, and is well  
242 characterized by the diagnosis and description presented above. The chaetotaxy is also very

243 similar to the fourth instar, other than in the setae arrangement presented in the  
244 description.

245 **Pupa** (Fig. 8).

246 **Description.** Length: 4.5–3mm; largest 3–5.1. Aedeagus and exarate. Body, including  
247 setae and spines, light yellow. Head covered by pronotum in dorsal view; each side with one  
248 vertical setae (*vs*), one supraorbital setae (*sos*), one orbital setae (*os*) and one rostral setae  
249 (*rs*). Rostrum surpassing anterior margin of abdominal segment IV. Rostrum of males and  
250 females of similar length.

251 Pronotum transverse, triangular-shaped, anterolateral margins strongly sinuous,  
252 posterior margin strongly curved; each side of pronotum with one discal seta (*ds*), one  
253 supracapital seta (*sas*), two pairs of lateral setae (*ls1-2*), and four posterolateral setae (*pls1-4*).  
254 Mesothorax with two fine and erect setae on each side, located on disc. Scutellum  
255 semicircular. Metathorax with a dorsal median longitudinal sulcus, which divides the discal  
256 area in two halves, each with two tergal setae. Abdomen: abdominal segments I–VII with  
257 four tergal setae forming a transverse row near posterior margin; segments VIII and IX  
258 reduced, segment IX only visible in ventral view, with pseudocerci (*pc*), each one with one  
259 pair of micro-setae inserted on small, acute processes. Gonotheca divided in females (Fig.  
260 8E), not divided in males (Fig. 8D). Pterotheca extending up to apex of fifth abdominal  
261 ventrite (Fig. 8B). Abdomen with seven pairs of annular spiracles.

262 **Life History and behavior**

263 **Oviposition**

264 Females lay eggs in female flowers. Oviposition starts as soon as female flowers begin to  
265 open during male anthesis and continues until the endocarp becomes hard. For that reason,

266 we will use the term “ovary” here to refer interchangeably to flowers or fruits consumed by  
267 larvae along this continuum. Instead of drilling a hole into the ovary with the rostrum as  
268 many species of Curculionidae do (Oberprieler *et al.*, 2007), females of *A. eriospathae* place  
269 the eggs externally between the bracts that surround the gynoecium (Video S1). Most  
270 flowers have approximately three eggs, usually in different stages of development  
271 (presumably laid on different days). While most eggs are deposited between bracts some  
272 were found underneath the bract cuticle.

### 273 **Egg**

274 Eggs are flat, elliptic, slightly curved following the bract curvature (Fig. 9A). This stage  
275 lasts approximately 11 days at room temperature, but we recorded up to 16 days in the  
276 field. Newly laid eggs are transparent, gradually becoming white after four days. By the fifth  
277 day, sclerotized mandibles are observable and by the ninth day the head is distinct. The  
278 larva seems to be fully formed by the tenth day. Approximately four hours prior to hatching,  
279 larvae begin to undulate, moving forward inside the egg. When mandibles touch the  
280 eggshell, the body movement stops and they start to move the mandibles, rupturing the egg.  
281 Once the eggshell is ruptured, the larva slowly moves forward through the hole.

### 282 **First instar**

283 This stage lasts approximately 3-3.5 days. After leaving the egg, the larva remains  
284 immobile for approximately three hours. After that, they do not consume the eggshell and  
285 instead rapidly move towards the base of the ovary. They slide between bracts and do not  
286 damage them. Most encounters between first-instar larvae take place while they move  
287 towards the base. Once a larva reaches the base of the flower or fruit, it drills a hole towards  
288 the seed (Fig. 9B). The larvae molt as soon as the gallery is large enough hold them, and  
289 exuviae can later be found in those galleries.

290 We offered eggs, first-instar larvae, second-instar larvae and larvae of pineapple beetles  
291 to first-instar weevil larvae. The weevil ignored eggs and pineapple beetles, even when  
292 bitten by the latter. They usually avoided second-instars, moving away from them and never  
293 attacking. Finally, first-instar larvae immediately attack conspecifics of the same instar (Fig.  
294 9C). Following a successful attack, they consume the killed larvae, which takes  
295 approximately 20 minutes (Video S2). We did not observe any obvious relationship  
296 between larval size or age and attacking success.

### 297 ***Second instar***

298 The second instar lasts approximately 3.5-4 days, and the larvae consume up to a third  
299 of the volume of the ovary (Fig. 10). Most of the larvae were found inside ovaries still  
300 attached to the inflorescence, although some second-instar larvae were found in aborted  
301 ovaries. Only a single second-instar larva was found in each flower and in most fruits.  
302 However, we sometimes did find a second-instar larva sharing a large non-aborted fruit  
303 with another second-instar or a third-instar larva. They would occupy opposite ends inside  
304 the fruit, seemingly to avoid contact with each other. Second-instar larvae are also able to  
305 colonize new fruits following abortion by making galleries between them.

306 We offered larvae from first to fourth instar and larvae of pineapple beetles to second  
307 instar larvae, and they only attacked and cannibalized first-instar larvae (Fig. 9D, Video S3).  
308 As soon as first-instar larvae approached their galleries, second-instar larvae attacked and  
309 consumed them.

### 310 ***Third instar***

311 The third instar lasts approximately five days. Third-instar larvae continue feeding on  
312 the ovary, consuming approximately two thirds of its volume (Fig. 10). Only single larvae  
313 were found in most fruits, although sometimes we found large non-aborted fruits shared

314 with second-instar larvae. Third-instar larvae were found both in aborted and non-aborted  
315 fruits. They are not aggressive and seem to avoid contact with other larvae.

#### 316 **Fourth instar**

317 Fourth-instar larvae continue feeding on the internal ovary tissues, leaving only a thin  
318 layer when finished (Fig. 9E, 10). Most of them are found in the aborted ovaries. Unlike the  
319 other instars, the fourth instar duration is highly variable. Once larvae finish feeding, they  
320 remain immobile for at least 30 days. Some larvae remained in this stage for more than 120  
321 days. They do not enter diapause, as they immediately respond when disturbed. Prior to  
322 pupation, larvae empty their guts and change color from translucent creamy-yellow to  
323 opaque yellow. Fourth-instar larvae are not aggressive and avoid contact with other larvae.

#### 324 **Pupa**

325 Pupation takes place inside the consumed flower/fruits (Fig. 10), many of them found in  
326 the persistent leaf bases or the base of the inflorescence (Figs. 9F-G). We have not found  
327 pupae in the soil surrounding trees. This stage lasts 5-8 days.

#### 328 **Adult**

329 Adults are diurnal and spend most of the time hidden at the base of inflorescences,  
330 resting and mating (Fig. 9H). They visit male flowers and feed on pollen from open and  
331 closed male flowers (Video S4). In the laboratory, we could keep them alive for up to 16  
332 days, if reared among freshly cut flowers that they used for feeding.

#### 333 **Body size**

334 Body size and fruit width are strongly correlated (Pearson correlation 0.746,  $p < 0.001$ ).  
335 The regression coefficient was highly significant (value = 0.1378,  $p < 0.001$ ), showing a clear  
336 linear relationship between fruit size and body size (Fig. 11).

337 **Taxonomy**

338 Bondar (1943) described *A. eriospathae* and *A. hatschbachi* from specimens collected  
339 from a single inflorescence of *Butia eriospatha*. The only difference between adults of the  
340 two species is the color pattern of the pronotum and the elytra (Figs. 12A-B). Even though  
341 he acknowledged they could represent color morphs of the same species, a more recent  
342 taxonomic revision maintained the species status (Vaurie, 1954). Here we found both color  
343 morphs together in the same trees, including intermediate morphs (Fig 12C). Additionally,  
344 we could not detect any difference in the larval morphology and behavior, or in the adult  
345 genitalia. We conclude therefore, that *A. eriospathae* and *A. hatschbachi* are the same species  
346 and should be synonymized. Since both were described in the same article, there is no  
347 priority, and we choose *Anchylorhynchus eriospathae* as the senior synonym because the  
348 name is biologically more informative.

349 **Discussion**

350 The peculiar morphology of the first instar larva of *A. eriospathae*, unique in the  
351 Curculionoidea, is an evident adaptation to a particular way of life. They slide between the  
352 sepals and petals of the female flower bud, mature flower, or developing fruit and only after  
353 the molt, the second subcylindrical instar digs deep inside the ovary tissue. The  
354 proportionately large and prognathous head, with a gula, has powerful muscles that move  
355 the falciform mandibles. These slender structures are adapted to predation, have a serrate  
356 retinaculum, and are used to fight with and eventually kill other first-instar larvae. Finally,  
357 the very long ventropedal lobe setae of thorax and abdomen are probably important  
358 structures able to detect the approximation of conspecific larvae.

359 First-instar larvae are even more distinct than those of *Revena rubiginosa*, in which the  
360 shape of the mandibles was the main difference between first- and late instars (Alves-Costa



361 & Knogge, 2005). *Anchylorhynchus* (Curculioninae, Acalyptini *sensu* Bouchard *et al.* (2011))  
362 and *Revena* (Baridinae) belong to very distinct groups in Curculionidae, and it seems that  
363 falcate mandibles in larvae are not common in either taxon.

364 Very few larvae of Baridinae have been described so far, but Pakaluk (1993) listed 13  
365 genera of Baridinae that had some larval stage described and we found descriptions of eighth  
366 additional genera since then (Nikulina, 2012; Epsky *et al.*, 2008; Ulmer *et al.*, 2007; Pakaluk,  
367 1994). Falcate mandibles are not mentioned in any description. In most cases, it is not clear  
368 which larval stage was described, but in a few cases the authors claim to have followed the  
369 development from the egg stage (Epsky *et al.*, 2008; Ulmer *et al.*, 2007). In those cases, we  
370 can infer that first-instar larvae were unexceptional.

371 *Anchylorhynchus* is the first genus of Acalyptini for which a detailed description of  
372 larvae is available. The fourth-instar larva of *A. eriospathae* agrees very well with the  
373 diagnosis of the Curculioninae larvae reported by May (1994), differing by the following:  
374 presence of only one pair of stemmata (instead of two); epipharyngeal lining reduced,  
375 anterior margin with only three pairs of spatulate setae and without setae and sensilla  
376 between labral rods (instead of modally arranged). It is important to highlight that  
377 Curculioninae is likely a polyphyletic assemblage (McKenna *et al.*, 2009), so more  
378 meaningful comparison should be done with Acalyptini, which is probably monophyletic  
379 (Franz, 2006). In spite of the lack of proper descriptions, several authors claim to have  
380 observed larvae of other genera in the Acalyptini (Tuo *et al.*, 2011; Franz & Valente, 2005;  
381 Bondar, 1943), including studies that followed the development from the egg stage (Tuo *et*  
382 *al.*, 2011; Franz & Valente, 2005). None of those studies mentions falcate mandibles or  
383 aggression between larvae. We have observed larvae of *Elaeidobius kamerunicus* (J. Faust,  
384 1898) and *E. subvittatus* (J. Faust, 1898)(a genus closely related to *Anchylorhynchus* (Franz,

385 2006)) of different sizes – presumably, different instars – and all of them seemed to have  
386 triangular mandibles. Even though more accurate studies are required, it is clear that the  
387 predatory morphology arose independently in *Anchylorhynchus* and *Revena*, and it seems  
388 that it is not a common feature among closely related genera of either group. The only other  
389 taxon with falcate mandibles in Curculionidae that we are aware of is *Ozopemon* M.  
390 Hagedorn, a genus in the subfamily Scolytinae. In this case, neotenic males have sclerotized  
391 heads with strong falcate mandibles used to kill other males (Jordal *et al.*, 2002).

392 *Revena rubiginosa* lays its eggs at a later stage in fruit development if compared to  
393 *Anchylorhynchus*, right before the endocarp becomes hard. After that, adults cannot drill  
394 through the endocarp, so the surviving larva does not have any more competitors (Alves-  
395 Costa & Knogge, 2005). *Anchylorhynchus*, on the other hand, starts oviposition during  
396 flowering and adults do not drill flowers. There is intense competition between first-instar  
397 larvae for access to the ovary, but the flower/fruit continues to be accessible to new first-  
398 instar larvae after the first larva molts. When larvae of different ages share a large enough  
399 fruit, they seem to switch to a scramble mode of competition and avoid direct interference  
400 with each other. Contrary to what was suggested by the biology of *R. rubiginosa*, a  
401 specialized first-instar killer can evolve and/or be maintained even if when later instars can  
402 experience competition with conspecifics.

403 The large time window of oviposition in *Anchylorhynchus eriospathae* also seems to be  
404 related to the great variation in body size in this species. Young and small flowers result in  
405 smaller individuals, suggesting that there are costs in being the first larva to occupy an  
406 ovary. On the other hand, the first occupants likely encounter and feed on more larvae,  
407 possibly complementing their diet. Further studies on this system could elucidate the trade-  
408 offs involved and the role of cannibalism in nutrition.

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409 In spite of all particularities in the life histories of *Anchylorhynchus* and *Revena*, species  
410 in both genera and other closely related weevils co-inhabit the same plants. For example,  
411 three other species of *Anchylorhynchus* feed on female flowers of *Syagrus romanzoffiana*  
412 (Vaurie, 1954), the host of *Revena rubiginosa*, and adults of both *Anchylorhynchus* and  
413 *Revena* emerge from fruits of *S. romanzoffiana* (da Silva *et al.*, 2012). Therefore, it is possible  
414 that larvae of all of the species encounter each other. In fact, bruchid beetles also develop  
415 inside fruits of *Syagrus* and other palms (Nilsson & Johnson, 1993), and we have observed  
416 larvae of bruchids attacking *B. eriospatha* as well. They are able to oviposit even later than  
417 *Revena*, and larvae enter the hard endocarp through the phloem (Bondar, 1937). The first-  
418 instar larvae of *Pachymerus cardo* (Fahraeus, 1839), a species that feeds on several palm  
419 species (including *S. romanzoffiana*), have mandibles that are more pointed than those of  
420 later instars (Prevett, 1968), but it is not known if they are used to kill other larvae. It is  
421 possible, therefore, that the morphologies of *Anchylorhynchus*, *Revena*, *Pachymerus* and  
422 other still unstudied beetles evolved in a complex scenario of intra- and inter-specific  
423 competition.

424 If cannibalism and contest competition is indeed common among concealed herbivores,  
425 it is possible that killer phenotypes at some larval stage are more common than currently  
426 acknowledged. In order to study that, we need to properly document the morphology and  
427 behavior of a greater diversity of larvae, including the often-overlooked first instars. With a  
428 greater knowledge of the frequency of killer larvae among herbivores, we would be better  
429 able to understand the trade-offs involved and the ecological contexts in which they evolve.

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431 We are grateful to José Inácio L. Moura for sending us spikelets of *Elaeis guineensis*  
432 inflorescences containing larvae of *Elaeidobius* and Ricardo Pinto da Rocha for access to the

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434 a previous version of this manuscript.

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508 der Unterfamilie Petalochilinae (Col., Curc.). *Entomol. Blätter* **39**: 60–64.
- 509



510

511 **Figure 1** *Butia eriospatha*, host plant of *Anchylorhynchus eriospathae*, in the main campus of  
512 the Universidade de São Paulo.

513

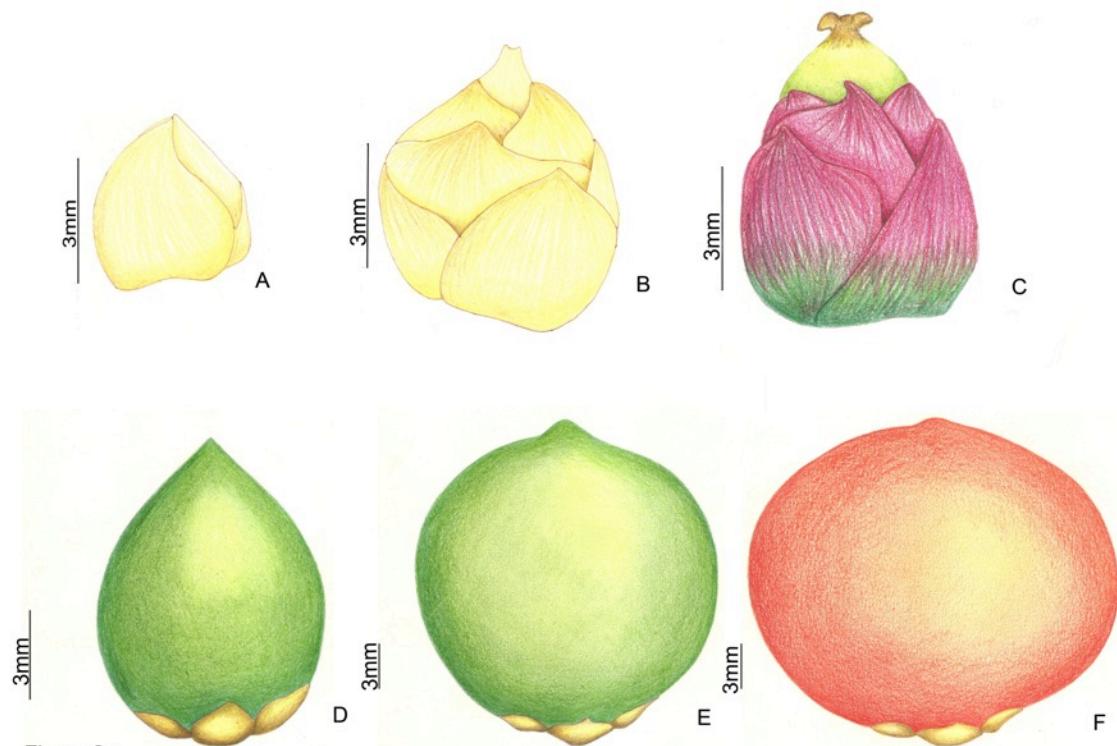
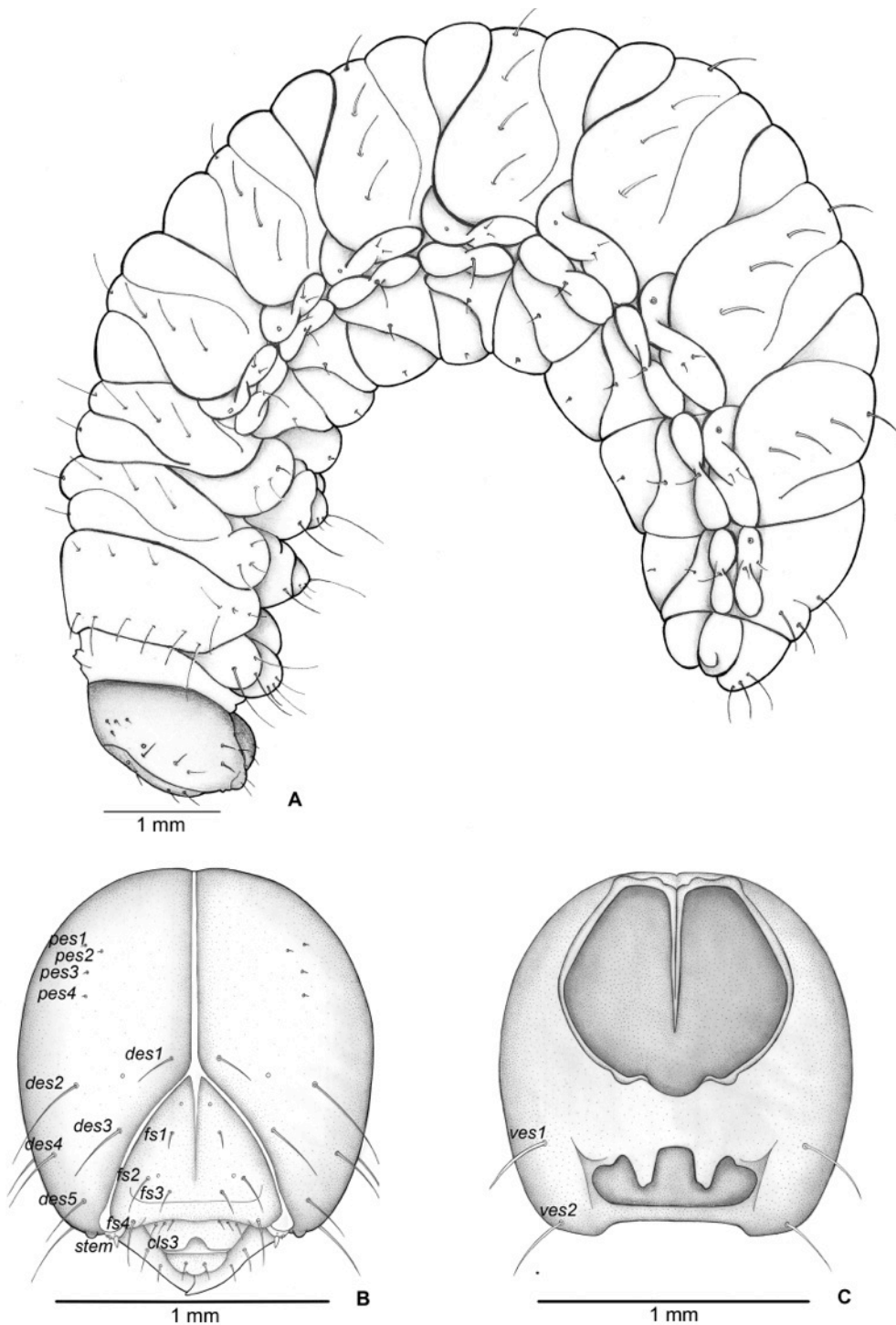


Figure 2

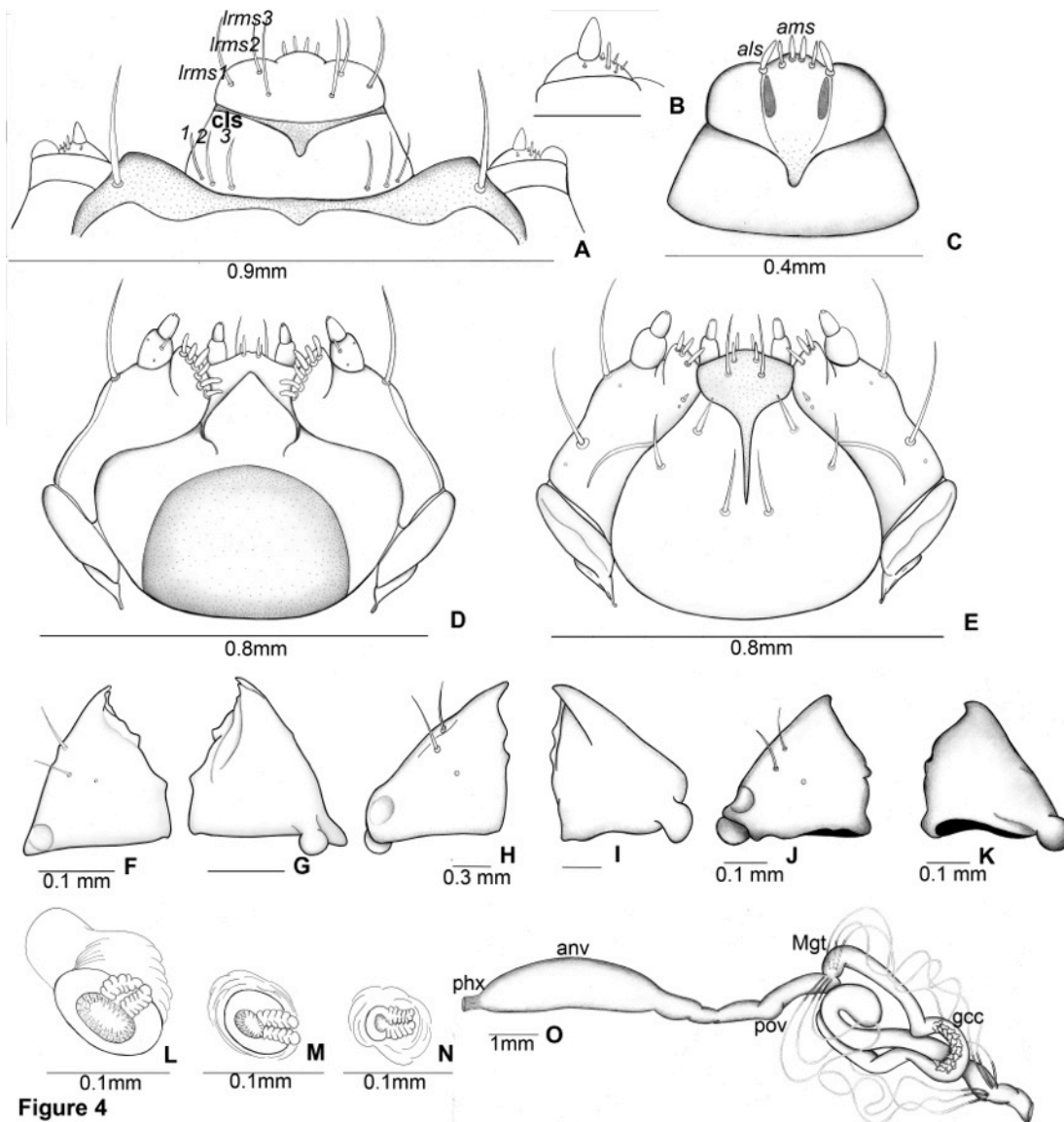
514  
 515 **Figure 2** Stages of fruit development in *Butia eriopatha*. **A** Closed female flower, right after  
 516 the spathe opens and flowers are exposed. **B** Opening female flower following male  
 517 anthesis. Oviposition starts at this phase. **C** Young fruit a few days after pollination. **D** Older  
 518 fruit before hardening of the endocarp. Some eggs are still laid during this phase. **E**  
 519 Maturing fruit with hard endocarp. Larvae of *A. eriopathae* cannot penetrate fruits that  
 520 reach this phase. **F** Ripe fruit.  
 521





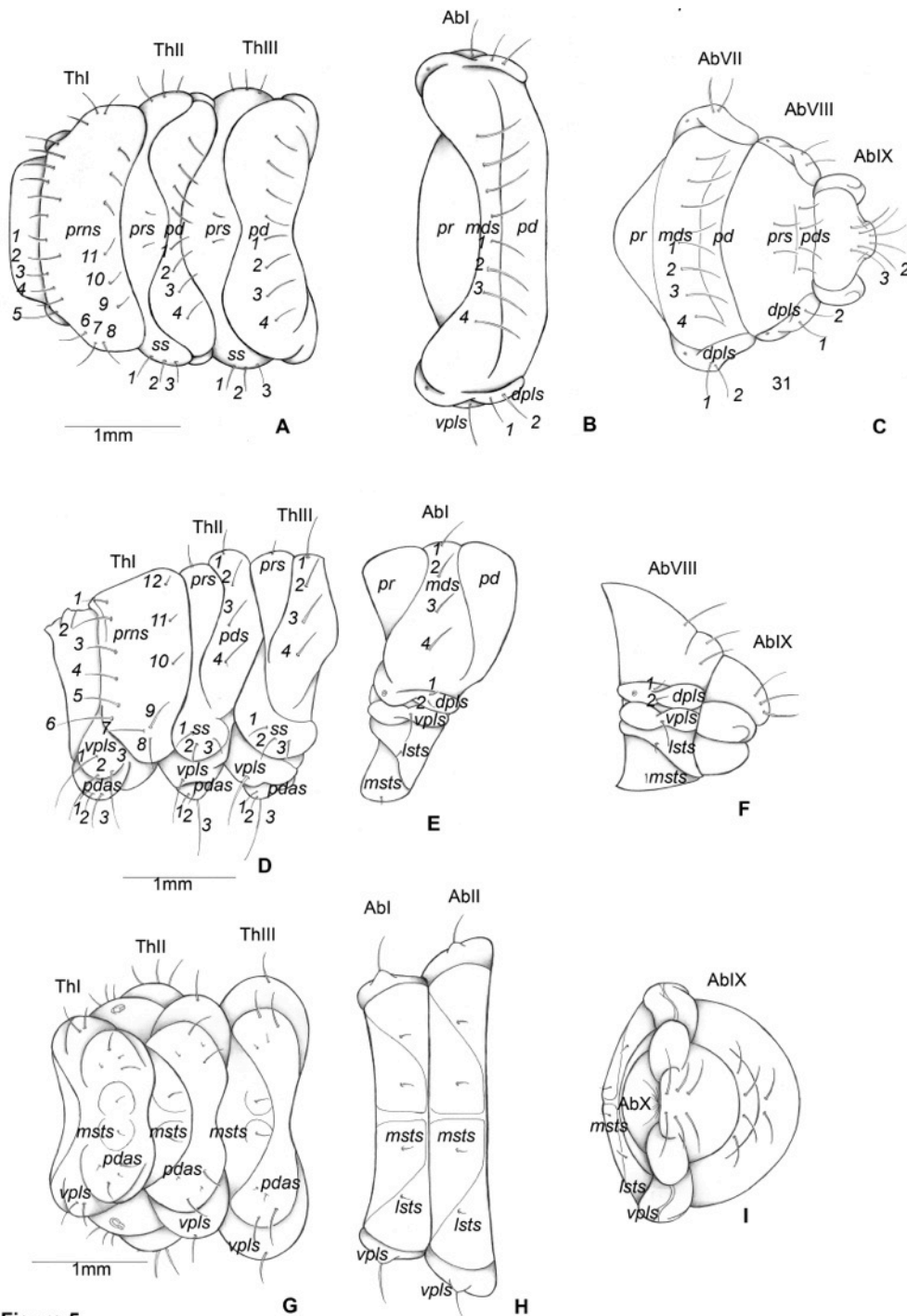
522 **Figure 3**

523 **Figure 3** *Anchylorhynchus eriospathae*, fourth-instar larva. **A** habitus (lateral view). **B** head  
 524 capsule (frontal view). **C** head capsule (posterior view). Abbreviations (s. = seta or setae):  
 525 *ant*—antenna; *cls*—clypeal s.; *des*—dorsal epicranial s.; *fs*—frontal s.; *lrms*—labral s.; *pes*—  
 526 posterior epicranial s., *ves*—ventral epicranial s.  
 527



**Figure 4**

528  
 529 **Figure 4** *Anchylorhynchus eriospathae* larvae (A–E, J–P fourth instar, F–G second instar, H–I  
 530 third instar). **A** clypeus and labrum. **B** antenna. **C** epipharynx. **D** maxillo-labial complex (dorsal  
 531 view). **E** maxillo-labial complex (ventral view). **F** mandible (2<sup>th</sup> instar, dorsal view). **G** mandible  
 532 (2<sup>th</sup> instar, ventral view). **H** mandible (3<sup>rd</sup> instar, dorsal view). **I** mandible (3<sup>rd</sup> instar, ventral  
 533 view). **J** mandible (4<sup>th</sup> instar, dorsal view). **K** mandible (4<sup>th</sup> instar, ventral view). **L** prothoracic  
 534 spiracle. **M** abdominal spiracle I. **N** abdominal spiracle VIII. **O** alimentary canal. Abbreviations  
 535 (s. = seta or setae): *als*—anterolateral s.; *ams*—anteromedian s.; *anv*—anterior ventriculus; *cls*—  
 536 clypeal s.; *gcc*—gastric caeca; *lrms*—labral s.; *Mgt*—Malpighian tubules; *phx*—pharynx; *pov*—  
 537 posterior ventriculus.  
 538



**Figure 5**

539  
 540 **Figure 5** *Anchylorhynchus eriospathae*, fourth instar larva. Thoracic segments: **A** dorsal, **D**  
 541 lateral, **G** ventral. Abdominal segment I: **B** dorsal, **E** lateral, **H** ventral. Abdominal segments  
 542 VIII-IX: **C** dorsal, **F** lateral, **I** ventral. Abbreviations (s. = seta or setae): *dpls*—dorsopleural s.;  
 543 *lsts*—laterosternal s.; *msts*—mesosternal s.; *pdas*—pedal s.; *pds*—postdorsal s.; *prns*—pronotal  
 544 s.; *prs*—pre dorsal s., *ss*—spiracular s.; *vpls*—ventropleural s.; *pd*—postdorsum; *pr*—predorsum;  
 545 *Ab*—abdominal segment; *Th*—thoracic segment.  
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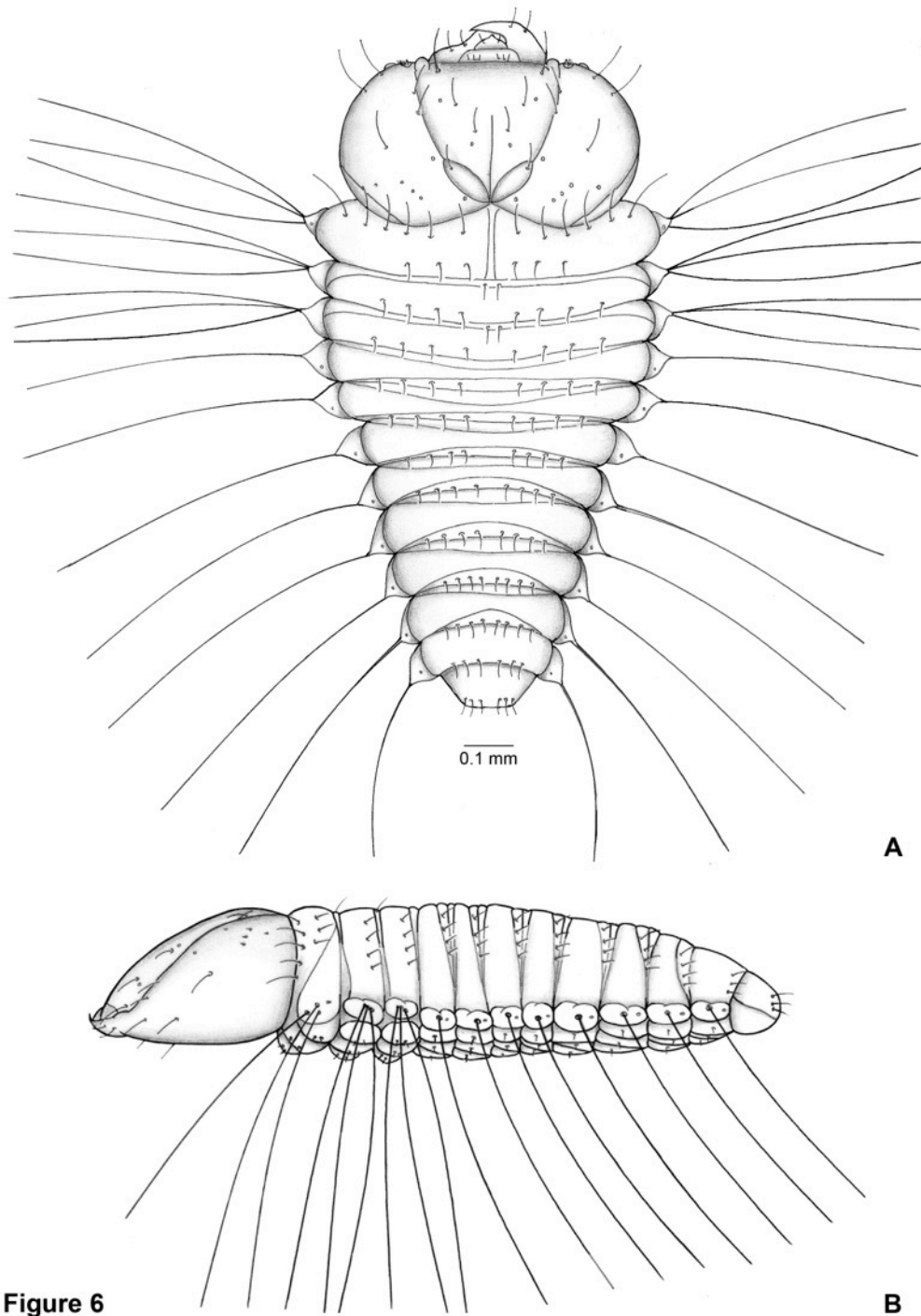
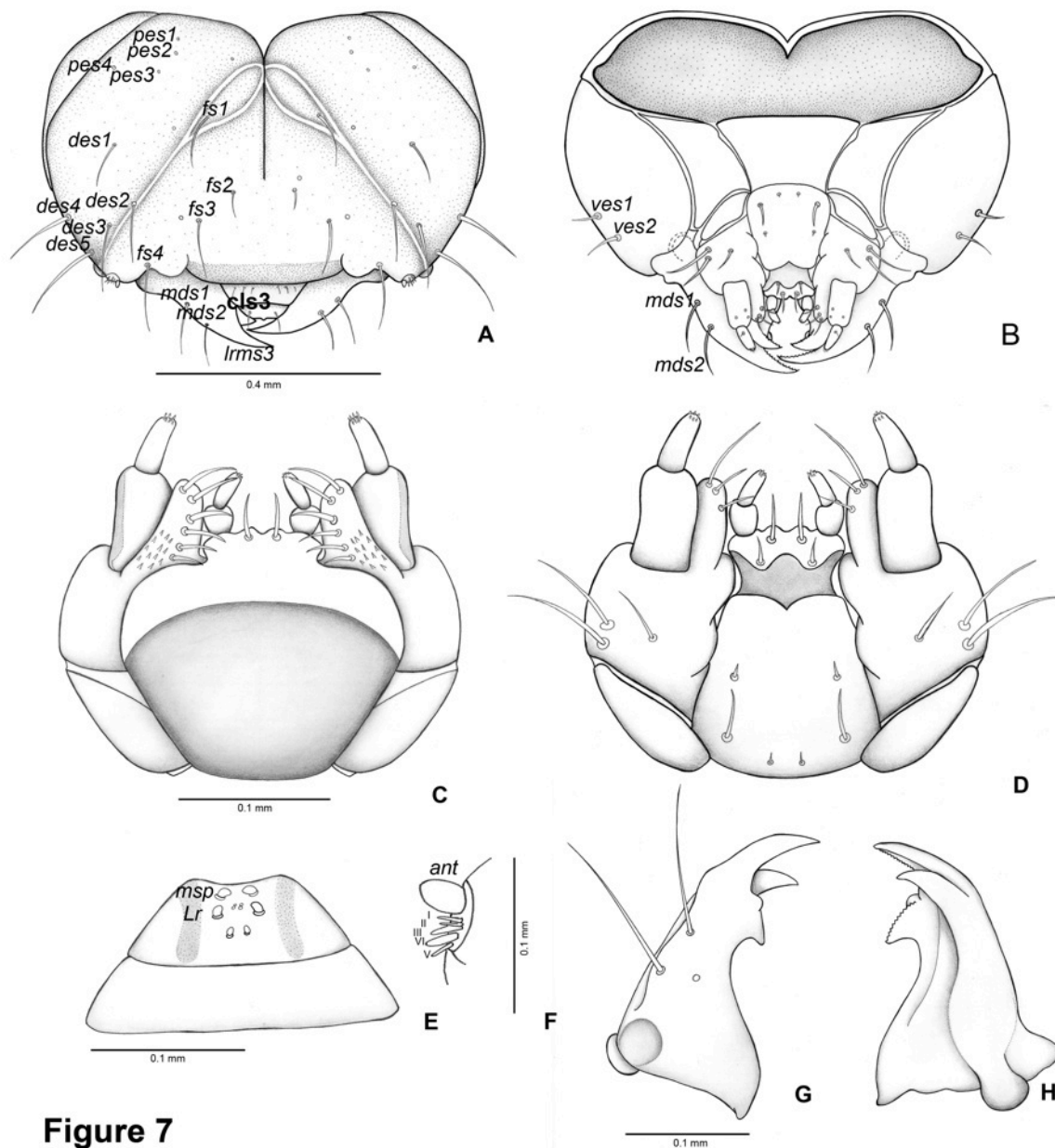


Figure 6

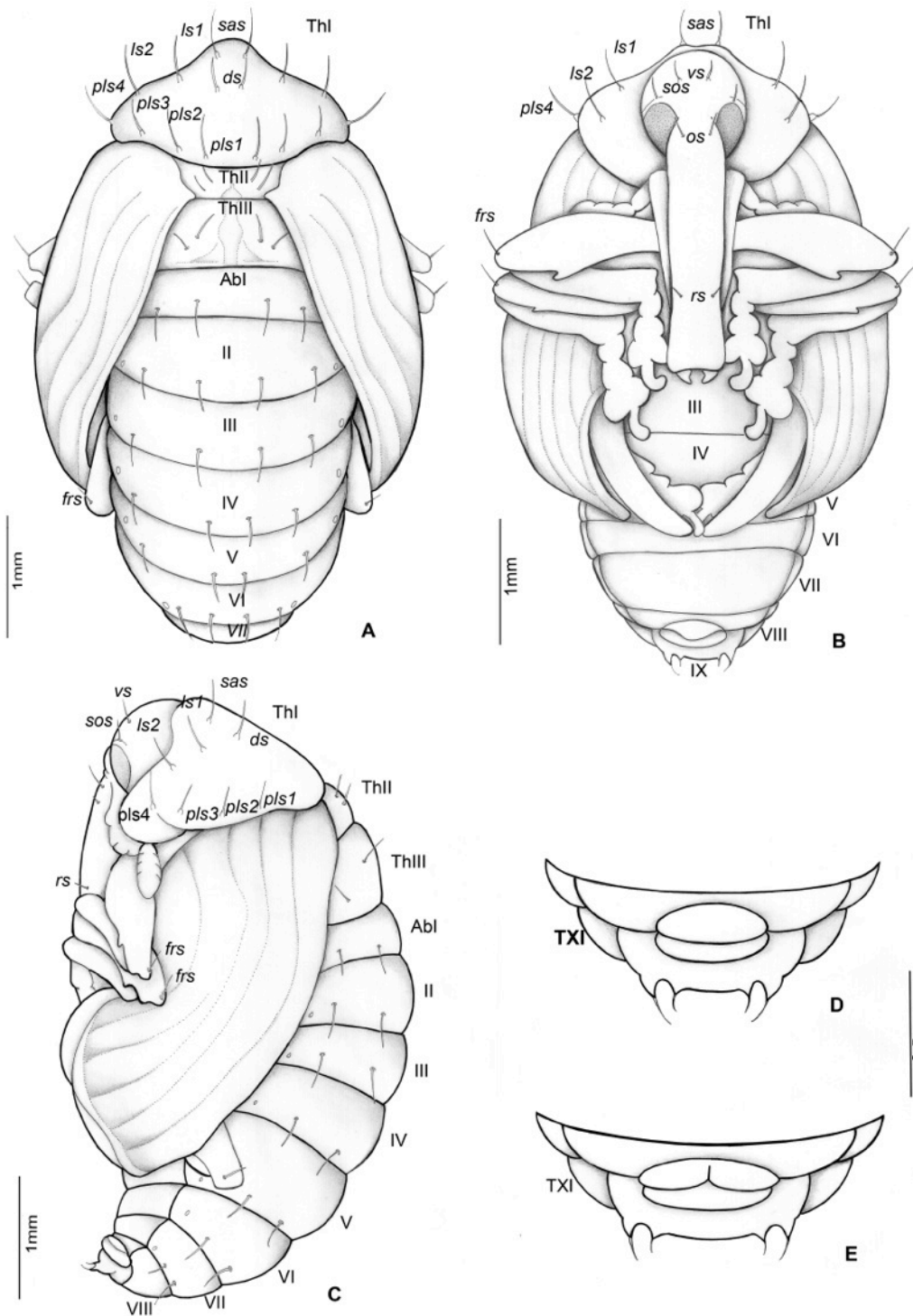
547  
548 **Figure 6** *Anchylorhynchus eriospathae*, first-instar larva. **A** habitus of larva (dorsal view). **B**  
549 habitus of larva (lateral view).  
550

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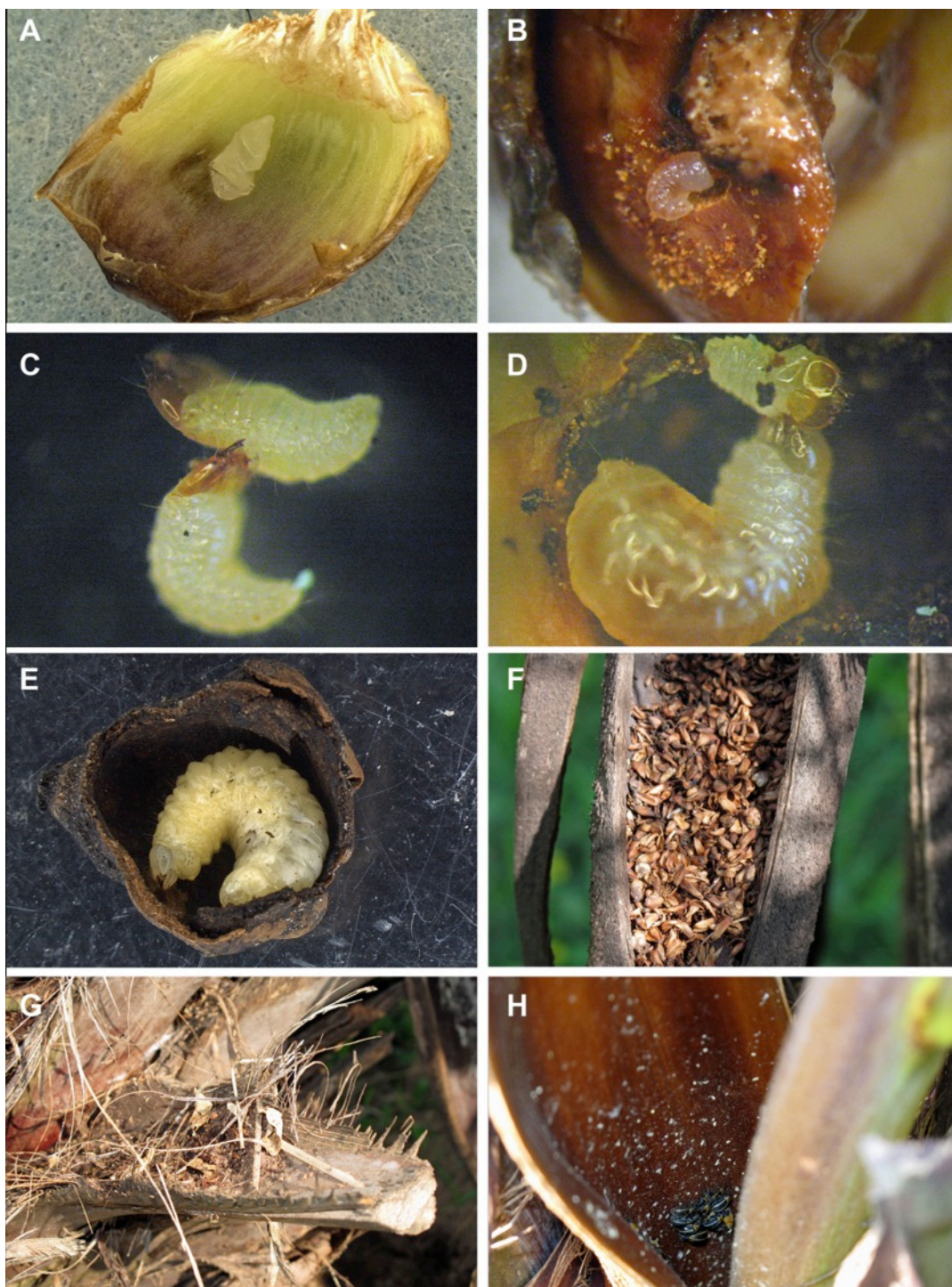
**Figure 7**

552 **Figure 7** *Anchylorhynchus eriospathae*, first-instar larva. **A** head capsule (frontal view). **B**  
 553 head capsule (posterior view). **C** maxillo-labial complex (dorsal view). **D** maxillo-labial  
 554 complex (ventral view). **E** antenna. **F** epipharynx. **G** mandible (dorsal view). **H** mandible  
 555 (ventral view). Abbreviations (s. = seta or setae): *ant*—antenna; *cls*—clypeal s.; *des*—dorsal  
 556 epicranial s.; *fs*—frontal s.; *lrms*—labral s.; *msp*—median spines; *ves*—ventral epicranial s.  
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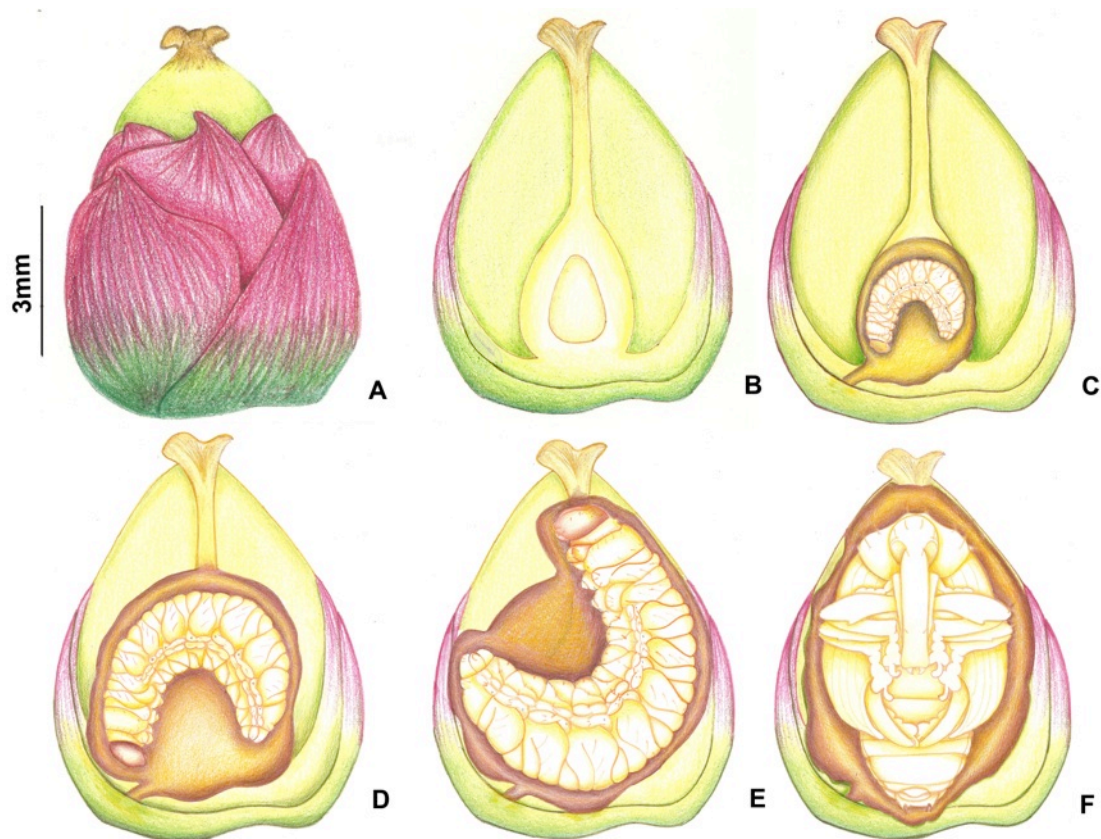


**Figure 8**

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 559 **Figure 8** *Anchylorhynchus eriospathae*, pupa. Habitus: **A** dorsal view, **B** ventral view, **C** lateral  
 560 view. Abdominal extremity, ventral view: **D** male, **E** female. Abbreviations (s. = seta or setae):  
 561 *ds*—discal s.; *frs*—femoral s.; *ls*—lateral s.; *os*—orbital s.; *pls*—posterolateral s.; *rs*—rostral s.;  
 562 *sas*—super apical s.; *sos*—superorbital s.; *vs*—vertical setae; *Th*—thoracic tergite; *Ab*—  
 563 abdominal tergite.



564  
 565 **Figure 9** Life history of *Anchyloerhynchus eriospathae*. **A** Recently laid egg over a bract  
 566 (transversal folds are an artifact of manipulation). **B** First-instar larva drilling the base of a  
 567 fruit. **C** First-instar larva attacking another first-instar larva. **D** Second-instar larva attacking  
 568 a first-instar larva. **E** Fourth-instar larva after having consumed almost all the internal part  
 569 of a fruit. **F** Aborted fruits and flowers trapped by old spathe. **G** Persistent leaf bases  
 570 trapping aborted flowers and fruits. **H** Adults of *A. eriospathae* hiding at the base of an  
 571 inflorescence.  
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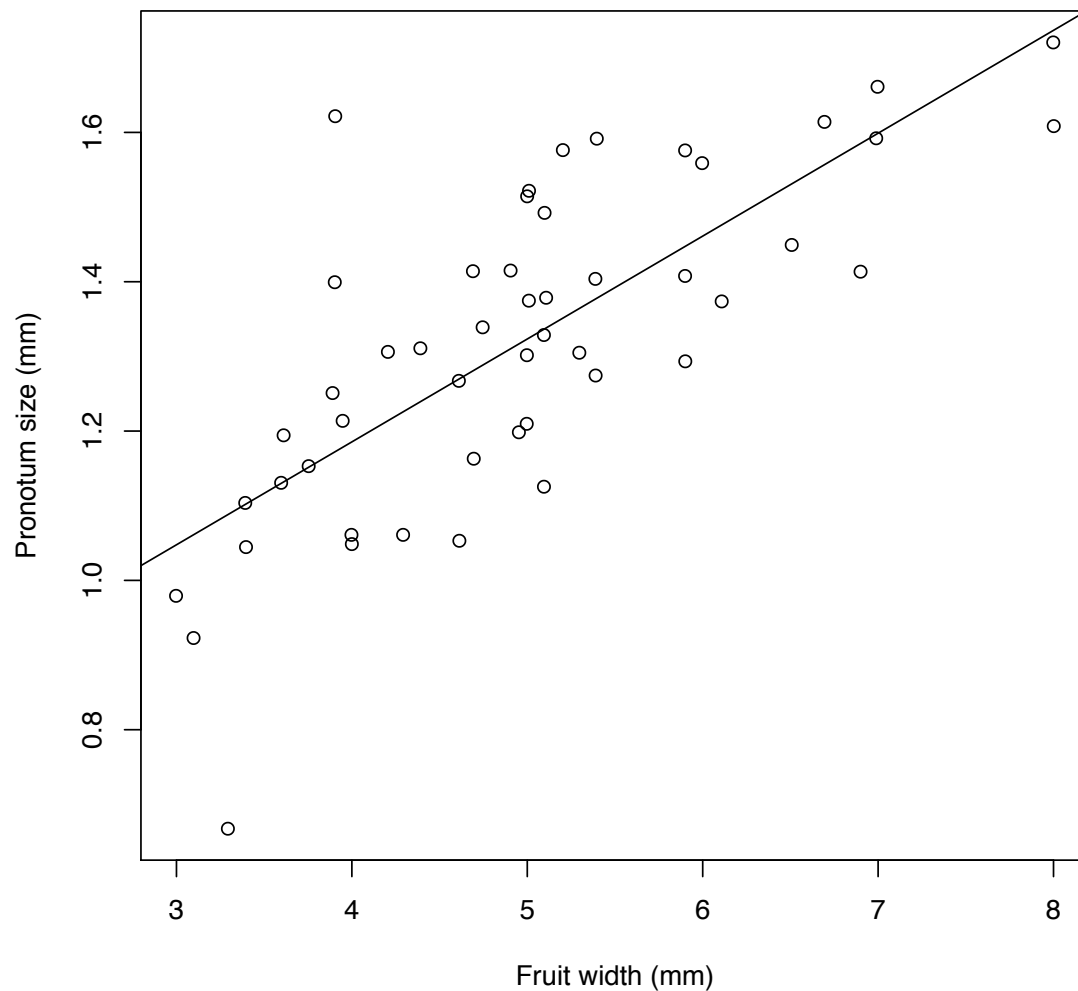


**Figure 10**

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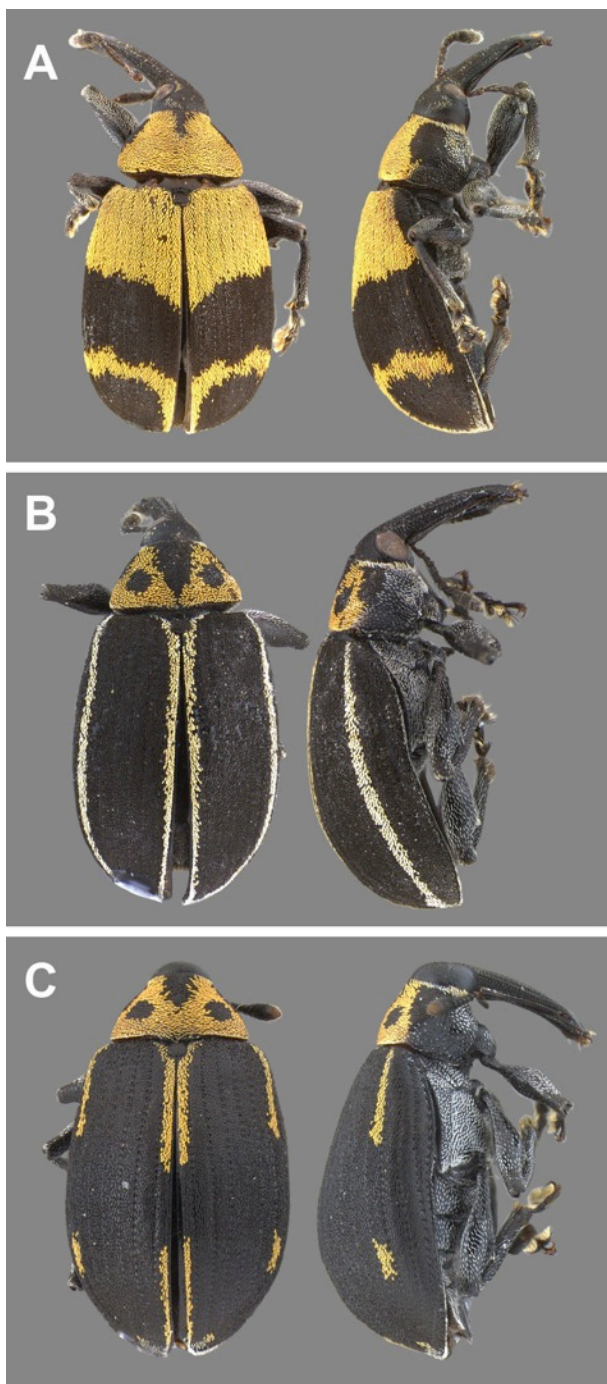
**Figure 10** Growth of immatures of *A. eriospathae* inside fruits of *B. eriospatha*. **A** external view of a recently fertilized fruit. **B** internal view of a non-infested fruit. **C** second-instar larva. **D** third-instar larva. **E** fourth-instar larva. **F** pupa.





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**Figure 11** Relationship between fruit width and size of emerged adult. Size of adults is the geometric mean between pronotum length and pronotum width. Line indicates the linear regression. A small random jitter (Normal with mean 0 and standard deviation 0.007) was added to each point to enhance visualization of overlapping records.



584  
 585 **Figure 12** Color morphs of adults of *Anchylorhynchus eriospathae* found in Cidade  
 586 Universitária. As defined by Bondar (1943), *Anchylorhynchus hatschbachi* (junior synonym)  
 587 has either the pattern shown in **A** or uniformly yellow dorsal surface of the elytra, and  
 588 *Anchylorhynchus eriospathae* has the pattern shown in **B** or additional yellow stripes on  
 589 intervals 3 and 5. **C** color pattern not described in the literature, intermediate between *A.*  
 590 *hatschbachi* and *A. eriospathae*.  
 591