

1 **A Novel Power-Amplified Jumping Behavior in Larval Beetles (Coleoptera:**  
2 **Laemophloeidae)**

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38 **Abstract**

39 Larval insects use many methods for locomotion. Here we describe a previously  
40 unknown jumping behavior in a group of beetle larvae (Coleoptera: Laemophloeidae). We  
41 analyze and describe this behavior in *Laemophloeus biguttatus* and provide information on  
42 similar observations for another laemophloeid species, *Placonotus testaceus*. *Laemophloeus*  
43 *biguttatus* larvae prelude jumps by arching their body while gripping the substrate with their legs  
44 over a period of  $0.22 \pm 0.17$ s. This is followed by a rapid ventral curling of the body after the  
45 larvae releases its grip that launches them into the air. Larvae reached takeoff velocities of  $0.47$   
46  $\pm 0.15$  m s<sup>-1</sup> and traveled  $11.2 \pm 2.8$  mm ( $1.98 \pm 0.8$  body lengths) horizontally and  $7.9 \pm 4.3$   
47 mm ( $1.5 \pm 0.9$  body lengths) vertically during their jumps. Conservative estimates of power  
48 output revealed that not all jumps can be explained by direct muscle power alone, suggesting  
49 *Laemophloeus biguttatus* uses a latch-mediated spring actuation mechanism (LaMSA) in which  
50 interaction between the larvae's legs and the substrate serves as the latch. MicroCT scans and  
51 SEM imaging of larvae did not reveal any notable modifications that would aid in jumping.  
52 Although more in-depth experiments could not be performed to test hypotheses on the function  
53 of these jumps, we posit that this behavior is used for rapid locomotion which is energetically  
54 more efficient than crawling the same distance to disperse from their ephemeral habitat. We  
55 also summarize and discuss jumping behaviors among insect larvae for additional context of  
56 this behavior in laemophloeid beetles.

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## 64 Introduction

65 The extraordinary evolutionary success of holometabolous insects can be partially  
66 attributed to their partitioned life history: immatures (larvae) are often soft-bodied and minimally  
67 mobile, adapted for feeding and growth, while adults are often highly mobile, enabling dispersal  
68 and mate seeking. This generally sedentary lifestyle exhibited by many larvae makes them  
69 highly attractive targets for predators and parasites. Holometabolous insects have evolved a  
70 number of solutions to the problem of larval self-defense (e.g. concealed habitats, chemical  
71 defense, parental care (Kim et al. 2016; Eisner 1970; Brandmayr 1992)), but few active means  
72 of escaping predators. Rapid locomotion is inherently difficult during this life stage; terrestrial  
73 larvae are typically plump and slow-moving, with short legs (e.g. Lepidoptera, most Coleoptera,  
74 and many Hymenoptera) or no legs at all (Diptera, most Hymenoptera, and some Coleoptera).  
75 Exceptions include a few groups containing active predators (e.g. Carabidae and Chrysopidae)  
76 and triungulin or planidial larvae, in which the highly mobile first instar locates a host before  
77 reverting to a largely immobile, parasitic form in subsequent instars (referred to as  
78 hypermetamorphosis) (Pinto 2009).

79 Despite these limitations, several insect lineages have evolved distinctive methods of  
80 rapid larval locomotion without using legs at all. The larvae of some dune-dwelling tiger beetles  
81 (Coleoptera: Cicindelidae) use wind-propelled, wheel-like locomotion (Harvey and Zukoff 2011),  
82 derived from an ancestral tendency to flip or somersault when attacked by parasitoid wasps  
83 (Harvey and Acorn 2019). The jumping ability of some fly larvae has been informally recognized  
84 for centuries, e.g. cheese skipper maggots (Piophilidae: *Piophila casei* (L.)), whose vigorous  
85 activity has long marked the quality of a Sardinian cheese known as *casu marzu* (Swammerdam  
86 1669; Gobbetti et al. 2018; Tunick 2014). Subsequent studies have found that larval jumping is  
87 widespread in holometabolous insects, including Lepidoptera, Hymenoptera, and Diptera.  
88 However, it was not until relatively recently that the actual physiological and kinematic  
89 mechanisms underlying larval jumping have begun to be adequately investigated.

90 Maitland (1992) described “the only known example of jumping by a soft-bodied legless  
91 organism,” in larvae of the Mediterranean fruit fly (Tephritidae: *Ceratitis capitata* (Wiedemann))  
92 (We note that, Swammerdam (1669) notwithstanding, the first detailed description of jumping  
93 locomotion in a fly larva seems to be Camazine’s 1986 study of *Mycetophila cingulum* Meigen).  
94 *Ceratitis* larvae achieve jumps of up to 150 times their body length by curling into a loop and  
95 pumping hemolymph into the abdominal segments until the resulting turgor pressure is sufficient  
96 for bodily propulsion when the loop is released (Maitland 1992). This body loop is anchored by  
97 attachment of the mandibles to the sclerotized anal plate, effectively forming a latch-mediated  
98 spring (Longo et al. 2019). All other reported cases of jumping in maggots appear to work in a  
99 similar way, albeit with varying attachment mechanisms: piophilids also form a ventral loop with  
100 mandibular-anal attachment, but mycetophilid larvae bend dorsally rather than ventrally,  
101 anchoring the thorax to the abdominal tergites with a velcro-like array of interlocking pegs  
102 (Camazine 1986). Farley et al. (2019) demonstrated that jumping larvae of the gall midge  
103 *Asphondylia* sp. anchor their body loop by connecting two regions of cuticle bearing velcro-like  
104 microstructures.

105 Certain case-bearing or enclosed Lepidoptera larvae are capable of short, rapid hops, by  
106 ventrally deflexing the body, increasing turgor pressure in selected body segments, then striking  
107 the interior of their case (Thyrididae: *Calindoea trifascialis* (Moore)) or seedpod (Tortricidae:  
108 *Cydia deshaisana* (Westwood); Pyralidae: *Emporia melanobasis* Balinsky) (Humphreys and  
109 Darling 2013). Hymenopteran “jumping galls” (Cynipidae: *Neuroterus saltatorius* Edwards) use a  
110 similar mechanism, although it is unclear whether the body loop is anchored or how the tightly-  
111 enclosed, conglobulated larva is able to displace enough hemolymph to store adequate energy  
112 for powering jumps (Manier and Deamer 2014). All evidence from the above cited studies  
113 indicates that these are not escape jumps meant to evade predators, but rather a means of  
114 dispersal toward optimal pupation sites, e.g. away from direct sunlight.

115           Only a few of these larval jumping behaviors have been recorded using high-speed  
116 videography, which allows for precise descriptions of takeoff sequences and measurements of  
117 kinematic performance such as acceleration and power output (e.g. jumping larvae of the gall  
118 midge *Asphondylia* sp. in Farley et al. (2019)). These kinematic measures can then be used as  
119 a metric to determine if jumps can be explained as the result of direct muscle movement alone,  
120 or if additional components, such as a latch-mediated spring actuation mechanism (abbreviated  
121 “LaMSA”; Longo et al. 2019), are involved. Some LaMSA systems are known or thought to  
122 utilize hydrostatic body deformations or deformations of a cuticular spring to amplify the power  
123 output of direct muscle action (Brackenbury and Hunt 1993; Burrows et al. 2008; Patek et al.  
124 2013; Tadayon et al. 2015; Farley et al. 2019). Typically, latches involving a mechanical  
125 interaction of one or more body components are employed to mediate the storage and release  
126 of this energy (Longo et al., 2019). However, our view of the diversity and functionality of  
127 LaMSA systems in larval insects is limited, as most examples remain undescribed and  
128 unresolved at the necessary level of mechanical detail.

129           Here we report and describe the mechanics of the first observation of latch-mediated  
130 escape jumping in a beetle larva (Coleoptera: Laemophloeidae: *Laemophloeus biguttatus*  
131 (Say)), using a novel mechanism that does not involve the looped body formation observed in  
132 many jumping insect larvae and appears to use attachment to the substrate as an anchor or  
133 latch. We also report observations of a similar behavior in another laemophloeid beetle larva,  
134 *Placonotus testaceus* (F.), and present a brief review of jumping behaviors in insect larvae.

135

## 136 **Materials and Methods**

### 137 *Specimen collection*

138           In October of 2019, beetle larvae were collected from under the bark of a standing, dead  
139 Darlington oak (*Quercus hemisphaerica* W. Bartram ex Willd.) exhibiting abundant growth of the  
140 fungus *Biscogniauxia atropunctata* (Schwein.) Pouzar (Figure 1). The tree was about twelve

141 (12) inches (30.5 cm) DBH and located on the South side of Governors Scott Courtyard on the  
142 main campus of North Carolina State University (35°47'15.0"N, 78°40'23.7"W). Numerous  
143 beetle larvae and adults (Laemophloeidae, Monotomidae, Mycetophagidae, Latridiidae, and  
144 others), flies and their larvae (Lonchaeidae, Ulidiidae), flat bugs (Aradidae), mites (Astigmatina),  
145 termites (*Reticulitermes*), ants (Formicidae, including *Brachymyrmex* and *Solenopsis invicta*  
146 Buren), and other arthropods were present. Various live insects were collected by MAB for  
147 photos and to preserve specimens for the NC State University Insect Museum. The insects  
148 were brought into the lab in small covered containers with some of the removed bark and kept  
149 moist with a damp paper towel until photos could be taken.

150 The larva of another cucujoid beetle was collected by TY from under the bark of a dead,  
151 broad-leaf tree with several conspecific adults and larvae on August 2nd, 2020. The tree had  
152 been cut down and lying in a place with good sunlight near the parking of Narukawa Valley,  
153 Kihokuchô Town, Ehime Pref., Japan (33°13'02.9"N 132°37'16.9"E). Several arthropods  
154 including non-laemophloeid beetles were also found associated with the dead tree.

155

#### 156 *Larval identification*

157 Larvae from the North Carolina site were presumed to be juveniles of one of the  
158 abundant beetle species associated with the tree, providing an initial starting point for  
159 identification. We used the Coleoptera keys in Stehr (1987) to initially identify larvae to family  
160 level. For species ID we amplified a 591 bp section of the mitochondrial cytochrome c oxidase  
161 subunit I (CO1), from a single larva, for comparison with published sequences. Primers used for  
162 amplification of this gene were based on Hebert et al. (2004). Voucher specimens of larvae are  
163 deposited in the NC State University Insect Museum (GBIF: <http://grbio.org/cool/ij62-iybb>).

164 Initially, the examined larva of the Japanese species was regarded as a laemophloeid  
165 based on overall morphological features: the flattened body, the well sclerotized, large,  
166 longitudinal abdominal segment VIII, and the small, well sclerotized, horn-like urogomphi

167 (Hayashi 1980; Thomas 1988). The species was identified based on the adult morphology after  
168 rearing the larva to the adult stage. The larva was transferred to a Petri dish (Becton Dickinson;  
169 diameter 50 mm, height 9 mm) for the rearing after transporting them into the laboratory. Some  
170 pieces of the bark of the dead tree, from which the larva was found, and a wet tissue squeezed  
171 tightly were placed in the dish to provide food for the larva and moisture. The dish was retained  
172 in the laboratory at room temperature until eclosion.

173

#### 174 *Jumping behavior*

175 To capture the jumping behavior for analysis, *L. biguttatus* larvae were placed on a  
176 60cm x 20cm acrylic platform affixed to a backing board with a 0.5cm<sup>2</sup> scale grid. Because  
177 larvae first proved unable to perform jumps on the smooth bare acrylic (see results), a single  
178 layer of standard 20 lb thickness copy paper was glued to the surface of the platform. Jumps  
179 were filmed at a rate of 3,200 frames s<sup>-1</sup> at a pixel resolution of 1280 x 720 using a Phantom  
180 Miro LC321s (Vision Research, Wayne, New Jersey, US), through a 60mm f/2.8 2X Ultra-Macro  
181 lens (Venus Optics, Hefei, Anhui, China). Image exposure time was 0.156 ms. The platform and  
182 insects were front-lit with an high-intensity LED light array (Visual Instrumentation Corporation,  
183 Lancaster, CA, US). The videos were captured at a frame wide enough to include the entire  
184 trajectory of the jumps. To confirm that 3,200 frames s<sup>-1</sup> was fast enough to resolve all jump-  
185 related rapid movements, an additional eight jumps from two individuals were filmed at 60,000  
186 frames s<sup>-1</sup>. To do so, we used a Photron FASTCAM SA-Z filming at a pixel resolution of 896 x  
187 368 with an image exposure time of 1.05 μs under the same conditions as described above.

188 The larvae were placed on the platform, unrestrained and filmed continuously until they  
189 performed a jump. Beyond being exposed to intense lighting, the larvae were not prodded or  
190 stimulated to perform jumps in any way. For analysis we filmed 39 jumps across 12 individuals.

191 Of those jumps filmed, 29 jumps from 11 of the 12 individuals were performed at an angle  
192 perpendicular to the camera, allowing us to perform the analyses described below. The  
193 remaining 10 jumps were excluded from additional analyses.

194 After all jumps were filmed, 15 beetle larvae (including the 12 that were filmed for  
195 analysis) were weighed using a balance sensitive to a tenth of a milligram (Denver Instruments  
196 PI-114N). Average mass was used for all jump calculations as due to the sensitivity of the  
197 balance used, mass values could not be associated with individual beetles.

198 The larva of *P. testaceus* was placed on a Petri dish (Becton Dickinson; diameter 50  
199 mm, height 9 mm) covered with a piece of wet tissue paper at room temperature. The larval  
200 jumping behavior was filmed at a rate of 30 frames s<sup>-1</sup> at a pixel resolution of 1920 x 1080 using  
201 a digital camera (Canon EOS 7D) fitted with a macro-objective (MP-E 65 mm), while illuminating  
202 the platform using an LED light (Hayashi-repic, HDA-TW3A). The larval locomotion was tracked  
203 with the camera by hand until successfully capturing the jumping behavior.

#### 204 *Video analysis*

205 Jumps of *L. biguttatus* were divided into four phases based on the movements, actions,  
206 and body positioning of the larval beetles: 1) the load phase (Figure 3A), which is thought to  
207 correspond to the contraction of muscles storing energy in the elastic components of the body,  
208 starts when the larvae first begin to arch their body dorsally, and ends when the larvae's  
209 prothoracic legs begin to lose contact with the ground; 2) the latch-decoupling phase (Figure  
210 3B), which occurs as the larvae release their grip on the substrate, starts immediately after the  
211 end of the loading phase and ends when the final proleg loses contact with the ground; 3) the  
212 launch phase (Figure 3C), which corresponds to the transfer of stored elastic energy within the  
213 bodies to kinetic energy of the jumps, begins during or after the end of the latch-decoupling  
214 phase and ends when all contact between the larvae's bodies and the substrate has ceased;

215 and 4) the airborne phase (Figure 3D), which begins after the end of the launch phase and is  
216 finished when the larvae land. The frames where phase transitions occurred were manually  
217 recorded for each video and used for temporal calculations of the jumps' phases.

218 Tracking of the larvae's movement was completed in ImageJ ver 1.52a (Rasband et al.  
219 2020). Videos were converted to 8-bit grayscale and thresholded to generate binary images.  
220 The movement of the larvae was then auto-tracked using the Multitracker plugin (Kuhn, 2001),  
221 which estimated the larvae's center of mass using the centroid of the converted images and  
222 traced movement of the centroid through each frame. The angles of the larvae's bodies at the  
223 end of the loading, latch-decoupling, and launch phases were measured using ImageJ's default  
224 angle tool.

225 The xy coordinates through time of each jump were imported into R ver 3.5.2 (R Core  
226 Team, 2020), where they were scaled using the 0.5cm<sup>2</sup> grid included in each video and  
227 reoriented so that each jump started at the origin of a cartesian grid and proceeded in the  
228 positive x and y directions. A parabola was fit to each trajectory using the *poly* function, and the  
229 maximum height (h) and horizontal distance (d) traveled over the course of the airborne phase  
230 of each jump were calculated as the y coordinate of the vertex and positive x-intercept,  
231 respectively, using the following equations:

232 
$$h = \frac{-b}{2a} \quad (1)$$

233 
$$d = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (2)$$

234 Where a, b, and c correspond to the coefficients of the fitted parabolic equation in the form of y  
235 = ax<sup>2</sup>+bx+c. the takeoff angle ( $\alpha$ ) was calculated as:

236 
$$\alpha = \tan^{-1}(b) \quad (3)$$

237 Cumulative displacement at each time point was calculated, and the amount of displacement  
238 occurring during each of the pre-airborne phases was estimated by dividing the cumulative  
239 displacement between the four phases. Displacement occurring during the latch-decoupling and  
240 launch phases were combined for later calculations, as the latch-decoupling phase varied  
241 widely in its duration and sometimes encompassed the entirety of the launch phase, and a non-  
242 negligible elevation of the larvae's centers of mass occurred during the latch-decoupling phase  
243 in these jumps. A spline function was fitted to the cumulative displacement data using the  
244 *smooth.ppline* function from the *pspline* package in R with a smoothing spar value of  $10^{-8}$   
245 (Ramsey and Ripley, 2017). This spar value was visually determined to fit the datasets  
246 sufficiently while not resulting in exceptionally noisy derivative curves. Velocity and acceleration  
247 curves were calculated by taking the first and second derivatives of the displacement splines,  
248 respectively, and takeoff velocities and accelerations were estimated as the maximum values of  
249 these curves. Jump energy (E) was calculated as:

$$250 \quad E = 0.5mv^2 \quad (4)$$

251 Where m is the body mass of the beetle and v is the takeoff velocity. Jump power (P) was  
252 calculated as:

$$253 \quad P = mL^2t^{-3} \quad (5)$$

254 Where L is displacement of the center of mass attributable to the latch-decoupling and launch  
255 phases and t is the combined duration of the latch-decoupling and launch phases. Power output  
256 (O) during the latch-decoupling and launch phase was estimated by assuming that a certain  
257 proportion of the beetles' body mass was contributing to energy input during this phase:

$$258 \quad O = Pm^{-1}c^{-1} \quad (6)$$

259 Where  $c$  is the assumed proportion of the beetles' body mass powering jumps. As the exact  
260 jumping mechanism (and the muscles powering it) is unknown, an upper bound for this value  
261 was estimated by measuring the total volume of all muscle within the beetle's body via microCT  
262 data (see below), and multiplying this by an assumed muscle density of  $1060 \text{ kg m}^{-3}$ , a value  
263 measured from mammalian muscle that has been previously used in calculations on insect  
264 muscle power (Mendez and Keys, 1960; Ellington, 1985; Tu and Daniel, 2004). This calculation  
265 revealed an estimated 9.78% of the beetles' total body mass to be composed of muscle. In  
266 addition to this estimate of  $c$ , power output calculations were also performed assuming 100%,  
267 75%, 50%, 32.31%, 19.60% and 4.89% of the beetles' body mass were powering jumps, to  
268 account for potential errors in muscle measurements from the CT data due to shrinkage, the  
269 likely possibility that not all of the beetles' muscles are powering jumps, and to calculate the  
270 maximum percentage of the beetles' body mass that can be powering jumps and still not be  
271 able to explain the power output of at least one of the measured jumps. For each set of power  
272 output calculations the estimates were compared to the highest known value from muscle  
273 (approximately  $400 \text{ W kg}^{-1}$ ; Askew and Marsh 2002) to determine whether muscle contraction  
274 without a spring-latch system could feasibly produce the performances measured.

275 To compare locomotory performance of jumping vs crawling in beetle larvae the energetic cost  
276 of transport ( $COT_{\text{jump}}$ ) of jumps was calculated following the methods of Farley et al. (2019):

$$277 \quad COT_{\text{jump}} = \frac{10E}{md} \quad (7)$$

278 This assumes an energetic efficiency of 10% for the muscles powering the jumps and has units  
279 of  $\text{J kg}^{-1} \text{ m}^{-1}$  (i.e. the amount of energy required to move one kilogram of the beetle's body mass  
280 one meter).  $COT$  for crawling was estimated by substituting the average beetle mass into the  
281 power regression equation determined for body mass vs crawling  $COT$  for legged arthropods by  
282 Full (1989):

283 
$$COT_{crawl} = 10.8m^{-0.32} \quad (8)$$

284 Where m is the average larval body mass in kilograms.

285         Uncertainty ratios for velocity, acceleration, energy and power were calculated using  
286 formulas provided in Longo et al. (2019). To reduce uncertainty attributable to length  
287 measurements, the 0.5cm<sup>2</sup> scale grid was measured more precisely using images of the grid  
288 taken with a Keyence VHX 5000 microscope and measured to a precision of 0.001mm. These  
289 calculations resulted in an average uncertainty of 8% for velocity, 16% for acceleration, 11% for  
290 kinetic energy, 26% for power, and 24% for mass-specific power.

291

#### 292 *MicroCT*

293         To estimate muscle mass for power output calculations, as well as examine internal  
294 morphology to uncover the mechanism powering the jumps, one *L. biguttatus* larva was  
295 scanned using microCT at the Imaging Technology Group, Beckman Institute for Advanced  
296 Science and Technology, University of Illinois at Urbana-Champaign. This specimen was killed  
297 by being placed directly into Brasil fixative (Electron Microscope Company, Hatfield, PA) and left  
298 for 24 hours. The larva was then washed several times with 70% ethanol to remove excess  
299 fixative and taken through an ethanol series to 100% ethanol (1 hour each at 80%, 90%, 95%  
300 and 100%). To improve contrast between the cuticle and muscle tissue the larva was stained  
301 overnight in I2E immediately prior to scanning (1% iodine in 100% ethanol) and then washed  
302 several times in 100% ethanol the following morning. The specimen was dried using an  
303 AutoSamdri-931.GL Supercritical Point Dryer (Tousimis Research Corporation, Rockville, MD)  
304 and scanned using an Xradia MicroXCT-400 scanner (Carl Zeiss, Oberkochen, Germany) with  
305 power settings of 25kV voltage and 5W power. 1441 images were taken at an exposure time of  
306 6 sec spanning a 360° view of the larva. A 4x lens was used and source and detector distances  
307 from the specimen were 59.1 mm and 15 mm, respectively. All reconstructions and

308 segmentations were done in Amira ver 5.4.5 (FEI, Hillsboro, OR). In this program the  
309 MaterialStatistics function was used to calculate total volume of the segmented muscle.

310

311 *SEM*

312 Three *L. biguttatus* larvae that were observed jumping were preserved and prepared for  
313 imaging through the following sequence. First, the live larvae were killed by a one-minute soak  
314 in boiling water. Next, they were transferred into 70% ethanol and stored for two weeks.  
315 Following this, the larvae were taken to the point of complete dehydration with 24-hour changes  
316 of room temperature 95% ethanol and three 100% ethanol changes. The larvae were then  
317 critical point dried in liquid CO<sub>2</sub> for 15 minutes at equilibrium using a Tousimis Samdri-795  
318 critical point dryer (Tousimis Research Corporation, Rockville MD) and then mounted on stubs  
319 with double-stick tape and careful application of silver paint to help prevent charging in the  
320 microscope. Larvae were sputter coated with approximately 50Å of gold-palladium in a Hummer  
321 6.2 sputtering system (Anatech USA, Hayward CA). Larvae were imaged using a JEOL JSM-  
322 5900LV at 10kV. Close inspection was done on parts of the body observed to make contact with  
323 the ground during the loading, latch, and launch phases of the jumps (specifically the ventral  
324 side of the head, the tarsi, and the ventral aspects of the terminal sections of the abdomen) to  
325 look for any potential morphological adaptations such as modified tarsal claws, friction patches,  
326 or modified setae that might aid the larvae in adhering to the ground during the loading and  
327 latch phases of jumps.

328

## 329 **Results**

### 330 *Identity of larvae*

331 The larvae collected in North Carolina, USA (Figure 2A), were initially identified as  
332 Laemophloeidae based on morphology and the abundance of adults (*Laemophloeus biguttatus*

333 (Say); Figure 2B) associated with the fungus. Identification was furthered by comparison to  
334 images from Bugguide.net (e.g. <https://bugguide.net/node/view/241687/bgimage>). A closer  
335 examination of morphology (including mouthpart dissection) and keys in Stehr (1987) confirmed  
336 that the larvae belonged to Laemophloeidae (as Cucujidae: Laemophloeinae in that reference).  
337 Comparison of the larval CO1 sequence to published sequences (NCBI Blast) resulted in a  
338 closest match (99.5%) with *L. biguttatus* (GenBank: XXXXXXXX).

339 The larva of the Japanese laemophloeid (Figure 2C) was successfully reared to  
340 adulthood (Figure 2D). The larva pupated on September 14th, 2020, and emerged on  
341 September 20th, 2020. The adult was conspecific with the laemophloeid adults collected with  
342 the larva and identified as *Placonotus testaceus* with reference to the taxonomic literatures  
343 (Lefkovitch 1958; Yablokov-Khinzoryan 1977; Hirano 2010).

344

#### 345 *Initial observations of jumps*

346 After collecting larvae from their habitat, *L. biguttatus* specimens were brought into the  
347 lab to photograph under fluorescent lighting and room temperature conditions. Placing larvae on  
348 bark collected from the larval site, MAB noticed that they would rapidly crawl a short distance  
349 before jumping a short distance (Supplemental video 1). Jumps were not instantaneous;  
350 instead, prior to jumping, the larvae stopped running or walking and flattened their head (the  
351 mouthparts in particular) and pygidial region against the substrate. Abdominal segments 1-6  
352 were then arched up off the substrate while keeping the distal portion of the abdomen and the  
353 urogomphi in contact with the ground. From that posture, they rapidly curled their bodies  
354 ventrally into a jump (Figure 3, Supplemental video 2). The larvae remain curled, in a complete  
355 circle, for the entirety of their jumps. After making impact with the ground in the curled posture,  
356 larvae bounced and rolled (if they didn't land on their side) before uncurling and resuming leg-  
357 powered movement. The initial observations of these behaviors, across several individuals, was  
358 the motivation for pursuing slow motion video capture of the larvae.

359 On the observation of the larva of *P. testaceus*, four jumps were filmed. As in the  
360 jumping behavior of *L. biguttatus*, the larva also crawled a short distance and took a posture  
361 flattening the head and distal abdominal segments against the substrate before each jump. In  
362 addition, at least three of the filmed jumping behaviors were observed just after the larva was  
363 dropped from a thin brush used for placing the larva on the platform. Although distances of all  
364 *Placonotus* jumps were not measured in detail, the longest jumping distance was about 5 cm,  
365 horizontally.

366 In preparation for slow motion video capture, *L. biguttatus* larvae were placed on smooth  
367 glass and acrylic platforms to test the suitability of each type as filming sets. On those  
368 substrates the larvae appeared to be unable to perform their jumps. Instead of jumping, larvae  
369 would struggle to grip the ground and attempts to arch their abdomen or ventrally-curl their  
370 bodies into the jumping posture would result in toppling onto their side and back. Successful  
371 jumps off of these smooth surfaces were never observed.

372

### 373 *Jump performance*

374

375 A summary of our analysis of high-speed video recordings of *L. biguttatus* jumps (n=11  
376 larvae, 29 jumps total) is included in Table 1. Jump sequences began when the larvae stopped  
377 walking and arched their abdominal segments off the substrate (as described above) in a  
378 'loading phase' which averaged  $0.22 \pm 0.17$  s (mean  $\pm$  standard deviation), and resulted in a  
379 change in body angle (head-to-posterior) from near horizontal to  $149.5 \pm 16.7$  degrees. From  
380 this arched stance, the rapid ventral curling of their body was initiated when their tarsal claws  
381 slipped or were released and lost grip with the substrate (Supplemental video 2). In all jumps  
382 where there was a clear view of the legs, the legs did not lose contact with the ground all at  
383 once; instead there was a 'latch-decoupling phase' between the first leg movement and the  
384 point at which the last legs left the ground averaging  $5.5 \pm 14.4 \times 10^{-3}$  s in duration. In 26 of the

385 29 analyzed jumps, the larva was angled so that the front, middle, and hind legs were visible  
386 during this period. In 23 of those jumps the front legs were the last to lose contact with the  
387 ground, two sequences had a combination of middle and front legs leaving the ground last, and  
388 one had middle legs losing contact last. In addition to the 29 jumps we filmed at 3,200 frames  
389 per second, we captured eight jumps at 60,000 frames per second in order to verify that there  
390 were no other rapid movements that set the latch release phase in motion, preceding the legs  
391 losing contact. These sequences confirmed that tarsal claws losing grip with the ground is the  
392 first observable motion in the latch-decoupling phase (Supplemental video 2). During the latch-  
393 decoupling phase the body of the larvae continues to arch further to  $124.1 \pm 29.6$  degrees.

**Table 1.** Jump kinematics based on high-speed imaging from several larvae of *Laemophloeus biguttatus*. Each row corresponds to jumps of individual larvae, with averages and ranges for all jumps from all larvae included in the final two rows.

No. of jumps	Body Length (x 10 <sup>-3</sup> m)	Est. body Mass (x 10 <sup>-6</sup> kg)	Loading phase (sec)	Latch release phase (x 10 <sup>-3</sup> sec)	Launch phase (x 10 <sup>-3</sup> sec)	Launch phase distance (x 10 <sup>-3</sup> m)	Avg. mass-specific power of launch (W kg <sup>-1</sup> )	Max takeoff speed (m s <sup>-1</sup> )	Kinetic energy at takeoff (x 10 <sup>-7</sup> J)	Takeoff angle (deg)	Total jump pitch (+/- %)	Total jump roll (+/- %)	Total jump yaw (+/- %)	Jump horizontal distance* (x 10 <sup>-3</sup> m)	Max jump height (x 10 <sup>-3</sup> m)
4	6.22	1.3	0.12 (0.05-0.16)	3.6 (1.9-5.9)	1.48 (0.31-3.44)	1 (0.8-1.4)	118 (44-258)	0.39 (0.33-0.48)	1.03 (0.73-1.51)	106.4 (65.8-146.4)	50 (0-100)	15.6 (-37.5-125)	28.3 (-25-138)	11.8 (6.5-17.5)	5.9 (2.3-13)
4	5.94	1.3	0.33 (0.26-0.41)	21.1 (1.3-79.7)	1.09 (0.94-1.25)	1.2 (0.6-2.4)	334 (0.11-693)	0.51 (0.44-0.54)	1.7 (1.25-1.91)	83.7 (49.9-104)	-37.5 (-150-0)	-93.75 (-200-0)	-37.5 (-150-0)	14 (5.9-19.7)	13.2 (11.5-14.8)
2	5.46	1.3	0.27 (0.25-0.3)	2 (1.9-2.2)	1.72 (1.56-1.88)	1.1 (1-1.2)	229 (202-257)	0.39 (0.36-0.42)	0.99 (0.84-1.15)	95 (93.9-96.2)	-50 (-100-0)	-12.5 (-50-25)	0 (0-0)	7.5 (5.6-9.4)	8.5 (7.1-9.9)
2	6.32	1.3	0.14 (0.11-0.17)	3.9 (2.8-5)	1.56 (0.63-2.5)	1.3 (1.3-1.3)	104 (99-108)	0.29 (0.28-0.29)	0.53 (0.51-0.55)	77.5 (64.1-90.9)	n/a**	n/a	n/a	7.9 (7.3-8.5)	3.9 (3.1-4.8)
4	5.53	1.3	0.51 (0.23-0.68)	3.2 (2.2-4.7)	0.86 (0.31-1.88)	0.9 (0.3-1.2)	159 (34-411)	0.32 (0.14-0.51)	0.78 (0.12-1.69)	56 (14.3-87.2)	25 (0-100)	-18.75 (-50-0)	70.75 (0-183)	10.3 (2.3-23.7)	4.6 (1.9-7.4)
3	5.86	1.3	0.1 (0.03-0.16)	1.1 (0.9-1.3)	1.46 (1.25-1.56)	1.3 (0.9-1.6)	913 (545-1206)	0.59 (0.5-0.63)	2.26 (1.64-2.62)	88.9 (68.7-100.4)	-33.33 (-50-0)	-12.5 (-37.5-0)	-8.33 (-25-0)	13.6 (12.6-14.3)	7.3 (3.1-9.8)
1	4.24	1.3	0.3	8.8	0.63	1.4	24	0.54	1.90	62.1	0	-75	-50	11.8	7.6
1	4.88	1.3	0.15	0.6	1.56	0.9	767	0.55	1.99	73.3	-50	37.5	0	8.7	8.5
3	5.22	1.3	0.1 (0.05-0.14)	3.2 (2.2-4.1)	1.77 (1.25-2.19)	1.2 (1-1.5)	121 (74-157)	0.46 (0.4-0.52)	1.38 (1.07-1.75)	74.5 (65-81.8)	0 (0-0)	16.67 (-50-50)	-50 (-100-0)	11.1 (9.4-14)	11.1 (8.6-14.2)
3	5.82	1.3	0.11 (0.06-0.17)	2.9 (1.9-4.7)	1.77 (0.31-2.81)	2.5 (1.3-4)	642 (259-1319)	0.72 (0.47-0.87)	3.62 (1.42-4.89)	95.6 (74.3-109.6)	16.67 (-100-100)	0 (-25-25)	-41.67 (-100-0)	14 (12.1-15.5)	3.6 (1.5-7.7)
2	5.58	1.3	0.13 (0.09-0.17)	3 (1.3-4.7)	2.19 (1.56-2.81)	2.1 (1.1-4)	213 (119-307)	0.63 (0.5-0.87)	2.74 (1.66-4.89)	58.9 (27.9-109.6)	-112.5 (-125-100)	0 (0-0)	0 (0-0)	9.8 (4-15.5)	11.2 (7.7-13.2)
<b>Avg±SD</b>	<b>5.55±0.6</b>	<b>1.3</b>	<b>0.22±0.17</b>	<b>5.5±14.4</b>	<b>1.4±0.8</b>	<b>1.3±0.7</b>	<b>323±353</b>	<b>0.47±0.15</b>	<b>1.59±1.05</b>	<b>79.6±28.2</b>	<b>-10.2±66.6</b>	<b>-16.2±63.4</b>	<b>-3.9±65.5</b>	<b>11.2±4.8</b>	<b>7.9±4.3</b>
Range			(0.03-0.68)	(0.63-79.69)	(0.31-3.44)	(0.32-4.02)	(0.11-1319)	(0.14-0.87)	(0.12-4.89)	(14.3-146.4)	(-150-100)	(-200-125)	(-150-183)	(2.33-23.75)	(1.51-14.83)

\* jumps were filmed from a single angle, so not all jumps were perfectly parallel to the plane of view and horizontal distance estimates may, therefore, be underestimated.

\*\* rotational data unavailable as beetle collided with the wall on descent, altering normal body rotation.

394 The launch phase, or the time from when all legs have released to when all contact between  
395 the body of the larvae and the substrate is gone, averaged  $1.4 \pm 0.8 \times 10^{-3}$  s. This phase  
396 corresponded to the elastic energy stored within the body being transferred to kinetic energy of  
397 the body jumping off of the substrate. The launch phase often began during the latch-decoupling  
398 phase when only some of the legs had lost contact with the substrate, and in at least one  
399 instance completely overlapped with the latch release phase such that the last point of contact  
400 with the ground was one of the larva's legs. During this phase larvae rapidly arched their body  
401 even further to  $79.6 \pm 28.2$  degrees prior to takeoff, reached a maximum acceleration of  $89.5 \pm$   
402  $34.5 \text{ m s}^{-2}$  and achieved a takeoff velocity of  $0.47 \pm 0.15 \text{ m s}^{-1}$  with the fastest takeoff velocity  
403 reaching  $0.87 \text{ m s}^{-1}$ , leaving the ground at an angle of  $79.6 \pm 28.2$  degrees. Over the course of  
404 the jump larvae were airborne for  $1.3 \pm 0.7 \times 10^{-3}$  s and covered distances of  $11.2 \pm 2.8$  mm  
405 horizontally and  $7.9 \pm 4.3$  mm vertically, equivalent to  $1.98 \pm 0.8$  and  $1.5 \pm 0.9$  body lengths,  
406 respectively, though jump trajectories were variable with the farthest horizontal jumper traveling  
407 23.75 mm (Figure 4). A cumulative displacement, velocity, and acceleration vs time plot for a  
408 representative jump is shown in Figure 5.

409 Results from the microCT scan revealed a total muscle volume of  $0.12 \text{ mm}^3$  in the  
410 specimen examined (Figure 6A-C). This volume had an estimated muscle mass of 0.12 mg,  
411 9.78% of the average total mass of the beetle larvae filmed. Assuming that all of this muscle  
412 mass is used to power jumps (likely an overestimate), the average power density during the  
413 launch phase of jumps was  $323 \pm 353 \text{ W/kg}$  muscle, with a maximum of  $1319 \text{ W/kg}$  muscle  
414 (Figure 6D). Five of the 11 larvae filmed had at least one jump with an estimated power density  
415 exceeding the maximum recorded power density for any muscle ( $400 \text{ W/kg}$ ), and three of those  
416 five had average power densities exceeding this value (Table 1). If only half of the total muscle  
417 mass (4.89% total body mass) was powering jumps, then eight of the 11 larvae had at least one  
418 jump with a power density that exceeded the  $400 \text{ W/kg}$  threshold (Figure 6D). It is possible that  
419 total percent muscle mass may be underestimated due to shrinkage occurring during the

394 fixation process, so additional calculations of power density were done assuming 50%  
395 shrinkage of muscle (19.6% body mass composed of muscle). This still resulted in three jumps  
396 from two larvae having power densities exceeding the 400 W/kg threshold (Figure 6D). Only  
397 when the estimated muscle mass exceeded 32.31% of total body mass were power densities  
398 estimates of all jumps below the 400 W/kg threshold (Figure 6D).

399 The average energetic cost of transport for jumping ( $COT_{jump}$ ) across all jumps was 110  
400  $\pm 74 \text{ J kg}^{-1} \text{ m}^{-1}$ , compared to an estimated cost of transport for crawling ( $COT_{crawl}$ ) of  $825 \text{ J kg}^{-1}$   
401  $\text{m}^{-1}$  based on the power regression function for crawling arthropods calculated by Full (1989).

402

#### 403 *External and internal morphology (SEM/MicroCT)*

404 SEM imaging of all body parts that were in direct contact with the substrate immediately  
405 prior to a jump did not reveal evidence of any micro- or nano-scale anatomical features which  
406 might be helping the larvae attach to the substrate during the loading phase of a jump (Figure  
407 7). Likewise, the microCT scan revealed muscle arrangements similar to those of other insect  
408 larvae (Snodgrass 1935), with the musculature of the abdominal segments consisting of a  
409 series of dorso-ventral, dorsal longitudinal, and ventral longitudinal fibers (Figure 6A-C). There  
410 did not appear to be any noticeable differences between abdominal segments in this  
411 arrangement.

412

#### 413 *Review of jumping behavior in insect larvae*

414 An extensive review of the literature was conducted in order to determine how common  
415 jumping behavior is within insect larvae; the results of this review are summarized in Table 2.  
416 Most authors provided a qualitative description of the jumping behavior without quantitative  
417 measurements of jump performance, but it is clear that some form of larval jumping is  
418 widespread in insects. This type of locomotion appears to have evolved in at least five orders of  
419 insects (as well as nematodes, not summarized), and is documented from at least 28 families,

394 including the Laemophloeidae documented herein. Given the phylogenetic distribution of  
395 jumping across unrelated orders and families, this behavior no doubt evolved repeatedly within  
396 holometabolous insects.

**Table 2.** Taxonomic distribution of jumping behavior among insect larvae.

Order	Family	Species	Life Stage	Maximum Distance	Speed	Mechanism	Host or substrate	Citation
Diptera	Acroceridae	<i>Ogcodes pallipes</i> Latreille	first instar/planidium			substrate-anchored cercal spring	active among host habitats; endoparasite of spider	Clausen 1940
		<i>Ogcodes rufoabdominalis</i> Cole	first instar/planidium			substrate-anchored cercal spring	active among host habitats; endoparasite of spider	Capelle 1966
		<i>Pterodontia</i> sp.	first instar/planidium			substrate-anchored cercal spring	active among host habitats; endoparasite of spider	Clausen 1940
	Cecidomyiidae	<i>Asphondylia</i> sp.	third instar	121 mm	0.85 m/s	self-anchored loop, ventral	galls	Camazine 1986, Farley et al 2019
		<i>Contarinia inouyei</i> Mani	third instar			various self-anchored loops	bud galls	Tokuhisa et al 1979
		<i>Contarinia tritici</i> Kirby	third instar			various self-anchored loops	bud galls	Barnes 1956
		<i>Tricholaba trifolii</i> Rùbsaamen	third instar			various self-anchored loops	inquilines in galls of <i>Dasineura</i> (Cecidomyiidae)	Milne 1961
	Chloropidae	<i>Cadrema pallida</i> (de Meijere)	unknown			self-anchored loop, ventral*	decaying organic matter	Bohart and Gressitt 1951
	Clusiidae	Unknown	unknown			self-anchored loop, ventral*	saproxyllic	Curran 1934
	Drosophiidae	<i>Drosophila cancellata</i> Mather	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Mather 1955a, b
		<i>Drosophila coracina</i> Kikkawa	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Kikkawa & Peng 1938
		<i>Drosophila enigma</i> Malloch	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Mather 1955a, b
		<i>Drosophila immigrans</i> Sturtevant	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015
		<i>Drosophila lativittata</i> Malloch	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Mather 1955a, b
		<i>Drosophila levis</i> Mather	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Mather 1955a, b
		<i>Drosophila maculosa</i> Mather	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Mather 1955a, b
		<i>Drosophila opaca</i> Mather	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Mather 1955a, b
		<i>Drosophila subtilis</i> Kikkawa & Peng	late instar			self-anchored loop, ventral	decaying fruit	Marinov et al. 2015, Kikkawa & Peng 1938
		<i>Scaptodrosophila kirki</i> (Harrison)	late instar			self-anchored loop, ventral	decaying fruit, fungus	Marinov et al. 2015
	Lonchaeidae	<i>Dasiops caustonae</i> Norrbom and McAlpine	late instar	100 mm		self-anchored loop, ventral*	fresh fruit of <i>Passiflora mollissima</i>	Causton & Rangel 2002
		<i>Dasiops vibrissata</i> (Malloch)	late instar			self-anchored loop, ventral	fungus under bark of dead tree	observations during this study
		<i>Lonchaea filifera</i> Bezzi	late instar			self-anchored loop, ventral*	decaying organic matter	Bohart and Gressitt 1951
	Mycetophilidae	<i>Mycetophila cingulum</i> Meigen	last instar	150 mm	~1.0 m/s	self-anchored loop, dorsal	polypore, <i>Polyporus squamosus</i>	Camazine 1986
	Phoridae	<i>Chonocephalus depressus</i> De Meijere	last instar			self-anchored loop, ventral*	decaying organic matter	Rao 1961
	Piophilidae	<i>Piophila casei</i> (Linnaeus)	unknown			self-anchored loop, ventral	cheese	Swammerdam 1669
		<i>Prochyliza xanthostoma</i> Walker	late instar	500 mm		self-anchored loop, ventral	carrion	Bonduriansky 2002
		<i>Stearibia nigriceps</i> (Meigen)	late instar			self-anchored loop, ventral*	carrion	Bonduriansky 2002
		<i>Liopiophila varipes</i> (Meigen)	late instar			self-anchored loop, ventral*	carrion	Bonduriansky 2002
		<i>Protopiophila latipes</i> (Meigen)	late instar			self-anchored loop, ventral*	carrion	Bonduriansky 2002
		<i>Parapiophila</i> spp.	late instar			self-anchored loop, ventral*	carrion	Bonduriansky 2002
	Pipunculidae	<i>Pipunculus annulifemur</i> Brunetti**	last instar			unknown	endoparasite of Auchennorhyncha	Subramaniam 1922, Clausen 1940

	Platystomatidae	<i>Scholastes aitapensis</i> Malloch	unknown			self-anchored loop, ventral*	decaying plant matter, dung	Bohart and Gressitt 1951
	Sepsidae	Unknown	unknown			self-anchored loop, ventral*	dung and decaying materials	Pont 1979
	Tephritidae	<i>Ceratitis capitata</i> (Wiedemann)	last instar	120 mm	0.5 m/s	self-anchored loop, ventral*	fruit	Maitland, 1992
	Ulidiidae	<i>Euxesta notata</i> Wiedemann	last instar			self-anchored loop, ventral*	decaying plant matter	Hutchison 1916
		<i>Notogramma camiciforme</i> Loew (as <i>N. stigma</i> )	last instar			self-anchored loop, ventral*	decaying plant matter	Bohart and Gressitt 1951
Lepidoptera	Pyralidae	<i>Emporia melanobasis</i> Balinsky	last instar			unknown	hollowed fruit	Krueger 1997
	Thyrididae	<i>Calindoea trifascialis</i> (Moore)	last instar			substrate-anchored loop	diptero carp leaf	Humphreys and Darling 2013
	Tortricidae	<i>Cydia saltitans</i> (Westwood)	last instar			substrate-anchored loop	hollowed seed	Westwood 1857, Gilligan et al 2020
Hymenoptera	Cynipidae	<i>Neuroterus saltatorius</i> Edwards	last instar larva	30 mm		unknown	hollowed gall	Kinsey 1923, Manier and Damier 2014
	Eucharitidae	<i>Dicoelothorax platycerus</i> Ashmead	first instar/planidium			substrate-anchored cercal spring	active among host habitats; feed on ant larvae	Torrens 2013
		<i>Galearia latreillei</i> (Guérin-Méneville)	first instar/planidium			substrate-anchored cercal spring	active among host habitats; feed on ant larvae	Torrens 2013
		<i>Latina rugosa</i> (Torréns, Heraty, and Fidalgo)	first instar/planidium			substrate-anchored cercal spring	active among host habitats; feed on ant larvae	Torrens 2013
		<i>Neolirata alta</i> (Walker)	first instar/planidium			substrate-anchored cercal spring	active among host habitats; feed on ant larvae	Torrens 2013
		<i>Neolirata daguerrei</i> (Gemignani)	first instar/planidium			substrate-anchored cercal spring	active among host habitats; feed on ant larvae	Torrens 2013
	Ichneumonidae	<i>Bathyplectes anurus</i> (Thomson)	last instar	50 mm (vertically)		substrate-anchored spring (?)	rigid cocoon; parasitoid of alfalfa weevil	Day 1970, Saeki et al 2016
	Perilampidae	<i>Monacon robertsi</i> Boucek	first instar/planidium			substrate-anchored cercal spring	active among host habitat; feed on beetle pupa	Darling and Roberts 1999
	Tenthredinidae	<i>Heterarthrus</i> spp.	last instar			unknown	flexible cocoon of leaf tissue	Liston et al 2019
Coleoptera	Brentidae	<i>Nanophyes</i> sp.	late instar			unknown	<i>Tamarix</i> seed capsules	Crowson 1981
	Carabidae	<i>Cicindela duodecimguttata</i> Dejean	third instar			unanchored loop, dorsal flexion followed by ventral flexion	sand, soil	Harvey and Acorn 2019
		<i>Cicindela lengi</i> Horn	third instar			unanchored loop, dorsal flexion followed by ventral flexion	sand, soil	Harvey and Acorn 2019
		<i>Cicindela tranquebarica</i> Herbst	third instar			unanchored loop, dorsal flexion followed by ventral flexion	sand, soil	Harvey and Acorn 2019
		<i>Habroscelimorpha dorsalis</i> Say	third instar			unanchored loop	sand	Harvey and Zukoff 2011
		<i>Omus dejeani</i> Reiche	third instar			unanchored loop, dorsal flexion followed by ventral flexion	sand, soil	Harvey and Acorn 2019
		<i>Tetracha carolina</i> (Linnaeus)	third instar			unanchored loop, dorsal flexion followed by ventral flexion	sand, soil	Harvey and Acorn 2019
	Curculionidae	<i>Conotrachelus anaglypticus</i> (Say)	unknown	89 mm		self-anchored loop, ventral	under bark of wounded trees	Brooks and Cotton 1924
	Laemophloeidae	<i>Laemophlous biguttatus</i> (Say)	late instars	11.2 mm	0.47 m/s	substrate-anchored loop	fungus under bark of dead tree	<i>this study</i>
		<i>Placonotus testaceus</i> (F.)	unknown			substrate-anchored loop	fungus under bark of dead tree	<i>this study</i>
Strepsiptera	Corioxenidae	<i>Corioxenos</i> sp.	first instar/planidium			substrate-anchored spring (?)	endoparasite of Hemiptera	Clausen 1940
	Mengenillidae	<i>Eoxenos laboulbeni</i> de Peyerimhoff	first instar/planidium			substrate-anchored spring (?)	endoparasite of Lepismatidae	Clausen 1940
	Myrmecolacidae	<i>Stichotrema dallatorreanum</i> Hofeneder	first instar/planidium			substrate-anchored spring (?)	endoparasite of Hymenoptera	Young 1987, Cook 2014

\*presumed based on other related taxa; \*\*Skevington and Marshall (1998) note that Subramaniam's observation of jumping *P. annulifemur* must have been another genus, as *Pipunculus* only parasitizes deltocephaline cicadellids.

## 397 **Discussion**

### 398 *Likelihood of power amplification and latch-mediated spring actuation*

399           The results of our power density calculations for jumps provide a reasonable case for  
400 direct muscle action alone being insufficient to explain jump power for these larvae. Although  
401 the majority of jumps fall beneath our established 400 W/kg cutoff point for power amplification  
402 in all scenarios examined, this cutoff point is based on measurements from muscles that have  
403 been naturally selected for extraordinarily high sustained power output (bird flight muscle;  
404 Askew and Marsh 2002), and it is unlikely that actual power output of the larvae's muscles are  
405 that high. Additionally, combining the latch-decoupling and launch phases for power calculations  
406 conservatively biased our estimates towards lower power densities, since the latch-decoupling  
407 phase did not always heavily overlap with the launch phase for all jumps examined. Finally, as  
408 the exact spring mechanism and the associated muscles that power the jump are currently  
409 unknown, our estimations of muscle mass for power density calculations are certainly  
410 overestimates, further biasing our power density towards conservatively low values. Even with  
411 these conservative estimates, the fact that a non-negligible number (24%) of observed jumps  
412 had power densities exceeding the 400 W/kg threshold strongly suggest that direct muscle  
413 action alone is not responsible for powering jumps.

414           This study is one of very few to describe jumping behavior of beetle larvae in the  
415 Polyphaga (a group of Coleoptera containing over 340,000 described species; McKenna et al.  
416 2015; Table 2) and, to the best of the authors' knowledge, is a unique example of a LaMSA  
417 mechanism in which the latching component requires interaction with the substrate to function  
418 properly. Furthermore, it is notable that we were unable to identify any morphological  
419 adaptations for latching or jumping from the SEM or microCT data, suggesting that adaptations  
420 for jumping in this species may be primarily behavioral (gripping the substrate prior to  
421 contracting abdominal muscles, and then releasing grip once enough energy has been  
422 elastically stored) rather than morphological. This may partially explain the low estimates of

397 power density for this species compared to other LaMSA systems, including other jumping  
398 larvae with highly derived jumping behaviors and morphologies (Patek et al. 2004; Burrows  
399 2006, 2009; Larabee et al. 2017; Gibson et al. 2018; Farley et al. 2019; Booher et al. 2021).  
400 Comparisons with closely related species that have conclusively been shown incapable of  
401 jumping to see what morphological characteristics, if any, are derived and may assist in jump  
402 performance in this species, as well as identifying and quantifying the characteristics of the  
403 spring mechanism, are important next steps in determining whether specific morphological  
404 adaptations for jumping are present that we did not detect in our current study.

405

#### 406 *Jumping Behavior in Insect Larvae*

407 Larvae that exhibit jumping behavior are found in dozens of species in a variety of  
408 ecological contexts (see Table 2 and references within), but there are three distinct  
409 circumstances under which the evolution of jumping larvae appears to be favored:

- 410 1. **Triungulin/planidial larvae**, i.e. the active, host-seeking first instar of parasitoid  
411 species. This includes those strepsipteran, dipteran, and hymenopteran larvae whose  
412 first instars appear to use their cercal bristles as a spring to launch themselves onto the  
413 host. This seems to be a particularly important strategy for the Acroceridae (Diptera) and  
414 Eucharitidae (Hymenoptera), both of which are larval parasites of well-defended  
415 predatory arthropods (spiders and ants, respectively). The ability to leap onto a host  
416 undetected may be a means of avoiding detection and attack during the larva's dispersal  
417 phase.
- 418 2. **Encapsulated larvae**, typically insects whose third instar or prepupal stage must seek  
419 an appropriate environment for pupation without leaving the seed or leaf envelope in  
420 which they have developed. This includes the "Mexican jumping bean" moth *Cydia*  
421 *saltitans*, as well as several other small moth species, sawflies in the genus  
422 *Heterarthrus*, cynipid gall wasps, and one species of ichneumonid wasp which

397 parasitizes encapsulated weevil larvae. In this type of legless leaping, the larva braces  
398 itself and strikes the inner wall of its gall, seed pod, or leaf envelope hard enough to  
399 move the entire capsule. Saeki *et al.* (2016) demonstrated that *Bathyplectes* larvae are  
400 able to direct this seemingly random jumping movement, increasing their activity in sun  
401 or heat and coming to rest in shady areas. Similar activity has been documented in other  
402 encapsulated species when exposed to light or heat; thus, this type of jumping is very  
403 likely a means of moving to a safe pupation habitat without exposing the larva itself to  
404 predators.

405 **3. Larvae at risk of [sudden] exposure**, including those who feed in concealed habitats  
406 that are at risk of being disturbed by predators or larger animals. This includes many  
407 mycophagous species, fruit and vinegar flies, and fungus gnats. Both the cheese  
408 skippers associated with *casu marzu* and their piophilid relatives feeding on vertebrate  
409 carcasses display this behavior as well. The common thread among these taxa is that  
410 their habitats – fermenting fruit, fungus, and carrion – are ephemeral and also likely to  
411 attract other scavengers and predators, particularly vertebrates. Jumping may represent  
412 a rapid means of escape from sudden exposure when the food source is disturbed (as  
413 described in Brooks and Cotton 1924 for larvae of *Conotrachelus anaglypticus*). It was  
414 also demonstrated by Bonduriansky (2002) that only later stage piophilid larvae jump, in  
415 an attempt to move from the food source to suitable pupation sites, thus reducing  
416 exposure time. Harvey and Acorn (2019) demonstrated that tiger beetle larvae,  
417 unearthed from their burrows in loose, sandy soil, react violently to a simulated  
418 parasitoid attack by performing “leaping somersaults.”

419

#### 420 *Function of jumping behavior in Laemophloeidae*

421 Due to the cryptic nature of insect larvae living under the bark of decaying plants, their  
422 patchy distribution due to ephemeral or sporadic food resources, and few researchers studying

397 their natural history, the behaviors of many subcortical insect larvae are not well known. In fact,  
398 while observing the fauna associated with the same tree in which *L. biguttatus* larvae were  
399 collected, we collected a number of maggots that also were observed to jump in a species that  
400 had not been recorded to do so (pers. obs. by MAB and AAS of *Dasiops vibrissata* Malloch,  
401 Lonchaeidae; Table 2).

402         Although we describe the mechanics of jumping laemophloeid larvae here, one  
403 important question remains: why do these larvae jump? It seems very unlikely that the jumping  
404 behavior of laemophloeids is used to routinely avoid or repel predators and parasitoids, because  
405 of the spatial constraints associated with living under bark or fungal structures. Another piece of  
406 evidence against predator/parasite avoidance is the fact that the larvae we observed did not  
407 jump when stimulated with forceps or other tools (simulating a predator attack, *cf.* Harvey and  
408 Acorn 2019), though they did flail and direct their sharp urogomphi towards the simulated  
409 attacker. This was also seen in the *Placonotus* larvae (Supplemental video1). The larvae  
410 instead stopped and jumped after crawling around, without any direct stimulus. The behavior of  
411 jumping laemophloeid larvae is most similar to that of mycophagous and saprophagous fly  
412 larvae associated with decaying wood and carcasses (Table 2) – a response to sudden  
413 exposure, intended to quickly move the insect to a safer microhabitat. Thus we speculate that  
414 the function of laemophloeid jumping behavior is to aid in rapid movement to suitable habitats  
415 as needed, avoiding predation or parasitism indirectly. We can envision cases where the bark of  
416 rotting trees sloughs off easily, exposing the larvae to the elements and attackers. Based on our  
417 COT calculations for crawling vs jumping in this species, jumping would result in a more rapid  
418 and energetically less costly locomotion compared to crawling (approximately 13% COT for  
419 jumping compared to crawling), and could also produce unpredictable trajectories by which the  
420 larvae can escape to new sites.

421         It is also possible that larval jumping is an artifact or exaptation of another behavior.  
422 During our (TY) observations of *Placonotus*, the larvae frequently exhibited a vertical prying

397 action in tight spaces, including subcortical habitats. This behavior appears to facilitate  
398 movement under bark or between fungal masses, similar to the “wedge-pushing” of carabid  
399 beetles (Evans 1977), and may use the same musculature as the jumping behavior documented  
400 in this study.

401 Unfortunately, due to the paucity of live specimens for our studies and inability to  
402 replicate more natural conditions for them to behave, we cannot fully address this point through  
403 experimentation. We encourage future research on this question by collecting larvae of these  
404 beetles and performing more experiments.

405

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416

#### 417 **Author Contributions**

418 Investigation: MAB, JCG, AAS; Methodology: MAB, JCG, AAS; Conceptualization: MAB, JCG,  
419 AES, TY, AAS; Formal Analysis: JCG, AAS; Writing – Original Draft Preparation: MAB, JCG,  
420 AES, TY, AAS; Writing – Review & Editing: MAB, JCG, AES, TY, AAS

421

422

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401

402 **Figure 1.** A: Location of the Darlington oak (*Quercus hemisphaerica* W. Bartram ex Willd.)  
403 exhibiting abundant growth of the fungus *Biscogniauxia atropunctata* (Schwein.) Pouzar where  
404 the beetles and larvae of *Laemophloeus biguttatus* were collected; B&C: Images showing the  
405 growth of the fungus and peeling bark (October of 2019; photos by MAB)

406

407 **Figure 2.** Habitus images of known Laemophloeidae with jumping larvae: A: larva of  
408 *Laemophloeus biguttatus*; B: same, adult; C: larva of *Placonotus testaceus*, D: same, adult.  
409 (A&B: taken by MAB; D&C: taken by TY)

410

411 **Figure 3:** *Laemophloeus biguttatus* jump sequences (A-C and D are separate jumps) taken  
412 from videos filmed at 3,200 frames per second. A-C: loading, release, and launch phases of a  
413 jump, times listed for the first and last pictured body position; D: complete jump trajectory. A:  
414 loading phase, 0.119 seconds elapse between top and bottom body postures. Bottom image is  
415 the frame directly preceding the top larval image in B. B: jump release sequence when legs lose  
416 grip on substrate, each larval image is a single sequential frame and only 0.625 ms separate the  
417 top and bottom image. C: launch phase capturing the moment the larva leaves the ground and a  
418 mid-air posture. 5.31 ms separate the bottom image from the top. The bottom image in panel C  
419 is 1.8 ms after the bottom image in panel B. D: the entire sequence spans 0.081 seconds, noted  
420 times pertain to the first, top, and last of the sequential images, and the scale bar pertains only  
421 to this panel.

422

397 **Figure 4:** trajectories of all observed jumps of *L. biguttatus*. Trajectories that share colors  
398 correspond to different jumps of the same larva.

399

400 **Figure 5:** Kinematic measurements of the jump of a beetle pictured in Figure 3D. Loading  
401 phase is shown in grey, latch-decoupling phase shown in light blue, and launch phase ending  
402 when the beetle loses all contact with the ground is shown in purple. Dark blue on the  
403 displacement graph denotes actual data points while the black line represents the fitted spline  
404 function.

405

406 **Figure 6:** Estimate of the contribution of muscle power in *L. biguttatus* jumps and evidence of a  
407 power amplification system. Panel A-C: MicroCT whole-body imaging and isolation of muscles  
408 throughout the body cavity used to calculate total muscle mass. Scale bars denote 1mm. Panel  
409 D: power density (W/kg muscle) of jumps assuming differing proportions of the beetles' total  
410 body mass is being used to power jumps. Total body muscle mass was estimated to be 9.78%  
411 of the beetle's total body mass based on microCT data. At that mass estimate, using an  
412 overestimate that all of beetles muscles are involved in powering a jump, the power density for 7  
413 of the 29 jumps we analyzed are beyond what can be explained by direct muscle contraction  
414 alone (those that are above the red dashed line), indicating the involvement of a power  
415 amplification mechanism. The red dashed line is reference to the 400 W kg<sup>-1</sup> high-power  
416 capability of vertebrate flight muscle (Askew & Marsh, 2002). If the muscles powering the jumps  
417 constitute more than 32.31% of the total body weight (left of the grey dashed line), then all  
418 analyzed jumps can be explained by direct muscle contraction alone.

419

420 **Figure 7.** Representative SEM images of *Laemophloeus biguttatus* body parts in direct contact  
421 with the substrate immediately prior to a jump. A: ventral surface of the head; B: detail of  
422 mouthparts; C: Ventral surface of the last abdominal segment and urogomphi; D: detail of last

397 abdominal segment and urogomphi; E: Ventral view of front and middle legs slightly bent  
398 inwards; F: Detail of front right tarsal claw. Body surface debris and fungal spores evident on all  
399 images.

400

401 **Supplemental Video 1:** Real-time (30 frames per second image capture and playback) of  
402 jumping behavior observed in *Laemophloeus biguttatus* and *Placonotus testaceus*. In order of  
403 appearance: 1: initial observation of *L. biguttatus* jumping on natural substrate; 2: full *L.*  
404 *biguttatus* jump sequence; 3: additional full *L. biguttatus* jump sequence; 4: closer view of an *L.*  
405 *biguttatus* jump; 5: series of *P. testaceus* jumps off of a tissue paper substrate, filmed from  
406 above.

407

408 **Supplemental Video 2:** Slow motion sequences of *Laemophloeus biguttatus* jumping behavior.  
409 In order of appearance: 1: 3,200 frames per second capture of the jump pictured in panels A-C  
410 of Figure 3; 2: 3,200 frames per second capture of the jump pictured in panel D of Figure 3; 3:  
411 60,000 frames per second capture of the initiation of jump showing the hind legs detaching from  
412 the substrate, as first body movement, when the jump sequence is set into motion.

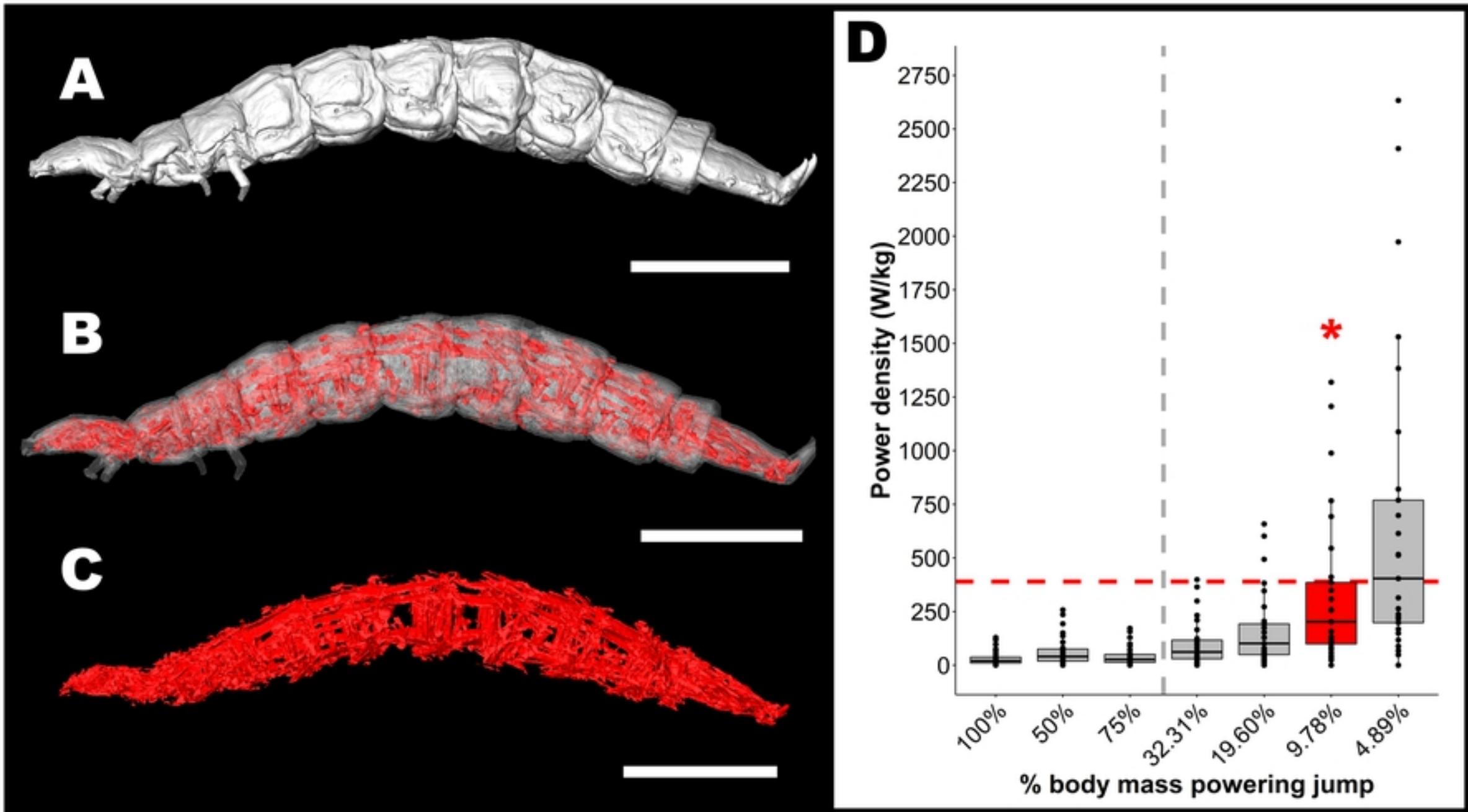


Figure 6



Figure 2

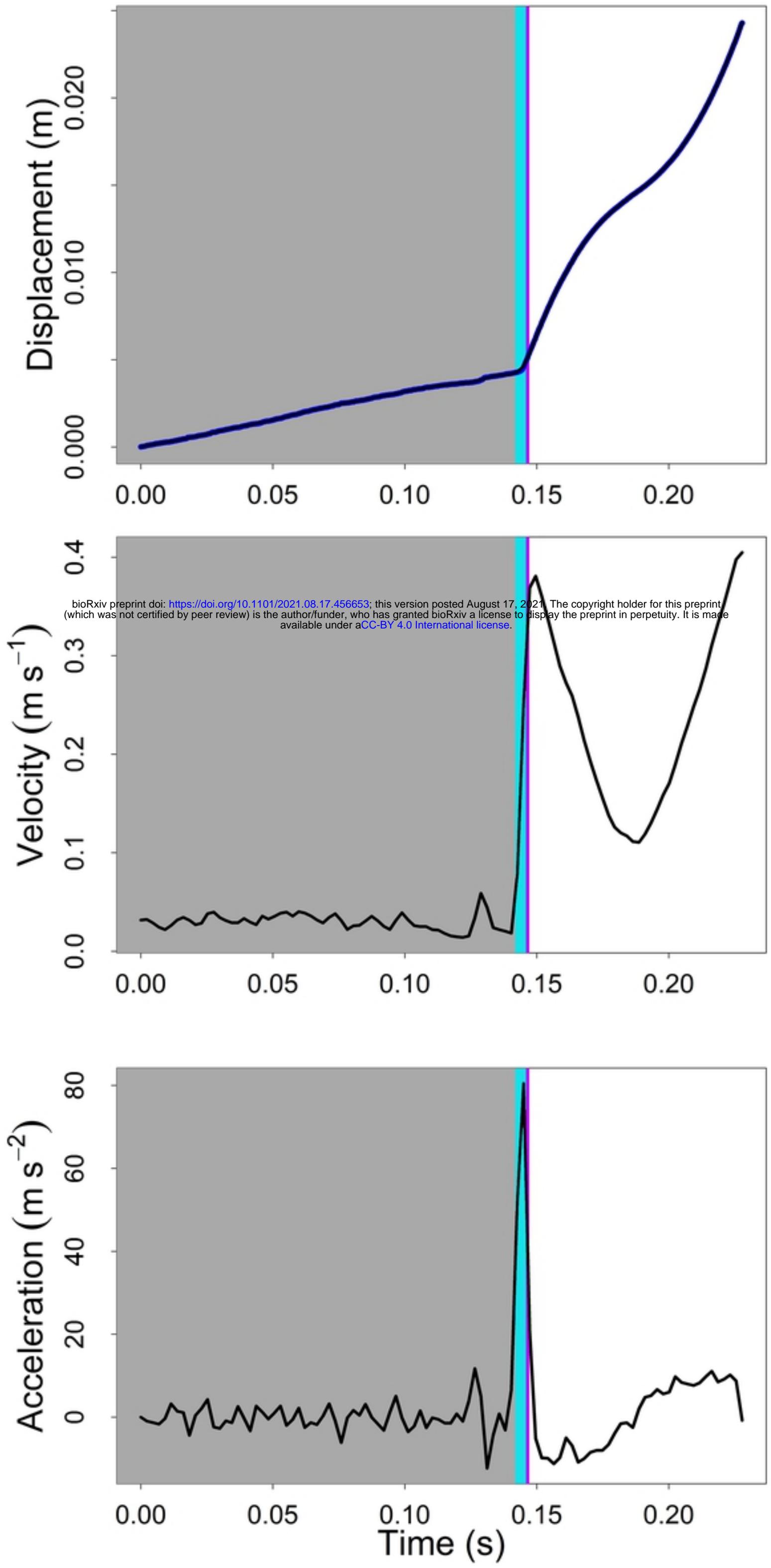


Figure 5

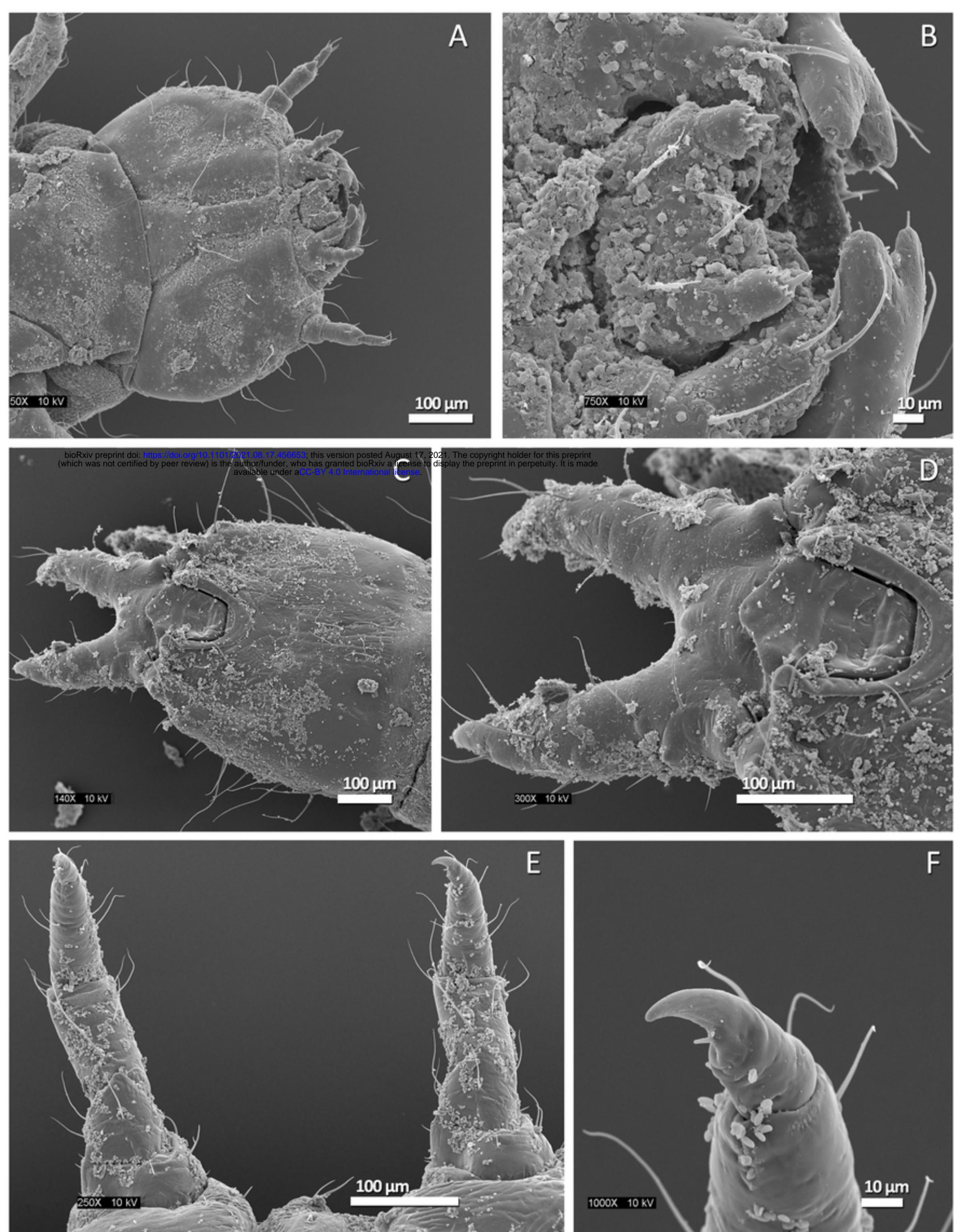


Figure 7



Figure 1

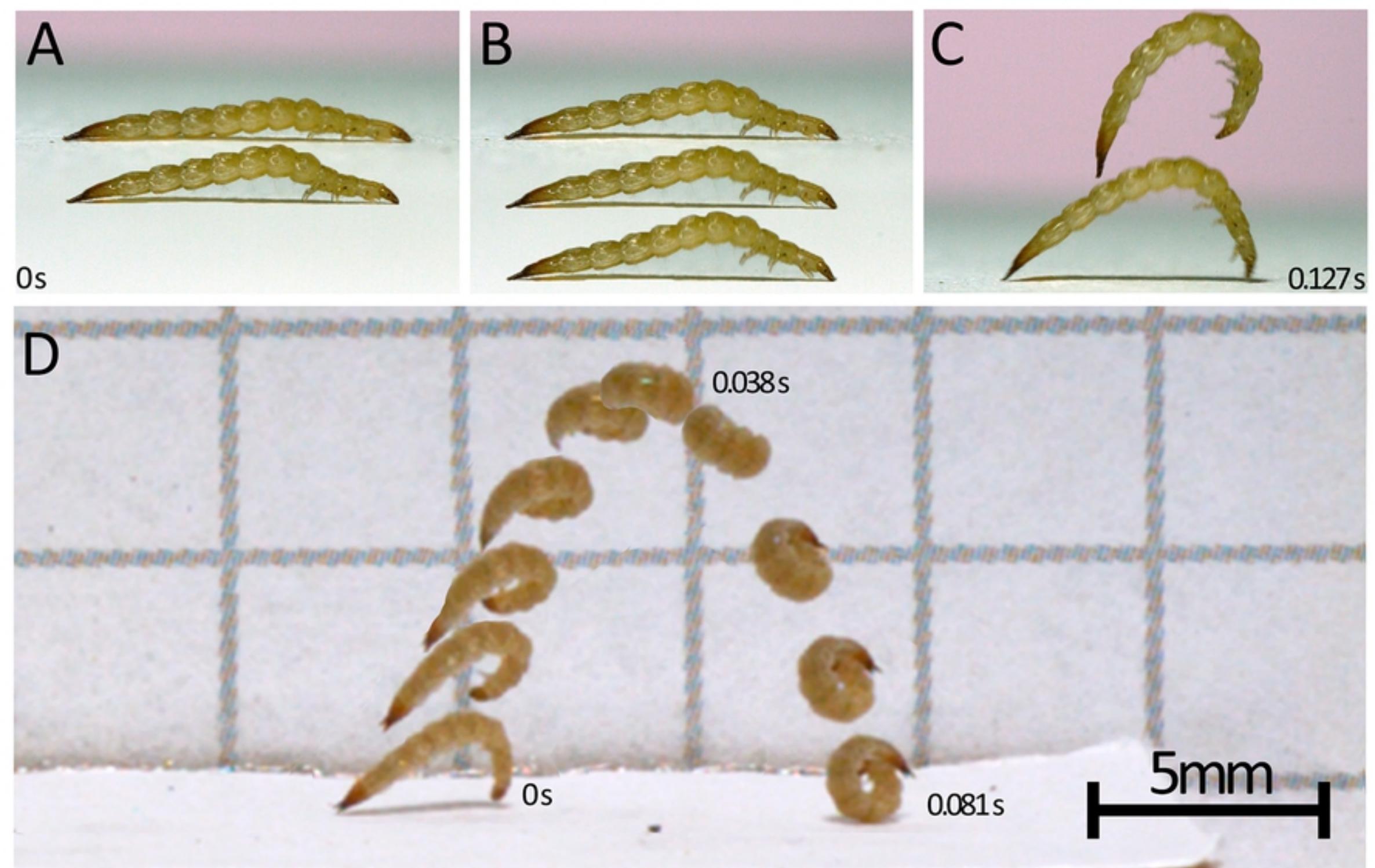


Figure 3

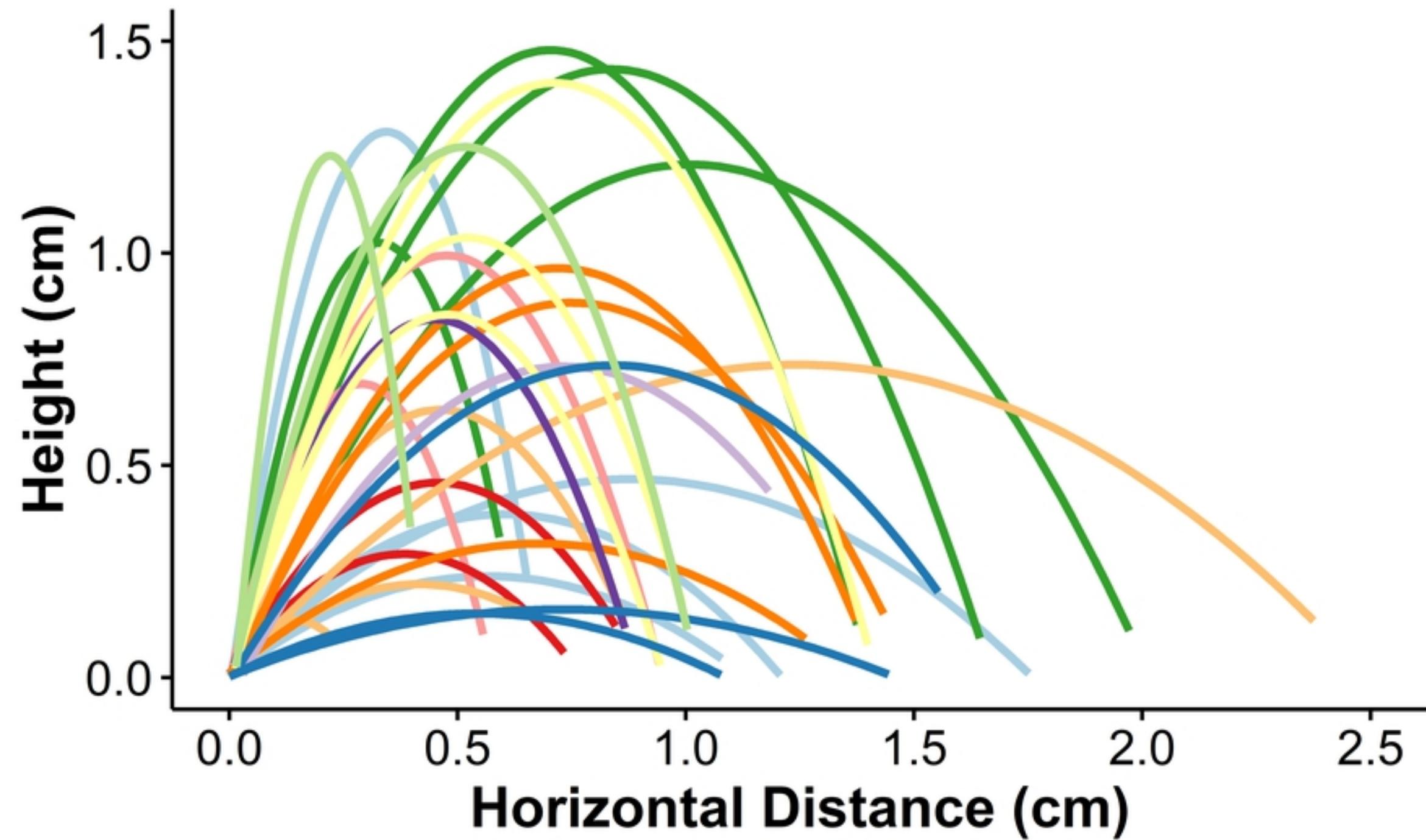


Figure 4