



Title	Exploration of the homology among the muscles associated with the female genitalia of the three suborders of Psocodea (Insecta)
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16 **ABSTRACT**

17 By using μ CT technology, we reconstructed 3D models of the female genital structures
18 and associated muscles of seven species from three suborders of Psocodea (free-living
19 species only, formerly known as independent insect order “Psocoptera”). The homology
20 of the female genital structures and associated muscles of different species is discussed.
21 A total of 21 muscle groups were observed, and except for one muscle, all were
22 homologized throughout the order. Moreover, some of the homologous muscles could
23 be identified confidently in holometabolan insects. Using the muscles as landmarks, we
24 discuss the homology of the ovipositor valves between Psocodea and other neopteran
25 insects. Most importantly, the ovipositor of the suborder Trogiomorpha was identified
26 to consist of the well-developed external valve (V3) plus a remnant of the dorsal valve
27 (V2). We also examined the phylogenetic information included in the female genital
28 muscles and found that certain muscles provide useful information and support deeper
29 nodes (e.g., monophyly of the suborder Psocomorpha). The present study of female
30 genital muscles not only helps us to better understand the phylogeny of Psocodea but
31 also provides a solid foundation for research on muscle evolution.

32 **KEYWORDS**

33 genitalia, muscles, Psocoptera, homology, phylogeny

34

35

36 1. INTRODUCTION

37 Insect genitalia are extremely diversified structures, and their morphology and
38 evolution have received great interest from variable perspectives (Boudinot, 2019),
39 such as taxonomic species recognition (e.g., Tuxen, 1970), lower- to higher-level
40 phylogenetic estimation (e.g., Yoshizawa & Johnson, 2006), selection forces acting on
41 these structures (e.g., Eberhard, 1985), or biomechanics (e.g., Matsumura *et al.*, 2017).
42 In most cases, male genital structures are the subject of these studies because male
43 genitalia are more strongly sclerotized than female genitalia, and their shapes and
44 variations are more easily observable and detectable. In contrast, insect female genitalia,
45 especially the structures having direct contact with male intromittent organ, are largely
46 membranous, and their importance has long been overlooked. However, recent studies
47 have shown that an accurate understanding of female genitalia, including the
48 membranous parts, is very important for elucidating genital evolution (Kamimura, 2016;
49 Muto, 2018; Simmons, 2019; Sloan, 2019; Genevcus, 2020; Onuma *et al.*, 2021) and
50 coupling mechanisms (Matsumura *et al.*, 2021).

51 Psocodea is one of the hemimetabolous insect orders composed of nonparasitic
52 barklice and booklice (formerly known as Psocoptera, hereafter called psocopterans)
53 and parasitic lice (Phthiraptera) (de Moya *et al.*, 2021). The order is subdivided into
54 three monophyletic suborders, Trogiomorpha, Troctomorpha (in which parasitic lice
55 are classified), and Psocomorpha. Over 6500 species in 41 families of psocopterans are
56 known to date (Lienhard & Smithers, 2002; Lienhard, 2016, 2021). As in the other

57 insect groups, male genitalia are the most important characters for diagnosing species
58 and recognizing some higher taxa (e.g., Smithers, 1972). Female genital characters are
59 also important taxonomically and phylogenetically. In particular, this character system
60 is known to be less homoplasious than male genital characters in estimating the
61 phylogenetic relationships of the suborder Psocomorpha (Yoshizawa & Johnson, 2014).
62 Moreover, two female genital apomorphies are the only morphological characters that
63 support the monophyly of the suborder Trogiomorpha (Yoshizawa & Lienhard, 2020).
64 These results suggest the potential importance of this character system for phylogenetic
65 estimations. However, ambiguities remain in the interpretation of homology among
66 certain key female genital structures; for example, simplified ovipositors of
67 Trogiomorpha have usually been interpreted as composed of external valves (Lienhard,
68 1998) but alternatively of ventral valves (Azar *et al.*, 2017).

69 In addition, female genitalia of Psocodea are of special interest. Recently, a penis-
70 like genital structure (termed gynosome) was discovered in females of the Brazilian
71 cave-dwelling psocopteran genus *Neotrogla* (Lienhard *et al.*, 2010). Subsequent studies
72 showed that the gynosome is inserted into the male genital chamber during copulation,
73 i.e., copulatory functions are completely reversed in *Neotrogla* (Yoshizawa *et al.*, 2014;
74 2019). This is a very prominent novel structure that has only been identified thus far in
75 this group of insects in the animal kingdom. However, the morphological origin and the
76 functional background of the coupling role reversals in *Neotrogla* are completely
77 unknown.

78 To elucidate the origin of the novel coupling structures and improve the utility of
79 the female genital character system for taxonomic, phylogenetic, evolutionary and
80 biomechanical studies, providing a detailed morphology and establishing a stable
81 homology interpretation of female genitalia, including both external and internal
82 structures, are essential. However, the detailed musculature related to female genital
83 structures has only been examined for a very limited number of species (Badonnel,
84 1934).

85 In this study, we examined the skeletal morphology and musculature of a wide
86 variety of psocopterans using the synchrotron micro-computed tomography (μ CT)
87 technique. Seven species from all three suborders were selected for examination. Based
88 on the examinations, we established a homology scheme of female genitalia throughout
89 psocopterans.

90

91 **2. MATERIALS AND METHODS**

92 **2. 1. Micro-CT imaging**

93 Seven species of psocopterans, *Prionoglaris stygia* Enderlein, 1909,
94 *Psyllipsocus clunijunctus* Lienhard, 2013 (Lienhard & Ferreira, 2013), *Lepinotus*
95 *reticulatus* Enderlein, 1904, Amphientomidae Gen. sp., *Archipsocus* sp., *Valenzuela*
96 *badiostigma* Okamoto, 1910 and *Trichadenotecnum pseudomedium* Yoshizawa, 2001
97 were examined (Table 1). All voucher specimens are stored in the Hokkaido University

98 Insect Collection. An uncopulated female of each species was used for μ CT
99 examination. Samples were fixed with FAA solution (formalin:alcohol:acetic acid =
100 6:16:1) or 80% ethanol and then preserved in 80% ethanol. Dehydration was conducted
101 in ascending order with 80–100% ethanol before drying them at the critical point (EM
102 CPD300, Leica, Wetzlar, Germany) to remove water without serious organ shrinkage.
103 Samples were then scanned using the synchrotron μ CT at the BL47XU (Uesugi *et al.*,
104 2012) beamline of the Super Photon ring-8 GeV (SPring-8; Hyogo, Japan) using a
105 stable beam energy of 8 keV in absorption-contrast mode. The tomography system
106 consists of a full-field X-ray microscope with Fresnel zone plate optics (Uesugi *et al.*,
107 2017). We used semiautomatic segmentation algorithms based on grey-value
108 differences in the software ITK-SNAP (Yushkevich *et al.*, 2006) to obtain 3D
109 representations of the terminalia of all six species.

110

111 **2. 2. Examination of phylogenetic signals**

112 We examined the phylogenetic significance of the female genital musculature by
113 reconstructing the character information on the phylogenetic tree that was previously
114 estimated using molecular and phylogenomic data (Yoshizawa *et al.*, 2006; Yoshizawa
115 & Johnson, 2014; Johnson *et al.*, 2018; de Moya *et al.*, 2021). The presence (coded as
116 1) or absence (0) of each muscle was used as the character state (Table 2), and this
117 dataset was parsimoniously reconstructed on the tree using MacClade version 4
118 (Maddison & Maddison, 2001). All characters were treated as unordered. Because the
119 homology of many muscles between psocopterans and outgroups was ambiguous (see

120 Discussion), no outgroup taxa were included in the analysis. Therefore, the character
121 changes at the deepest node (between Trogiomorpha and Troctomorpha + Psocomorpha)
122 could not be reconstructed.

123

124 **3. RESULTS**

125 **Skeletal and muscle structures of female terminalia.**

126 We describe the basic structure of the female terminalia and muscles related to them. We
127 grouped those muscles according to their origin as follows: muscles of the epiproct [ep];
128 paraproct [pa]; subgenital plate [sg]; ventral valve [ve]; dorsal valve [do]; external valve
129 [ex]; spermapore plate or the membrane surrounding it [sp]. The distribution of the
130 terminal muscles is summarized in Table 2.

131 Abbreviations: O – origin; I – insertion; and F – assumed function (based on
132 morphological conditions).

133 3. 1. Clunium

134 The clunium is composed of the fused tergites of segments IX and X and houses many
135 attachments of muscles originating from the epiproct, paraproct, gonapophyses,
136 subgenital plate, and spermapore plate (e.g. Fig. 7). Three valves of gonapophyses arise
137 from the ventral end of the clunium and articulate posteriorly with the epiproct and
138 paraproct.

139 3. 2. Epiproct and paraproct

140 The epiproct is a structure located dorsal to the anus. The paraproct is a paired structure
141 located ventrolateral to the epiproct and involves a reduced cercus (trichobothrial field).
142 These two structures are thought to represent segment XI and together surround the
143 anus. Males also have these two structures, usually with some characteristic protrusions
144 on them, which play an important role in the copulation process. The paraproct and
145 epiproct of females is usually simpler and less variable, with sparse bristles and no
146 protrusions (Fig. 7C, D).

147 Muscle originating from the epiproct

148 **01** epX01 (Fig. 2B); O: posterior end of the epiproct; I: mid-dorsal site of clunium
149 (segment IX); and F: closure and/or flipping of the epiproct.

150 This is the only muscle originating from the epiproct observed throughout Psocoptera.
151 However, this muscle is absent in *Prionoglaris* (Fig. 1B). In *Lepinotus*, additional
152 paired muscles (paX02: see below; Fig. 3B) are also inserted into the epiproct.

153 Muscles originating from the paraproct

154 **02** paX01 (Fig. 8); O: anterodorsal end of the paraproct, very close to the posterolateral
155 margin of epiproct; I: anterolateral region of clunium (segment X); and F: involved in
156 opening the paraproct.

157 **03** paX02 (Fig. 8); O: anterodorsal end of the paraproct, very close to the anterolateral

158 corner of epiproct; I: mediodorsal region of clunium (segment X); and F: involved in
159 opening the paraproct.

160 **04** paX03 (Fig. 9); O: internal margin of the paraproct near the anal opening; I:
161 mediolateral region of clunium (segment X); and F: involved in opening the paraproct.

162 **05** paX04 (Fig. 9); O: anterolateral margin of the paraproct; I: mediolateral region of
163 clunium (segment IX); and F: involved in restoring the paraproct.

164 **06** paX05 (Fig. 10); O: anteroventral end of the paraproct; I: anteroventral margin of
165 clunium (segment IX), near the base of the dorsal valve; and F: involved in opening the
166 paraproct.

167 The paX01–05 muscles are widely observed throughout psocopterans, although
168 paX03 is absent in *Prionoglaris*, *Lepinotus*, *Amphientomidae*, *Archipsocus* and
169 *Valenzuela* (Fig. 9), while paX05 is absent in *Psyllipsocus* (Fig. 2B).

170 **07** papa01 (Fig. 9); O: internal margin of the paraproct near the anal opening; I:
171 anterolateral margin of the paraproct; and F: involved in opening the anus.

172 **08** papa02 (Fig. 9); O: internal margin of the paraproct near the junction of the two
173 paraprocts; I: internal margin of the anteroventral region of the paraproct; and F:
174 involved in opening the anus.

175 The papa01 muscle is widely observed throughout psocopterans but is absent in
176 *Trichadenotecnum*. The papa02 muscle is only observed in *Prionoglaris* and coexists
177 with papa01 (Fig. 9A).

178

179 3. 3. Gonapophyses

180 The female gonapophyses arise from sternum VIII (ventral valve) or the IX segment
181 (dorsal and external valves), and the following three pairs of valves are tightly
182 associated to each other: the most external valve on the outside, the dorsal valve at the
183 middle and the most internal ventral valve on the inside. The shape and degree of
184 development vary greatly among different taxa. A reduction of the gonapophyses is
185 especially distinct in Trogiomorpha: in most members, the ventral and dorsal valves are
186 highly reduced or completely absent. In contrast, in *Valenzuela* (Psocomorpha), the
187 external valve is greatly reduced, although the ventral and dorsal valves are well
188 developed (Fig. 6A). In *Archipsocus* (Psocomorpha), all gonapophyses are greatly
189 reduced (Fig. 5A).

190 Muscle originating from the ventral valve

191 **09** veVII01 (Fig. 11A, B); O: base of the ventral valve; I: ventral region of segment
192 VII; and F: involved in opening the ventral valve.

193 This is the only muscle in psocopterans that originates from the ventral valve. The
194 muscle is observed in *Amphientomidae* and *Valenzuela* but is absent in all other taxa
195 examined here, which is partly due to the reduction of the ventral valve
196 (Trogiomorpha and *Archipsocus*). However, in *Trichadenotecnum*, this muscle is
197 absent, despite the species possessing a well-developed ventral valve.

198

199 Muscles originating from the dorsal valves

200 **10** dosp01 (Fig. 11C, D); O: base of the dorsal valve; I: on the membrane connected to

201 the spermapore plate, near the posterior tip of the spermapore plate; and F: involved in
202 restoring the position of the spermapore plate.

203 **11** doIX01 (Fig. 11C, D); O: anterior end of the dorsal valve; I: posterolateral margin
204 of segment IX; and F: involved in opening the dorsal valve.

205 These two muscles originate from the dorsal valve and are inserted into segment
206 IX at either the spermapore plate (dosp01) or the clunium (doIX01). They are only
207 observed in *Valenzuela* and *Trichadenotecnum* (Psocomorpha) but are absent in all
208 other taxa examined.

209

210 Muscles originating from the external valves

211 **12** exsp01 (Fig. 12); O: base of the external valve; I: on the membrane connected to the
212 spermapore plate, near the posterior tip of the spermapore plate, and partially
213 overlapping dosp01; and F: involved in restoring the position of the spermapore plate.

214 **13** exIX01 (Fig. 12); O: lateral margin of the external valve; I: posterolateral margin of
215 segment IX, and partially overlapping doIX01; and F: involved in opening the external
216 valve.

217 **14** exIX02 (Fig. 12); O: base of the external valve, near the middle of the junction
218 with the clunium; I: anterolateral margin of the clunium; and F: involved in opening
219 the external valve.

220 These three muscles originate from the external valve and are inserted on segment
221 IX at either the spermapore plate (exsp01) or the clunium (exIX01–02). These
222 muscles are widely observed throughout psocopterans but completely absent in

223 *Archipsocus* and *Valenzuela*. The exIX01 muscle is absent in *Prionoglaris*,
224 *Archipsocus* and *Valenzuela*. In addition, the exIX02 muscle is absent in Psocomorpha
225 (Fig. 12).

226

227 **15** doex01 (Fig. 11E, F); O: base of the dorsal valve; I: base of the external valve; and
228 F: involved in opening the dorsal valve.

229 This is the only muscle connecting two gonapophyses (dorsal and external)
230 observed in psocopteran. The muscle is only observed in *Lepinotus* (Trogiomorpha)
231 and Amphientomidae (Troctomorpha).

232

233 3. 4. Subgenital plate

234 The subgenital plate is formed by sternum VIII and covers the gonapophyses
235 ventrally. It is variable in shape between taxa, and it frequently bears a posterior
236 extension (egg guide). In contrast, the subgenital plate is greatly reduced in
237 Trogiomorpha and only covers the anterior part of the gonapophyses.

238 **16** sgVIII01 (Fig. 13); O: middle of the dorsal surface of the subgenital plate; I: middle
239 of the ventral surface of the egg guide; and F: involved in closing the subgenital plate

240 **17** sgVIII02 (Fig. 13); O: middle of the dorsal surface of the subgenital plate; I: anterior
241 margin of sternum VIII, and partially overlapping sgVIII01; and F: involved in opening
242 the subgenital plate.

243 These two groups of muscles originate from the subgenital plate and are only
244 observed in Psocomorpha. No muscles are attached on the subgenital plate in the
245 suborders Trogiomorpha and Troctomorpha. The subgenital plate of Trogiomorpha is
246 greatly reduced and the absence of the subgenital muscles may be partly linked to this
247 condition. The sgVIII02 muscle was present in all Psocomorpha taxa examined,
248 whereas sgVIII01 was absent in *Archipsocus* (Fig. 13).

249 3. 5. Spermapore plate

250 The spermapore plate is a sclerite bearing the spermapore (e.g., Figs. 1B and 12).
251 According to Bitsch (1979) the spermapore plate is situated on the posterior part of
252 sternum VIII (see Lienhard *et al.*, 2010). However, by observing the 3D modeling, in
253 most cases, the spermapore plate is located behind the subgenital plate, but is not
254 connected to it. The skeletal part of the spermapore plate is not connected to the
255 surrounding structures directly but is connected to the genital structures of the segment
256 IX through the spermapore membrane, so we judged it to be a part of the sternum IX.
257 Its degree of sclerotization is highly variable, and it is almost completely membranous
258 in some taxa (*Archipsocus* and *Valenzuela*; Figs. 5B and 6B). Its lateral margins loosely
259 articulate with surrounding structures (paraproct, gonapophyses, and subgenital plate)
260 via the spermapore membrane.

261 In addition to the muscles described below, the muscles dosp01 and exsp01, both
262 originating on the gonapophyses, are also associated with the membranous part of the
263 spermapore plate in many taxa examined (see above).

264 **18** spVIII01 (Fig. 14C, E); O: on the spermapore membrane; I: anteroventral margin
265 of segment VIII, near the junction of the clunium; and F: involved in restoring the
266 position of the spermapore plate.

267 This is the only muscle that originates from the spermapore plate; it is inserted
268 onto sternum VIII the examined if present. It is present in *Lepinotus* (Trogiomorpha)
269 and *Valenzuela* (Psocomorpha).

270 **19** spIX01 (Fig. 14); O: on the spermapore membrane; I: mediolateral region of the
271 clunium; and F: involved in restoring the position of the spermapore plate.

272 **20** spIX02 (Fig. 14); O: on the spermapore membrane; I: mediolateral region of the
273 clunium, and partially overlapping with exIX01; and F: involved in restoring the
274 position of the spermapore plate.

275 **21** spIX03 (Fig. 14); O: the membranous part of the spermapore plate, near the
276 internal part of the dorsal valve; I: anterolateral margin of segment IX, and partially
277 overlapping with doVIII02 and exIX02; and F: involve in stretching the spermapore
278 plate.

279 The spIX03 muscle is often present but missing in *Prionoglaris* and
280 Amphientomidae. In contrast, muscle spIX02 is restricted to Trogiomorpha and spIX01
281 was only found in *Psyllipsocus* and *Lepinotus*.

282

283 **4. DISCUSSION**

284 **4. 1. Homology of the muscles**

285 Apart from the ovipositor valves (discussed below), the homology of the female
286 terminal sclerites (i.e., clunium, epiproct, paraproct, subgenital plate and spermapore
287 plate) is almost unambiguously identifiable throughout psocopterans (Figs. 1–7).
288 Therefore, based on the origin and insertion points of each muscle, the homology of the
289 female genital muscles of psocopterans can be established confidently throughout the
290 order (Table 2). Badonnel (1934) also examined the female genital musculature of
291 *Stenopsocus stigmaticus*, a close relative of *Valenzuela* examined here, and the muscles
292 that he observed can be homologized with the muscles identified here (see also the
293 ovipositor section below). However, the homology of several muscles is debatable.

294 For example, paX02 (Fig. 8) was consistently observed throughout psocopterans
295 and almost always originates from the dorsal margin of the paraproct, very close to the
296 lateral margin of the epiproct. However, in *Lepinotus*, the site of origin of the
297 corresponding muscle is changed to the epiproct (Fig. 3B). Therefore, judging only
298 from origin, this muscle may be regarded as an epiproctal muscle. However, in
299 *Lepinotus*, no other muscle corresponding to paX02 could be detected. In addition, its
300 site of origin is very close to that of paX02 observed in other psocopterans, and its
301 insertion site is consistent with that of paX02. Therefore, although its origin and
302 probably also its function are different from that of paX02 in other psocopterans, the
303 muscle originating from the lateral margin of the epiproct of *Lepinotus* was identified
304 here as paX02 (Table 2).

305 A similar situation was observed for spIX02 (Fig. 14A–C), which only occurs in

306 Trogiomorpha. The insertion site of the muscle in *Psyllipsocus* and *Lepinotus* is the
307 mediolateral region of the clunium. The spIX02 muscle of *Lepinotus* consists of a pair
308 of bundles, whereas spIX02 of *Prionoglaris* and *Psyllipsocus* consists of a single bundle.
309 Moreover, the insertion site of spIX02 of *Prionoglaris* is the middle region of the
310 external valve. Except for spIX02, no other muscles of *Prionoglaris* are connected to
311 the spermapore membrane, and no muscles similar to those on the external valve are
312 found in other psocopteran. In *Prionoglaris*, the external valve is significantly enlarged
313 and thus expands dorsally in comparison to other trogiomorphan genera. The
314 orientation of the muscles (horizontally directed towards its origin on the spermapore)
315 is similar in *Prionoglaris* and *Psyllipsocus/Lepinotus*. Therefore, the muscle originating
316 from the spermapore plate and inserted on the middle region of the external valve of
317 *Prionoglaris* was identified as spIX02 (Table 2).

318 In addition, some muscles are confirmed only in few distantly related taxa. For
319 example, veVII01 (Fig. 11A, B) was only observed in Amphientomidae (Troctomorpha)
320 and *Valenzuela* (Psocomorpha), and doex01 (Fig. 11E, F) was only found in *Lepinotus*
321 (Trogiomorpha) and Amphientomidae (Table 2). As discussed below (see the
322 phylogenetic significance section), independent gains of these muscles are the most
323 parsimonious interpretation (i.e., Fig. 15), but corresponding muscles are also observed
324 in some holometabolans (Table 2: Hünefeld *et al.*, 2012). It is generally regarded that
325 the loss of a character occurs much more frequently than the independent gain of a
326 novel feature (e.g., Trueman *et al.*, 2004). Therefore, although this remains ambiguous,
327 we tentatively recognized the muscles only observed in distantly related psocopteran

328 taxa and holometabolans as homologous (Table 2), suggesting multiple loss rather than
329 more parsimonious independent gain.

330 Homology identification of the female genital muscles between psocopterans and
331 other insects is complicated. Psocopterans are traditionally included in the superorder
332 Paraneoptera (this view is still most strongly supported morphologically and also could
333 not be rejected also by phylogenomics: Hennig, 1969; Yoshizawa & Lienhard 2016;
334 Johnson *et al.*, 2018), but the order Psocodea was interpreted as the sister taxon of the
335 Holometabola by phylogenomics (Misof *et al.*, 2014; Johnson *et al.*, 2018). The ground
336 plan condition of the female genital musculature in Holometabola has been estimated
337 by Hünefeld *et al.* (2012). Therefore, we tried to homologize the psocopteran female
338 genital muscles according to the system of Hünefeld *et al.* (2012). As shown in Table
339 2, homology of only a few muscles (e.g., epiproctal epX01 muscle) can be identified
340 confidently between psocopterans and Holometabola. In contrast, although
341 homologous muscles are probably present in Holometabola (e.g., paraproctal muscles
342 paX01–05), the homology of many psocopteran muscles cannot be specified with
343 reasonable certainty, for which more than one candidate homologous muscle is
344 indicated in Table 2. Examination of the other paraneopteran and holometabolan taxa
345 may improve these ambiguities.

346 **4. 2. Homology of the psocopteran ovipositor valves**

347 In the neopteran ground plan condition, the ovipositor is composed of three sets of
348 elongated valves derived from postabdominal appendages, namely, V1 (segment VIII),

349 V2 and V3 (both segment IX). Frequently, a short valvifer (or gonocoxite) is also
350 present basal to the valves of each segment (VIII and IX) (Beutel *et al.*, 2014).

351 The psocopteran ovipositor valves are also usually composed of three valves,
352 namely, ventral (segment VIII), dorsal and external valves (both segment IX), which
353 are generally considered to be homologous to V1–V3 of the neopterans groundplan.
354 When all valves are well developed, they can be addressed very clearly. Even though
355 the dorsal and external valves are fused with each other, they can be clearly identified
356 as elongated dorsal valve and basally bulged external valve (e.g., Yoshizawa, 2005). In
357 contrast, the valvifer is never developed as a separated sclerite in psocopterans.

358 Hünefeld *et al.* (2012) examined the musculature of the female postabdomen of a
359 wide variety of taxa and estimated the ground plan of the abdominal musculature of the
360 Holometabola. According to the interpretation presented in Hünefeld *et al.* (2012), all
361 intersegmental and dorsoventral muscles associated with the ovipositor valves are
362 inserted on the valvifer (i.e., the coxal segment of the appendage).

363 In psocopterans, one intersegmental muscle (veVII01: homologous to the muscle
364 “c” of Badonnel, 1934: fig. 69) is inserted to the base of the ventral valve (segment VIII:
365 Fig. 11A, B). This muscle is apparently homologous to either muscle 08 or 09
366 (intersegment VII) of Hünefeld *et al.* (2012) (Table 2), suggesting that the psocopteran
367 ventral valve is composed of fused valvifer VIII and V1. In psocopteran segment IX,
368 three dorsoventral muscles are observed (Figs 11C, D and 12): one is inserted on the
369 dorsal valve (doIX01: muscle “e” of Badonnel, 1934) and the other two on the base of

370 the external valve (exIX01 and 02: muscle “f” of Badonnel, 1934). They very likely
371 correspond to either the muscles 50, 51, 52 or 53 (dorsoventral IX) of Hünefeld *et al.*
372 (2012), which are usually inserted on valvifer IX in neopterans. Therefore, it is also
373 likely that the psocopteran dorsal and external valves are possibly a composite structure
374 formed by fusion of V2, V3, and valvifer IX.

375 In addition, a muscle named doex01 was observed in *Lepinotus* and
376 Amphientomidae, which connect the 65s (Fig. 11E, F). In the holometabolan ground
377 plan condition, two muscles connecting valvifers IX and V2 (57 and 58) are present
378 (Hünefeld *et al.*, 2012), and doex01 is very likely homologous to either 57 or 58 of
379 Hünefeld *et al.* (2012) (Table 2). In Amphientomidae (with three well-developed
380 valves), this muscle originates from the base of the dorsal valve and is inserted to the
381 external margin of the external valve (Fig. 11F). It also provides additional support that
382 valvifer IX is fused to V3 in psocopterans, together forming the external valve.

383 The presence of doex01 also provides interesting insights for the morphological
384 interpretation of ovipositor valves in psocopterans. In the suborder Trogiomorpha, they
385 are greatly reduced and are usually represented by a single pair of valves (Figs 1–3). It
386 is generally assumed that these single pair of valves are homologous to the external
387 ones. However, the presence of the doex01 muscle (= either 57 or 58 of Hünefeld *et al.*,
388 2012) strongly suggests that the well-developed valve of *Lepinotus* involves at least a
389 vestigial element of the dorsal valve as the insertion site of the muscle doex01 (Fig.
390 11E).

391 Different morphological interpretations for the female ovipositor valves were also
392 proposed for the mid-Cretaceous trogiomorphan genus *Palaeosiamoglaris* by Azar *et*
393 *al.* (2017). *Palaeosiamoglaris* is thought to be most closely related to the extant genus
394 *Siamoglaris* from Thailand, and they together form a monophyletic subfamily
395 *Prionoglaridinae* with *Prionoglaris*. The basic female genital structures of *Siamoglaris*
396 and *Prionoglaris* (examined here) are very similar. Azar *et al.* (2017) identified the most
397 well-developed ovipositor valve of *Palaeosiamoglaris* as the ventral valve, and opaque
398 structures presented next and dorsal to these structures were identified as external and
399 dorsal valves, respectively (Azar *et al.*, 2017: figs 5B, 15B). However, as discussed
400 above, the most distinct ovipositor valve of *Prionoglaris* and other trogiomorphan taxa
401 should be homologized with the external valve (with some vestigial element of the
402 dorsal valve), and reduction of the ventral and dorsal valves should be considered as an
403 autapomorphy of Trogiomorpha (Yoshizawa & Lienhard, 2020). As mentioned above,
404 *Palaeosiamoglaris* is placed "within" a subfamily of a trogiomorphan family and is
405 very closely related to *Prionoglaris*. Under such conditions, it is less likely that a well-
406 developed ventral valve is retained in *Palaeosiamoglaris*; thus, the structure is
407 interpreted here as the external valve (plus some vestigial element of a dorsal valve), as
408 also seen in *Prionoglaris*.

409 **4. 3. Phylogenetic significance of the musculature**

410

411 The data presented in Table 2 were treated as a character state matrix and
412 reconstructed on the phylogenetic tree of psocopterans estimated based on multiple

413 genes (Yoshizawa *et al.*, 2006; Yoshizawa & Johnson, 2014) or transcriptomes (Johnson
414 *et al.*, 2018; de Moya *et al.*, 2021) to visualize the phylogenetic signal contained in the
415 female genital musculature (Fig. 15). The consistency index (CI) was calculated as 0.69,
416 and the retention index (RI) was 0.53. This CI value is relatively high compared to other
417 morphological character system used for the phylogenetic reconstruction of
418 Psocomorpha, e.g., 0.41 for the total morphology, 0.36 for male external genitalia, and
419 0.45 for female external genitalia (Yoshizawa & Johnson, 2014), suggesting that the
420 characteristic system includes useful phylogenetic signals with relatively low levels of
421 homoplasies.

422 The most parsimonious reconstruction of the character matrix on the tree yielded
423 some useful characters supporting deep psocopteran phylogenetic splits (Fig. 15): the
424 absence of exIX01 (14) and the presence of sgVIII01 (16) support the monophyly of
425 the suborder Psocomorpha, and the presence of dosp01 (10), doIX01 (11) and sgVIII02
426 (17) (all non-homoplasious) support the monophyly of Psocomorpha excluding
427 Archipsocetae. In addition, the condition of spIX02 (20) was consistently different
428 between Trogiomorpha (present) and Troctomorpha + Psocomorpha (absent) (Table 2).
429 Therefore, this muscle likely contains a signal to support either of these clades
430 (depending on the condition of the outgroup, but if the reduction of muscles is a general
431 tendency, this may rather support Troctomorpha + Psocomorpha: Table 2). Some
432 homoplasious and nonhomoplasious character states are only identified at the terminal
433 branches (i.e., phylogenetically noninformative) (Fig. 15). However, with denser
434 taxonomic sampling, these characters may be phylogenetically informative on a lower

435 taxonomic level (i.e., infraorder, family, or genus).

436 It has to be noted that the female genital musculature may contain more
437 homoplasies than discussed above. As already mentioned, independent losses of a
438 character are generally considered to be much more frequent than independent gains of
439 a novel feature. The present results suggested several independent gains of new muscles
440 (Fig. 15), for which the alternative interpretation (multiple loss of the muscles) should
441 also be considered. For example, exIX01 is absent in *Valenzuela* but was observed in
442 *Stenopsocus* (a close relative of *Valenzuela*) by Badonnel (1934: fig. 69). Although the
443 ancestral condition of this muscle is ambiguous for *Valenzuela* (Fig. 15), the presence
444 of exIX01 in *Stenopsocus* strongly suggests that the absence of this muscle is an
445 apomorphic condition independently derived in *Prionoglaris*, *Archipsocus*, and
446 *Valenzuela*. Much denser sampling of psocopterans is needed to accurately identify the
447 evolutionary trend of the female genital muscles. The present study provides the
448 foundation for such a future project.

449

450

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459

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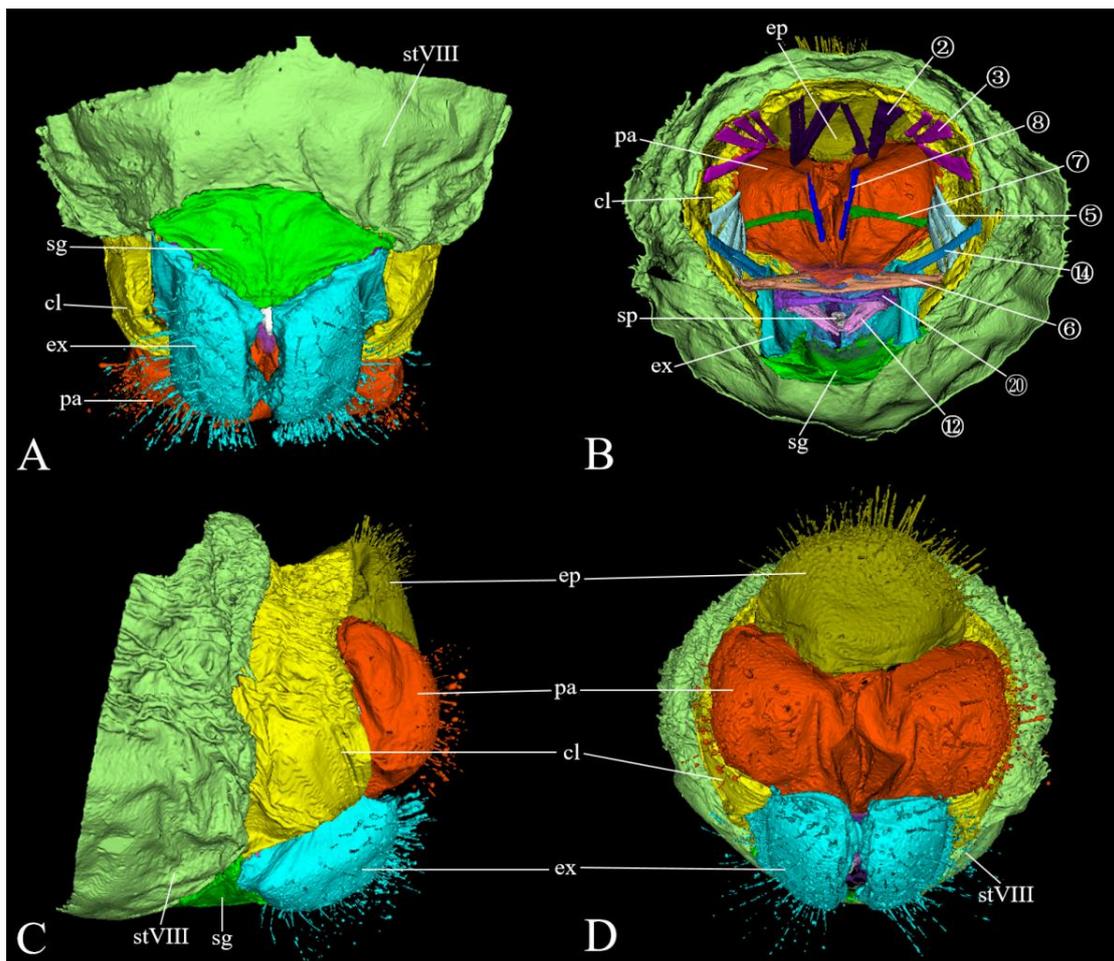
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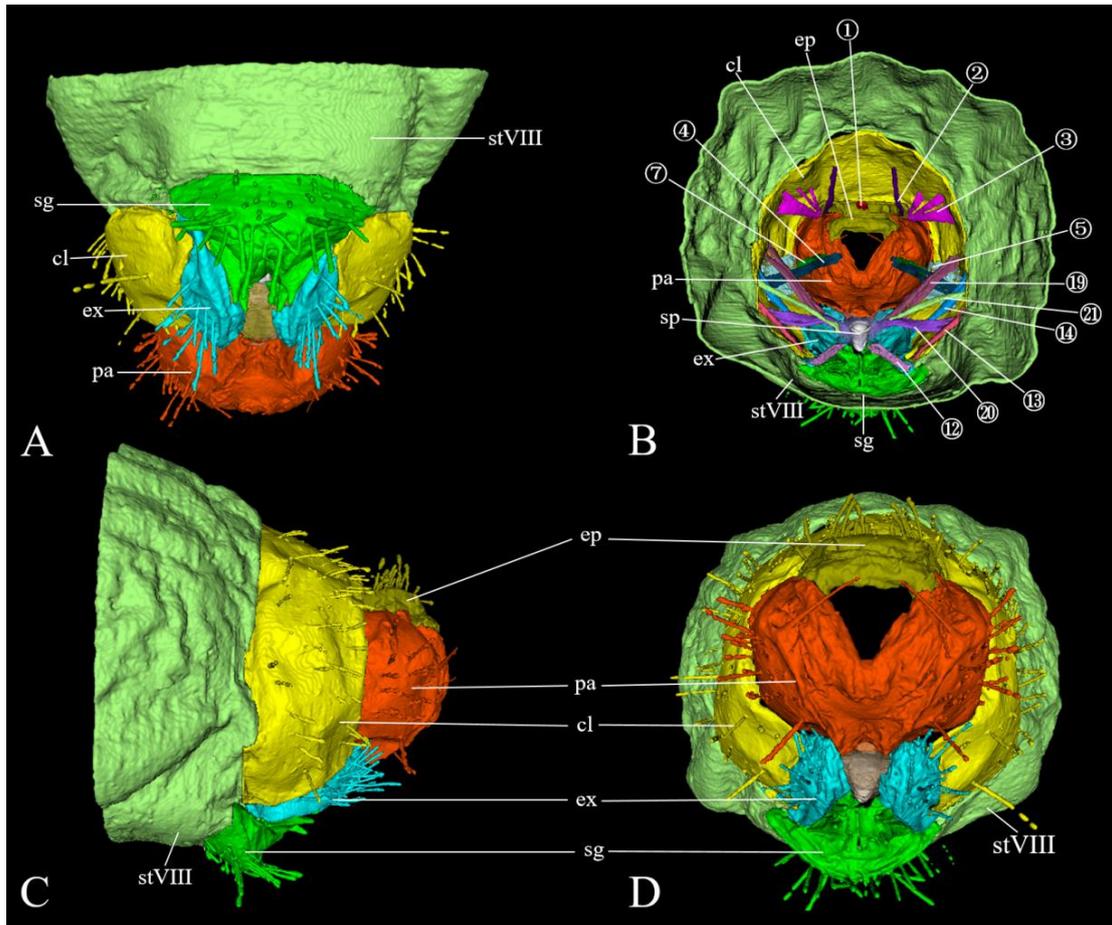


575

576 **Fig. 1.**

577 3D reconstruction of the female terminalia of *Prionoglaris stygia*: (A) ventral view; (B)

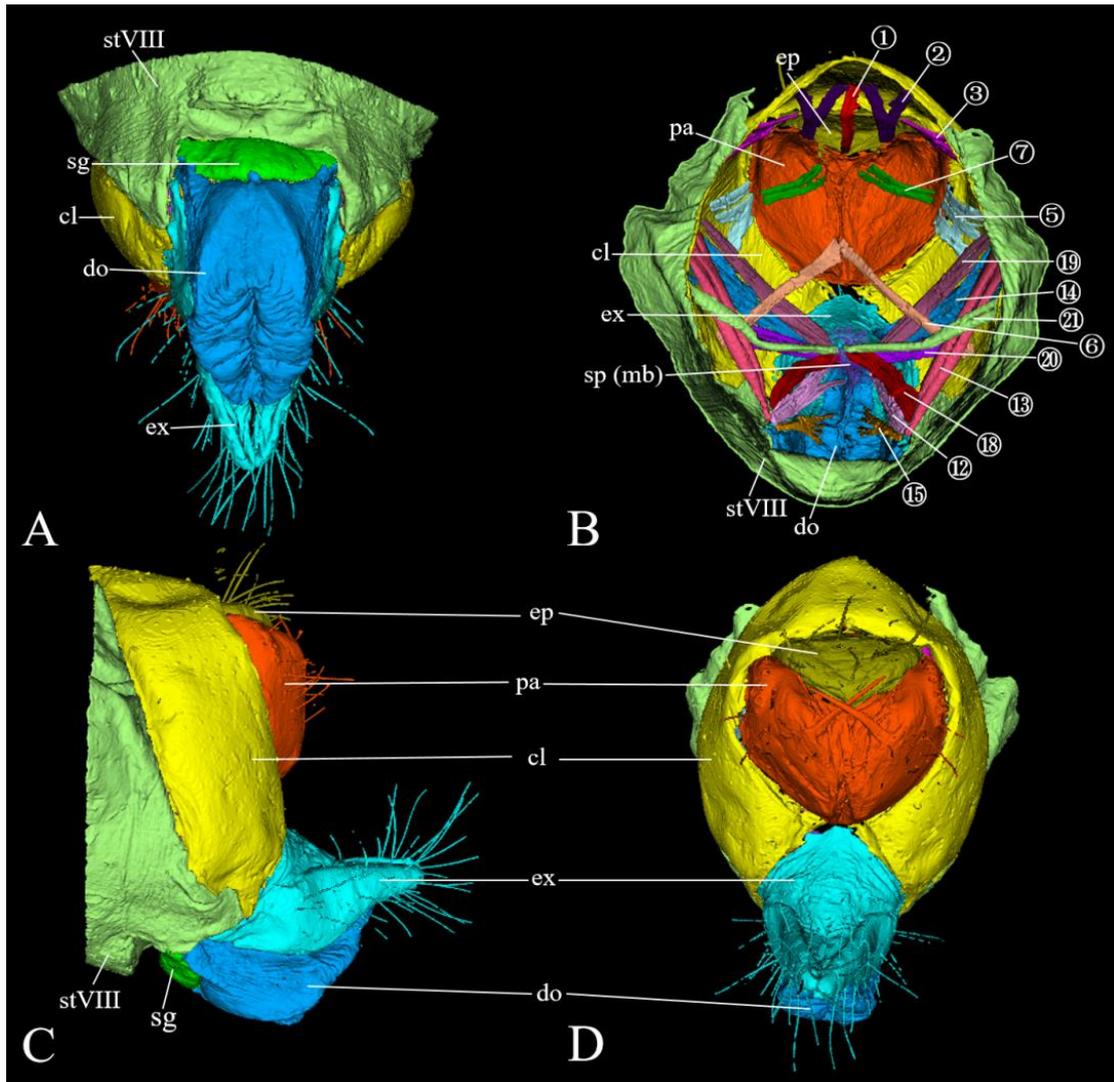
578 internal view: (2) paX01; (3) paX02; (5) paX04; (6) paX05; (7) papa01; (8) papa02;
 579 (12) exsp01; (14) exIX02; (20) spIX02; (C) lateral view; and (D) posterior view.
 580 Abbreviations: cl = clunium; ex = external valve; sg = subgenital plate; ep = epiproct;
 581 pa = paraproct; sp = spermapore plate; mb = membrane; st = sternum.



582

583 **Fig. 2.**

584 3D reconstruction of the female terminalia of *Psyllipsocus clunijunctus*: (A) ventral
 585 view; (B) internal view: (1) epX01; (2) paX01; (3) paX02; (4) paX03; (5) paX04; (7)
 586 papa01; (12) exsp01; (13) exIX01; (14) exIX02; (19) spIX01; (20) spIX02; (21) spIX03;
 587 (C) lateral view; and (D) posterior view. See Fig. 1 for abbreviations.



588

589 **Fig. 3.**

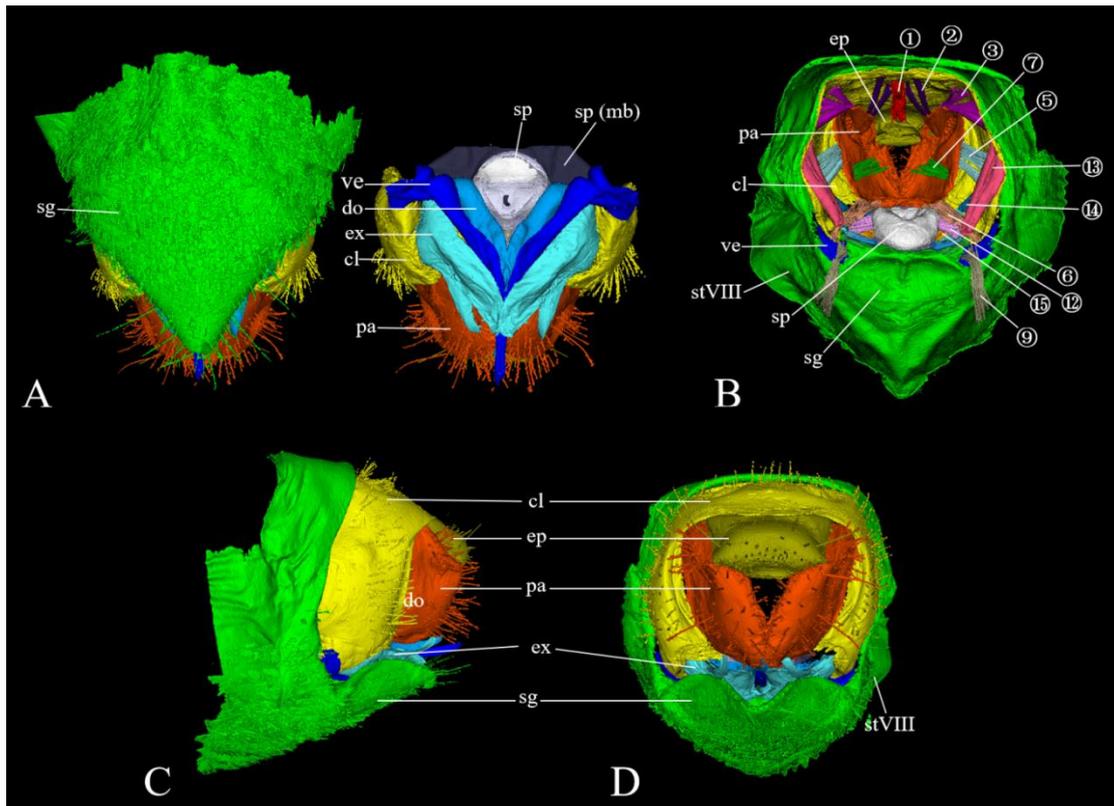
590 3D reconstruction of the female terminalia of *Lepinotus reticulatus*: (A) ventral view;

591 (B) internal view: (1) epX01; (2) paX01; (3) paX02; (5) paX04; (6) paX05; (7) papa01;

592 (12) exsp01; (13) exIX01; (14) exIX02; (15) doex01; (18) spVIII01; (19) spIX01; (20)

593 spIX02; (21) spIX03; (C) lateral view; and (D) posterior view. See Fig. 1 for

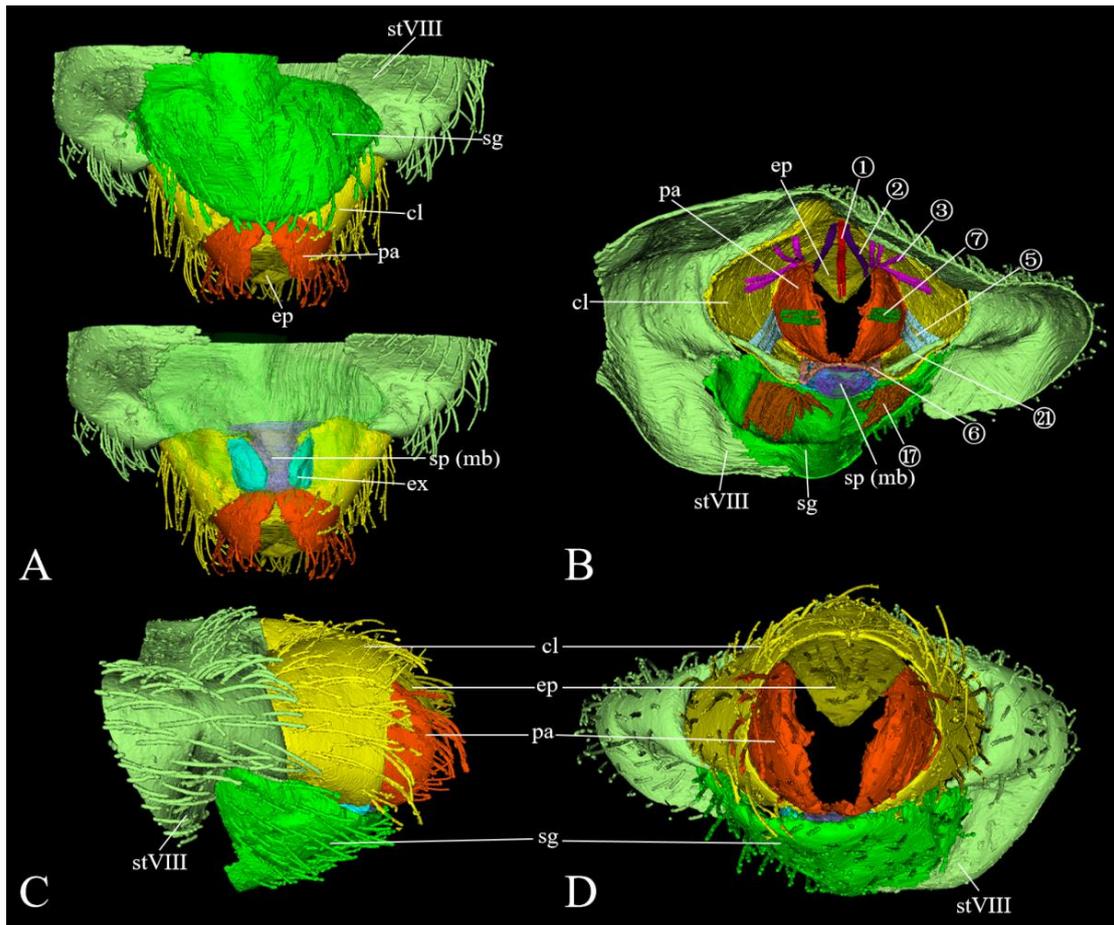
594 abbreviations.



595

596 **Fig. 4.**

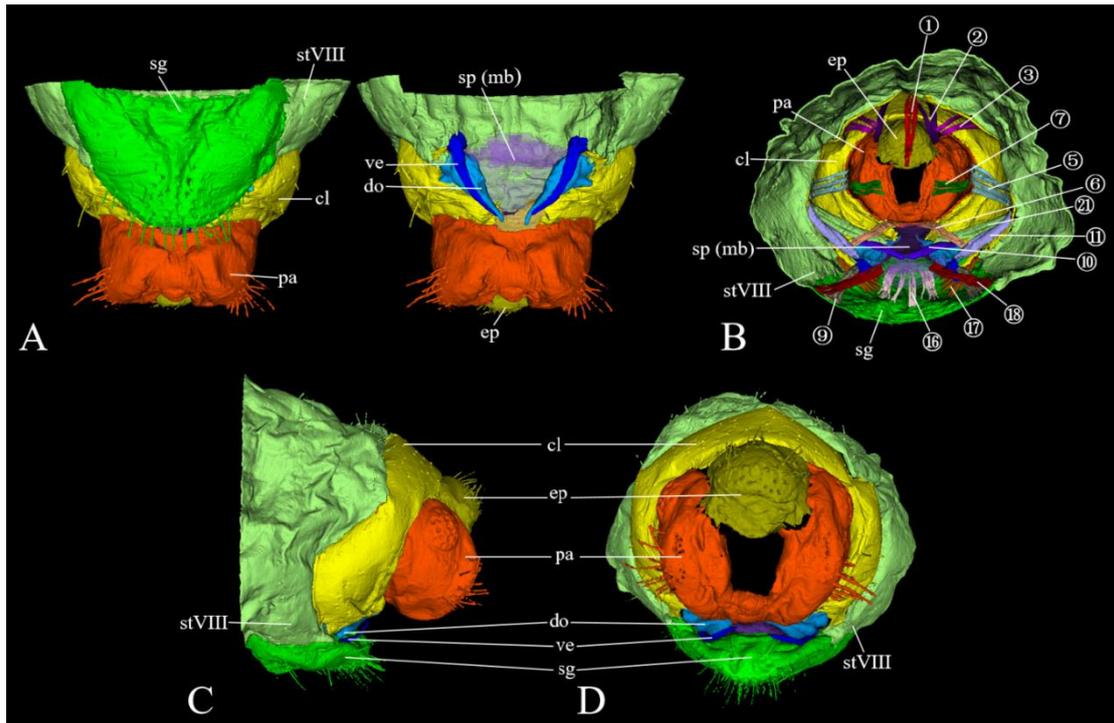
597 3D reconstruction of the female terminalia of Amphientomidae Gen. sp.: (A) ventral
 598 view (right: the subgenital plate is invisible); (B) internal view: (1) epX01; (2) paX01;
 599 (3) paX02; (5) paX04; (6) paX05; (7) papa01; (9) veVII01; (12) exsp01; (13) exIX01;
 600 (14) exIX02; (15) doex01; (C) lateral view; and (D) posterior view. See Fig. 1 for
 601 abbreviations.



602

603 **Fig. 5.**

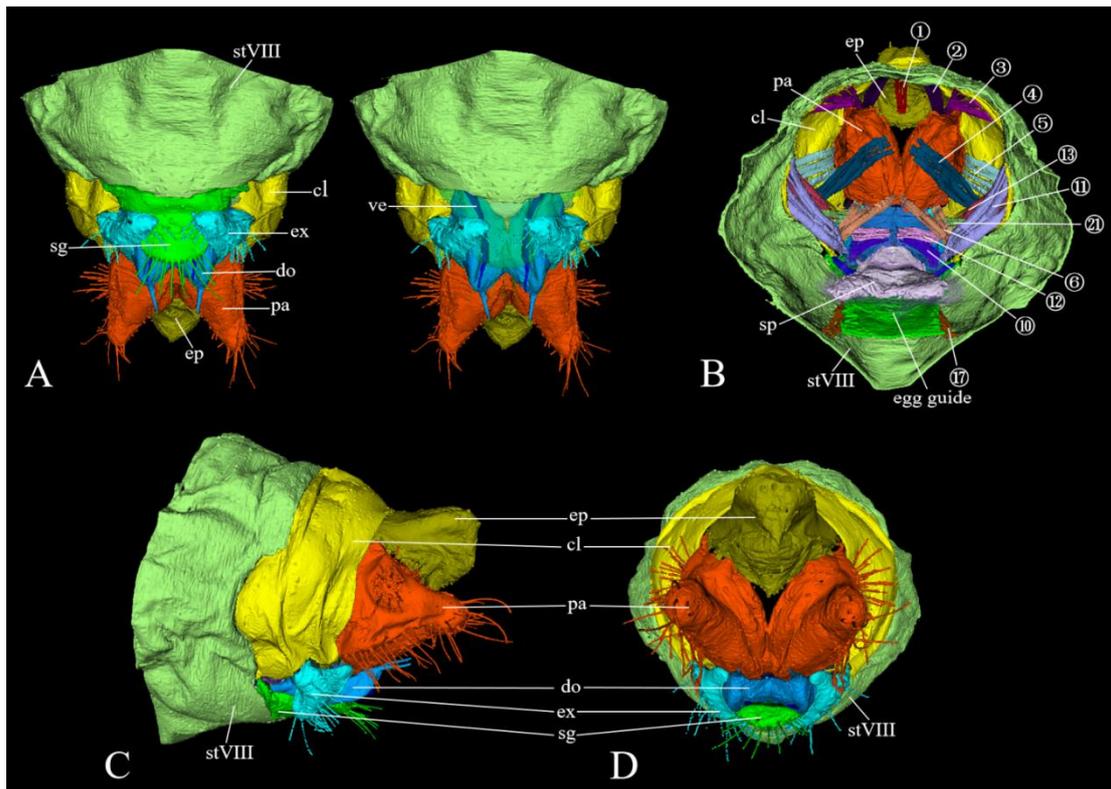
604 3D reconstruction of the female terminalia of *Archipsocus* sp.: (A) ventral view (below:
 605 the subgenital plate is invisible); (B) internal view: (1) epX01; (2) paX01; (3) paX02;
 606 (5) paX04; (6) paX05; (10) dosp01; (11) doIX01; (12) exsp01; (13) exIX01; (17)
 607 sgVIII02; (21) spIX03; (C) lateral view; and (D) posterior view. See Fig. 1 for
 608 abbreviations.



609

610 **Fig. 6.**

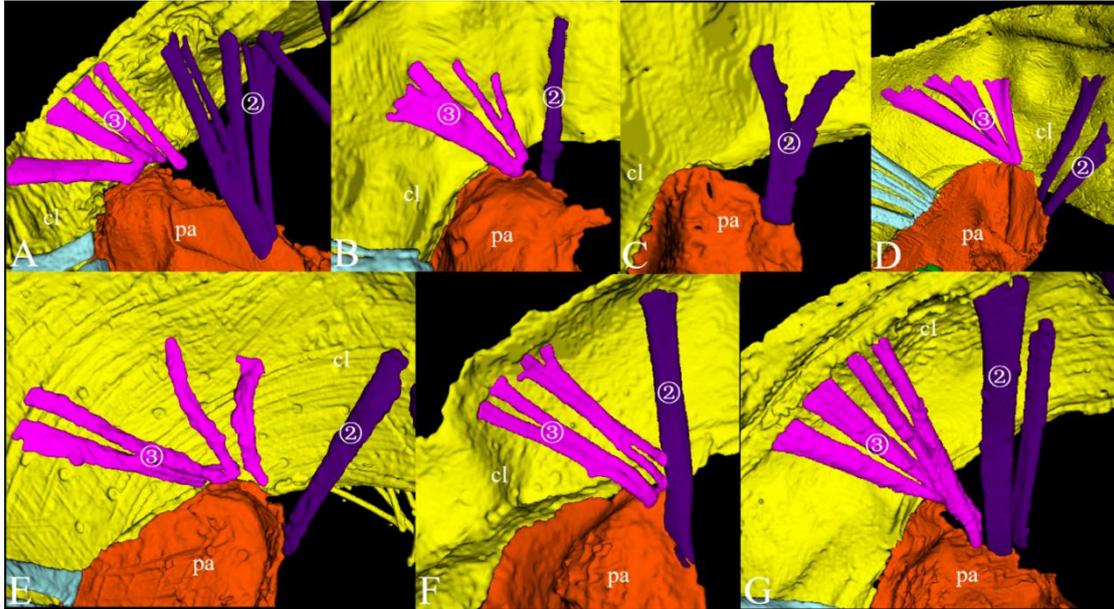
611 3D reconstruction of the female terminalia of *Valenzuela badiostigma*: (A) ventral view
 612 (right: the subgenital plate is invisible); (B) internal view: (1) epX01; (2) paX01; (3)
 613 paX02; (5) paX04; (6) paX05; (7) papa01; (9) veVII01; (10) dosp01; (11) doIX01; (16)
 614 sgVIII01; (17) sgVIII02; (18) spVIII01; (21) spIX03; (C) lateral view; and (D) posterior
 615 view. See Fig. 1 for abbreviations.



616

617 **Fig. 7.**

618 3D reconstruction of the female terminalia of *Trichadenotecnum pseudomedium*: (A)
 619 ventral view (right: subgenital plate is invisible); (B) internal view: (1) epX01; (2)
 620 paX01; (3) paX02; (4) paX03; (5) paX04; (6) paX05; (10) dosp01; (11) doIX01; (12)
 621 exsp01; (13) exIX01; (17) sgVIII02; (21) spIX03; (C) lateral view; and (D) posterior
 622 view. See Fig. 1 for abbreviations.



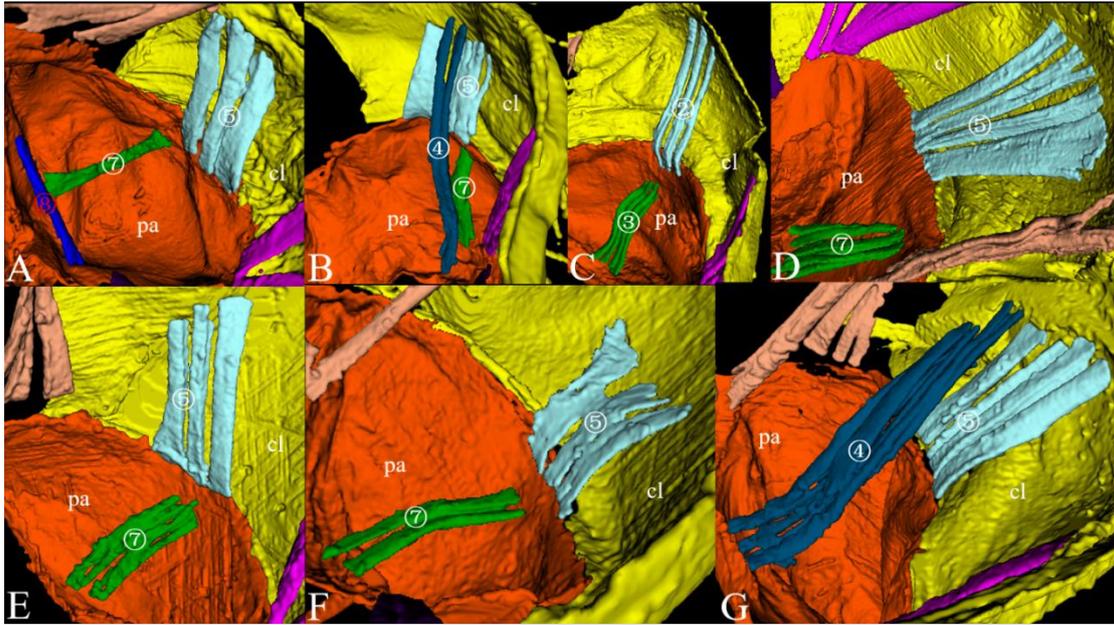
623

624 **Fig. 8.**

625 Close up of the paraproct (dorsal part) and clunium, internal view: (A) *Prionoglaris*
 626 *stygia*; (B) *Psyllipsocus clunijunctus*; (C) *Lepinotus reticulatus*; (D) Amphientomidae
 627 Gen. sp.; (E) *Archipsocus* sp.; (F) *Valenzuela badiostigma*; and (G) *Trichadenotecnum*
 628 *pseudomedium*. (2) paX01; (3) paX02. See Fig. 1 for abbreviations.

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631

632 **Fig. 9.**

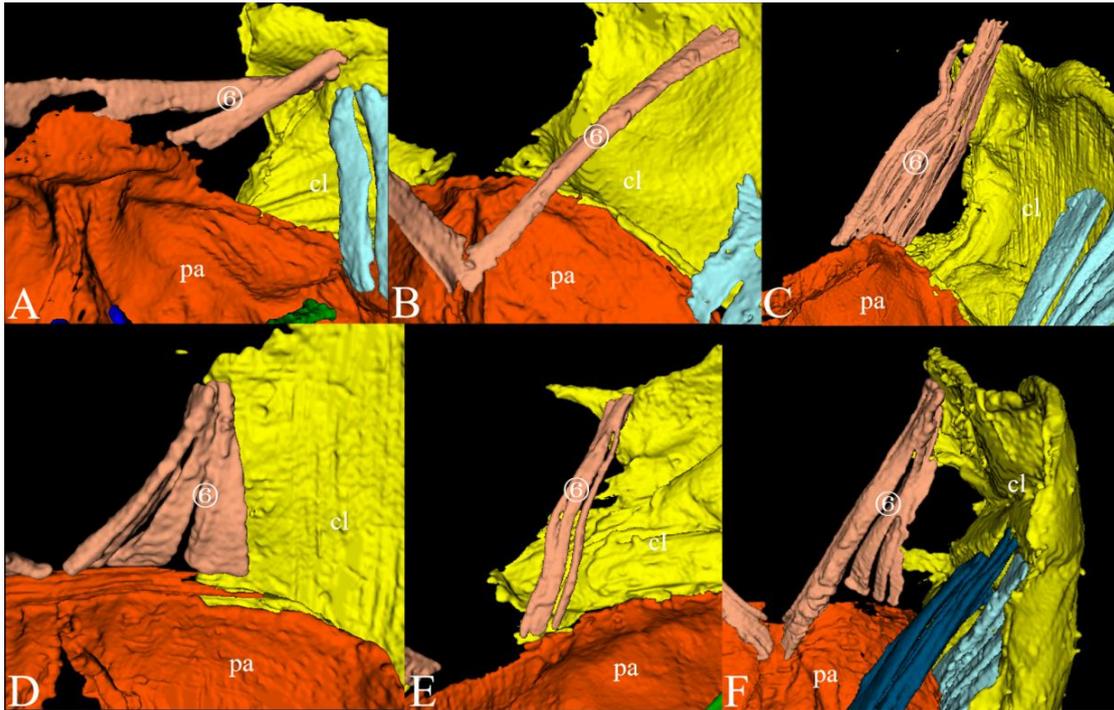
633 Close up of the paraprot (lateral part) and clunium, internal view: (A) *Prionoglaris*

634 *stygia*; (B) *Psyllipsocus clunijunctus*; (C) *Lepinotus reticulatus*; (D) Amphientomidae

635 Gen. sp.; (E) *Archipsocus* sp.; (F) *Valenzuela badiostigma*; and (G) *Trichadenotecnum*

636 *pseudomedium*. (4) paX03; (5) paX04; (7) papa01; (8) papa02. See Fig. 1 for

637 abbreviations.



638

639 **Fig. 10.**

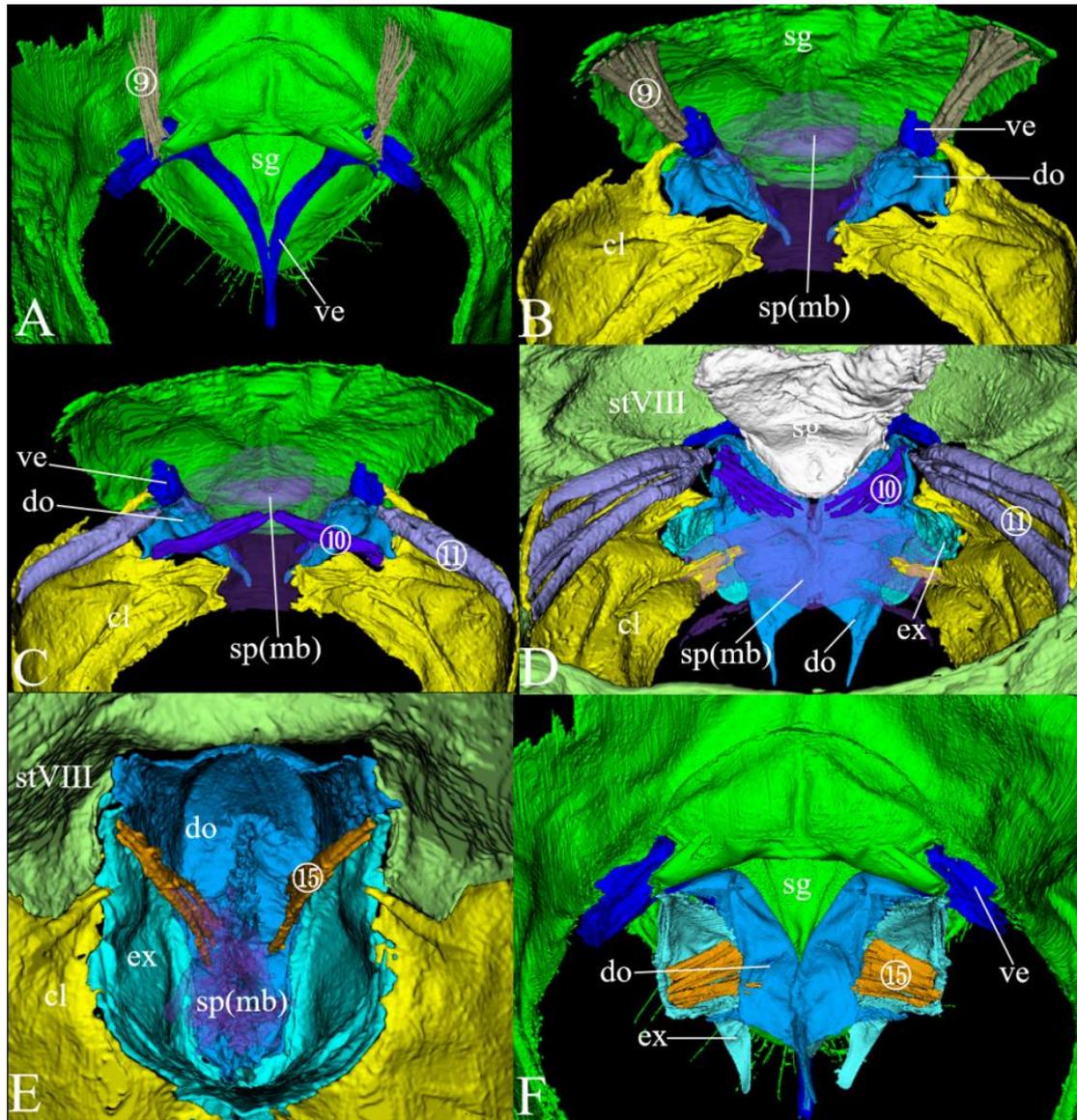
640 Close up of the paraproct (ventral part) and clunium, internal view: (A) *Prionoglaris*

641 *stygia*; (B) *Lepinotus reticulatus*; (C) Amphientomidae Gen. sp.; (D) *Archipsocus* sp.;

642 (E) *Valenzuela badiostigma*; and (F) *Trichadenotecnum pseudomedium*. (6) paX05.

643 Abbreviations: cl = clunium; pa = paraproct.

644



645

646 **Fig. 11.**

647 Close-up of the gonapophyses and related structures, internal view: (A)

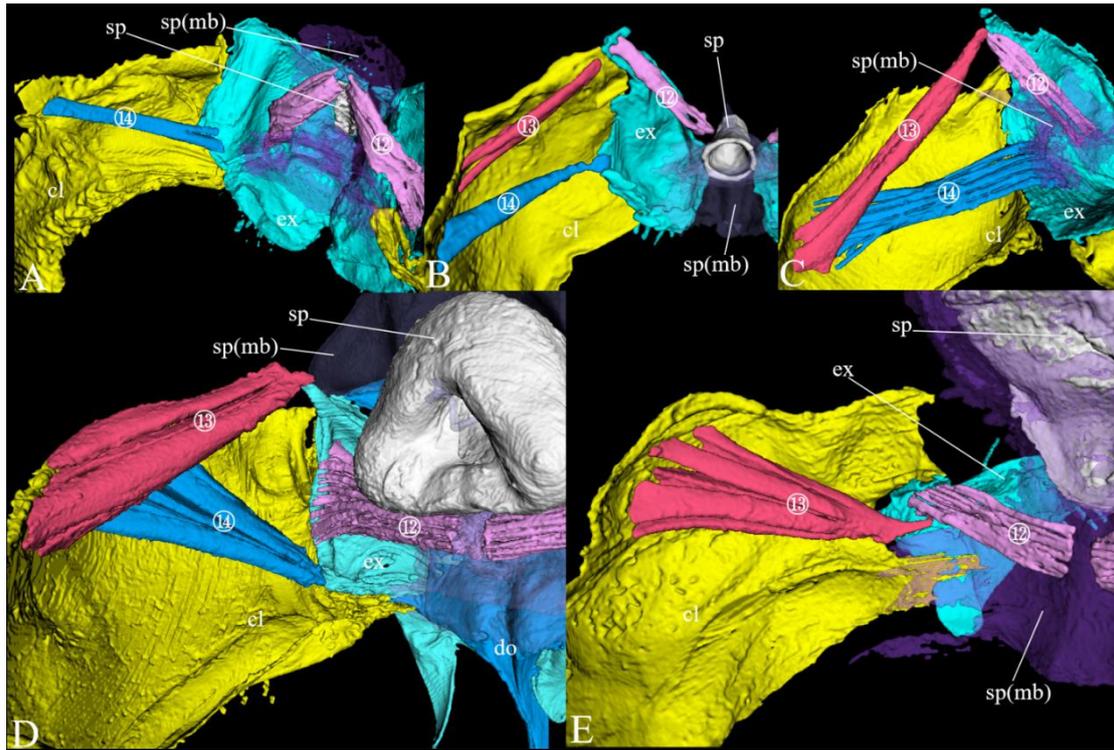
648 Amphientomidae Gen. sp.; (B) *Valenzuela badiostigma*; (C) *Valenzuela badiostigma*;

649 (D) *Trichadenotecnum pseudomedium*; (E) *Lepinotus reticulatus*; and (F)

650 Amphientomidae Gen. sp. (9) veVII01; (10) dosp01; (11) doIX01; (15) doex01. See

651 Fig. 1 for abbreviations.

652



653

654 **Fig. 12.**

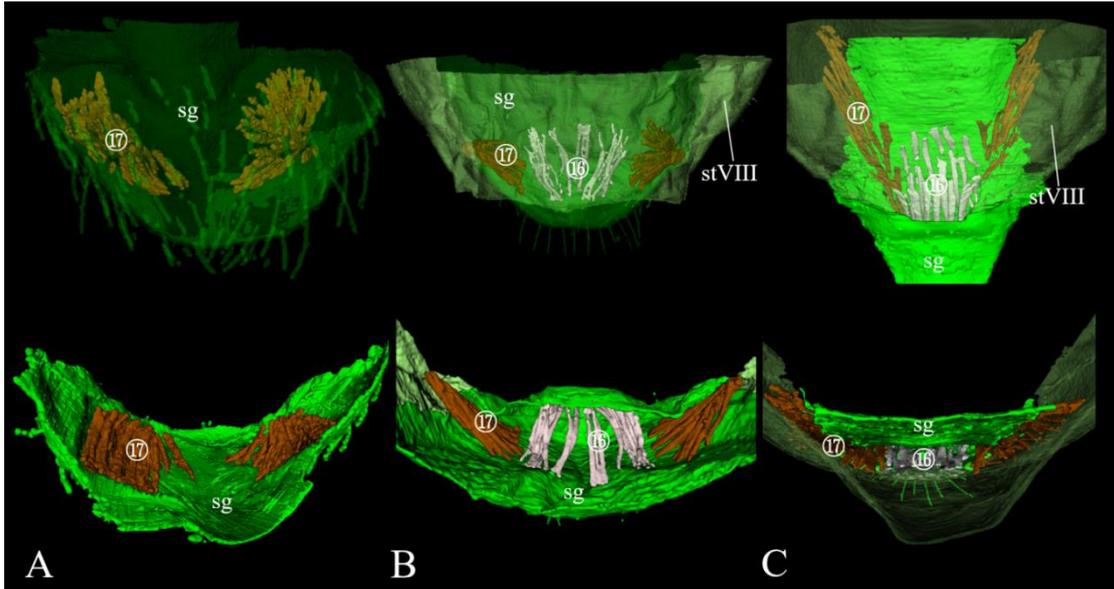
655 Close-up of the gonapophyses and related structures, internal view: (A) *Prionoglaris*

656 *stygia*; (B) *Psyllipsocus clunjunctus*; (C) *Lepinotus reticulatus*; (D) Amphientomidae

657 Gen. sp; and (E) *Trichadenotecnum pseudomedium*. (12) exsp01; (13) exIX01; (14)

658 exIx02. See Fig. 1 for abbreviations.

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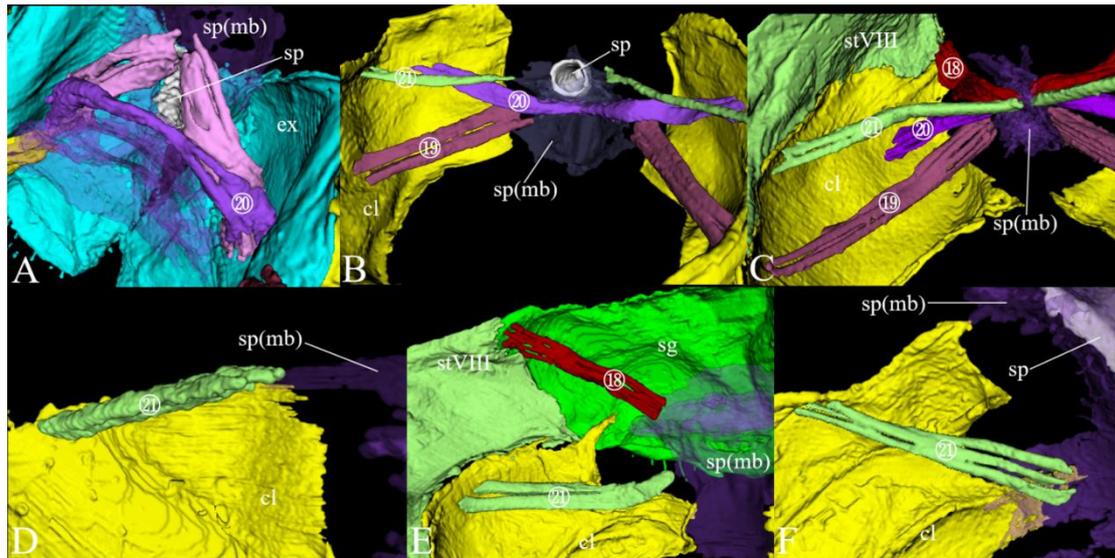
661 **Fig. 13.**

662 Subgenital plate, internal view: (A) *Archipsocus* sp. (top: ventral view; below: internal
 663 view); (B) *Valenzuela badiostigma* (top: ventral view; below: internal view); and (C)
 664 *Trichadenotecnum pseudomedium* (top: ventral view; below: internal view). (16)
 665 sgVIII01; (17) sgVIII02. See Fig. 1 for abbreviations.

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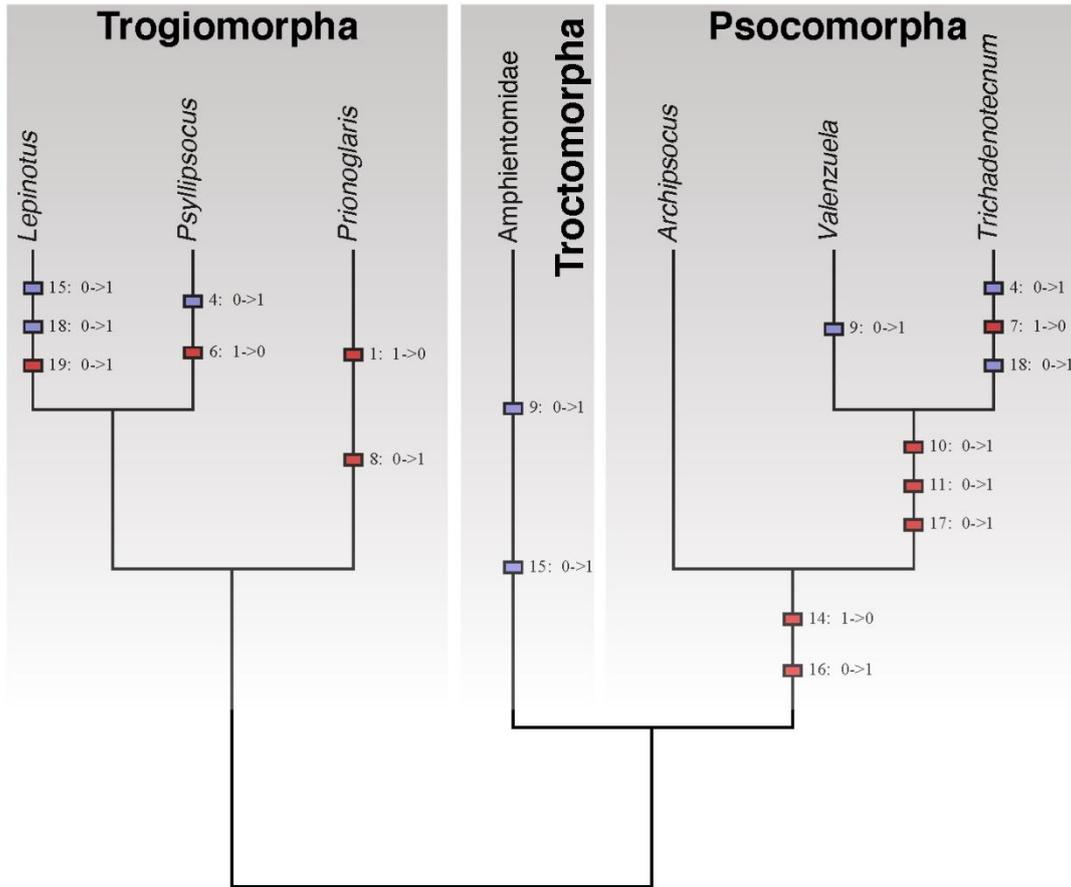
670 **Fig. 14.**

671 Muscles related to the spermapore membrane, internal view: (A) *Prionoglaris stygia*;

672 (B) *Psyllipsocus clunjectus*; (C) *Lepinotus reticulatus*; (D) *Archipsocus* sp.; (E)

673 *Valenzuela badiostigma*; and (F) *Trichadenotecnum pseudomedium*. (18) spVIII01; (19)

674 spIX01; (20) spIX02; (21) spIX03. See Fig. 1 for abbreviations.



675

676 **Fig. 15.**

677 Most parsimonious reconstruction of the presence (1) or absence (0) of the female
 678 genital muscles. Tree topology is from the phylogenomic tree estimated by de Moya *et*
 679 *al.* (2021). See Table 2 for the character table. Red bars indicate nonhomoplasious
 680 characters, and blue bars indicate characters that include homoplasy.

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Suborder	Infraorder	Family	Species	Locality	Specimen ID	Collector
Trogm orpha	Prionogharidetae	Prionogharididae	<i>Prionogharis stygia</i>	France	S8KY37	B. Horser
	Psyllipsocetae	Psyllipsocidae	<i>Psyllipsocus chinjunctus</i>	Brazil	S8KY10	R. Ferreira
	Atropetae	Trogidae	<i>Lephotus reticulatus</i>	Japan	S8KY32	K. Yoshizawa
Troctom orpha	Amphientometae	Amphientomidae	Genus sp.	Malaysia	S8KY14	K. Yoshizawa
Psocom orpha	Archipsocetae	Archipsocidae	<i>Archipsocus</i> sp.	Mexico	S8KY75	K. Yoshizawa
	Caeciliuseae	Caeciliusidae	<i>Vaenzueh badonneti</i>	Japan	S8KY18	K. Yoshizawa
	Psocetae	Psocidae	<i>Trichadenotecnum pseudomedium</i>	Japan	S8KY28	K. Yoshizawa

687

688 **Table 1**

689 Taxa examined in this study.

690

Muscle #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
	epX01	paX01	paX02	paX03	paX04	paX05	papa01	papa02	veV ID1	dosp01	doR01	exp01	exR01	exR02	doex01	sgV ID1	sgV ID2	spV ID1	spK01	spK02	spK03	
<i>Prionogharis</i>	0	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	
<i>Psyllipsocus</i>	1	1	1	1	1	0	1	0	0	0	0	1	1	1	1	0	0	0	0	1	1	
<i>Lephotus</i>	1	1	1	0	1	1	1	0	0	0	0	1	1	1	1	0	0	1	1	1	1	
Amphientomidae	1	1	1	0	1	1	1	0	1	0	0	1	1	1	1	0	0	0	0	0	0	
<i>Archipsocus</i>	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	
<i>Vaenzueh</i>	1	1	1	0	1	1	1	0	1	1	1	0	0	0	0	1	1	0	0	0	1	
<i>Trichadenotecnum</i>	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1	0	0	1	
# in Hünefeld et al. (1934)	59			65, 67-69			66	64	08, 09	c	d	e		f		50-52	57, 58	27, 33	30, 31	70		75-77

691

692

693 **Table 2**

694 List of the muscles identified in the psocopteran female terminalia and their distribution

695 throughout the taxa examined (present 1/absent 0). The muscle numbers correspond to

696 the character numbers shown in Figure 15. Homology with the holometabolan female

697 genital muscles (as proposed in Hünefeld *et al.* 2012) and homology of the

698 gonapophyses muscles observed by Badonnet (1934) are also shown at the bottom of

699 the table (for holometabolan muscles, more than one candidate homologous muscle is

700 listed for many cases).