

1 **Selection for imperfection: A review of asymmetric genitalia**
2 **in araneomorph spiders (Araneae: Araneomorphae).**

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19 **Running Title:** Asymmetric genitalia in spiders

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23

24 **Abstract**

25

26 Bilateral asymmetry in the genitalia is a rare but widely dispersed phenomenon in the
27 animal tree of life. In arthropods, occurrences vary greatly from one group to another
28 and there seems to be no common explanation for all the independent origins. In
29 spiders, genital asymmetry appears to be especially rare. Few examples have been
30 studied in detail but isolated reports are scattered in the taxonomic literature. Based on a
31 broad literature study, we found several species in thirteen families with evidence of
32 genital asymmetry, mostly expressed only in females. Our review suggests that spider
33 genital asymmetries, although rare, are more common than previously thought and
34 taxonomic descriptions and illustrations are a useful but not entirely reliable tool for
35 studying them. Here we also document thoroughly the case of the liocranid spider
36 *Teutamus politus*. We collected live specimens to observe male-female interactions and
37 document their genital morphology. We consider *T. politus* to be the first known case of
38 directional asymmetry and the first report of developmentally asymmetric male genitalia
39 in Entelegynae spiders. Generalities, evolution and categorization of asymmetry in
40 spiders are further discussed.

41

42 **Keywords:**

43 Chirality, sexual selection, antisymmetry, Araneae, Synspermiata, Entelegynae, RTA,
44 Liocranidae.

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49 **Introduction**

50 Genital asymmetry is a trait that has evolved independently several times in many
51 animal groups. Invertebrates show a wide range of genital asymmetries with probably
52 thousands of independent origins. Many, sometimes not mutually exclusive,
53 explanations have been proposed, namely: i) morphological compensation for selected
54 changes in mating position; ii) sexually antagonistic co-evolution; iii) cryptic female
55 choice for asymmetric male genitalia; iv) different functions for the left and right side;
56 v) one-sided reduction to save space and resources; vi) functional constraints: to
57 function properly, the separate parts of the genitalia need to connect in an asymmetric
58 fashion; vii) efficient packing of internal organs in the body cavity [1–4].

59

60 Asymmetries are often classified as fluctuating (FA), antisymmetry (AS) or directional
61 (DA) [3,5,6]. This categorization is based on the degree and relative frequencies of the
62 different chiral forms found in a population. FA describes slight asymmetric variation
63 around a symmetrical mean; the appearance of this type of asymmetry is usually related
64 to environmental or developmental constraints [5,7]. AS describes cases where two
65 mirror image forms, dextral and sinistral, are identifiable and within a population,
66 occurring usually in equal or similar proportions [3]. Finally, DA refers to cases where
67 only one asymmetric form is virtually always present [3]; this might be associated with
68 mechanical, behavioral, or functional differentiation and selection of one asymmetrical
69 form of the structures or organs [3,8].

70

71 Genital asymmetry, although rare as a whole, is a recurring phenomenon in a few
72 groups of arthropods like mites, crustaceans, opiliones, and very common several insect
73 orders. However, in spiders (Fig. 1a), sexual asymmetries seem to be rather an

74 uncommon exception [1–4,9,10]. In insects, copulatory mechanics and the presence of a
75 single male genital structure located at the posterior end of the abdomen might explain
76 the great incidence of genital asymmetry in this group [1,3,11]. In contrast, spiders have
77 two male copulatory organs derived from a modified pair of leg-like appendages (Fig.
78 1b). These are normally both used sequentially for sperm transfer during copulation
79 [12]. The presence of these paired structures has been hypothesized to act as an
80 “evolutionary buffer” to the development of genital asymmetry, especially on male
81 genitals [1,3,10].

82

83 **Figure 1.- Spider relations and spider genitalia.** a) Schematic tree based on a
84 comprehensive spider phylogeny by Wheeler et al. [13]. Number of families per
85 clade are indicated between parentheses; approximate percentage of species per
86 clade relative to the Order Araneae is also given. Family name tags indicate the
87 ones with known asymmetric species. b) Ventral view of spider copulatory organs:
88 ♀ Epigynum (E) and ♂ Pedipalp bulb (P); modified from Foelix [12].

89

90 Most cases of asymmetry in spiders have not been studied in detail or even discussed,
91 with the notable exception of pholcids and theridiids [1,3]. Nevertheless, taxonomic
92 illustrations and descriptions give evidence of the existence of this phenomenon in other
93 families. Genital asymmetry has been documented in females, males or both sexes, with
94 seemingly several independent origins in the spider tree of life. All known cases have
95 been reported in two major clades: Synspermiata and Entelegynae that include about
96 13% and 80% of known spider diversity, respectively (Fig. 1a); within the Entelegynae,
97 asymmetries have been documented in the clades Araneoidea and RTA.
98 Morphologically, Synspermiata spiders tend to have structurally simpler genitalia than
99 entelegyne spiders in both sexes. Asymmetries in Synspermiata have been properly
100 documented in two families: Pholcidae (Fig. 2 a, h) and Oonopidae (Fig. 2 , h); but
101 taxonomic descriptions of some Ochyroceratidae (Fig. 2 b, d), Telemidae (Fig. 2 f) and

102 Sicariidae depict female genital asymmetry too. In Entelegynae, examples appear more
103 scattered with most cases being found in the family Theridiidae (Fig. 3a-c) and some
104 more documented in at least six families of the RTA clade (Fig. 3b, d-h). Explanations
105 for genital asymmetry in spiders are diverse and could include individual variation,
106 natural selection, or sexual selection [1,3,10,14,15].

107

108

109 **Figure 2.- Examples of genital asymmetry in Synspermiata.** a, e) male pedipalps,
110 lateral view. b-d, f-h) vulva, dorsal view. a) Pholcidae: *Metagonia mariquitarensis*;
111 modified from Huber [8]. b) Ochyroceratidae: *Althepus naphongensis*; modified
112 from Li *et al.* [16]. c) Sicariidae: *Hexophthalma albospinosa*; modified from
113 Magalhaes and Brescovit [17]. d) Ochyroceratidae: *Speocera cattien*; modified
114 from Tong, *et al.* [18]. e) Oonopidae: *Paradysderina righty*; modified from Platnick
115 and Dupérré [19]. f) Telemididae: *Telema exiloculata*; modified from Lin and Li
116 [20]. g) Oonopidae: *Triaeris stenaspis*. h) Pholcidae: *Metagonia delicate*; modified
117 from Huber [21].

118

119 **Figure 3.- Examples of genital asymmetry in Entelegynae.** a, b, d-h) vulva, dorsal
120 view. c) male and female during copulation. a) Theridiidae: *Asygyna*
121 *coddingtoni*; modified from Agnarsson [22]. b) Phrurolithidae: *Scotinella*
122 *fratella*;; modified from Dondale and Redner [23]. c) Theridiidae: *Tidarren*
123 *sisyphoides*. Arrow shows the presence of only one pedipalp; modified from
124 Knoflach [24]. d) Gnaphosidae: *Apopyllus gandarella*; modified from Azevedo
125 *et al.* [25]. e) Hahnidae: *Neoantistea agilis*; modified from Opell and Beatty
126 [26]. f) Trachelidae: *Trachelas ductonuda*; modified from Rivera-Quiroz and
127 Alvarez-Padilla [27]. g) Liocranidae: *Jacaena mihun*. h) Cithaeronidae:
128 *Cithaeron praedonius*;; modified from Ruiz and Bonaldo [28].
129

130 Spider genital asymmetry can be classified as follows: Fluctuating asymmetry (FA) is
131 probably the most common type and has been properly documented in some Lycosidae
132 [29–32], Pholcidae [33], and Oxyopidae [10,34]. Other examples of seemingly
133 asymmetric structures like the pedipalps of the one known specimen of *Pimoa petita*
134 [35] or the numerous documented anomalies and deformities [36–39] might easily be
135 explained by developmental malformations (Fig. 4).

136

137 **Figure 4.- Examples of genital malformation in spiders.** a,c) male pedipalps,
138 posterior-lateral view. b) vulva, ventral view. a) *Lycosa ammophila*; modified
139 from Kaston [37]. b) *Pardosa sagei*; modified from Kaston [37]. c) *Pimoa*
140 *petita*; modified from Hormiga [35].
141

142

143 Antisymmetry (AS) is the second most common form of asymmetry in spiders and has
144 been documented in three genera of the Theridiidae (*Asygyna*, *Echinotheridion*, and
145 *Tidarren*) (Fig. 3a, c) [22,40,41]; one genus of Pholcidae (*Metagonia*) (Fig. 2a, h) [21];
146 one genus of Phrurolithidae (*Scotinella*) (Fig. 3b) [42] and scattered cases such as in
147 Trachelidae (Fig. 3f) [27,43,44], Cithaeronidae (Fig. 3h) [45] and other RTA families.

148 Directional asymmetry (DA) is the rarest type and, until now, it had only been reported
149 in the pholcid *Metagonia mariguitarensis* (Fig 2h) [8]; DA has also been implied some
150 descriptions within the Oonopidae (Fig 2e) [19,46], and in the liocranid *Teutamus*
151 *politus* female genitalia [47]. All of these, other isolated reports, and scattered
152 descriptions and illustrations suggest that genital asymmetries in spiders have originated
153 independently several times and their study might give better insights into how and
154 when this phenomenon has evolved and the selective mechanisms behind it.

155

156 A particularly interesting example are the Liocranidae where two different types of
157 asymmetry are present [47–49]. For example, *Jacaena mihun* (Fig. 3g) shows no
158 external chirality, but internally the asymmetric copulation ducts are highly variable
159 among individuals. Another example, *Teutamus politus* (Figs 5-7), shows external
160 asymmetry in the female genitalia with both copulatory openings fused together in one
161 atrium placed on the left side of the epigyne (see Deeleman-Reinhold [47]: fig 800,
162 801). Deeleman-Reinhold [47] mentioned female asymmetry as a diagnostic character
163 for this species and noted that in all six of the specimens available for examination, the

164 atrium is located in the left side. A revision of the genus *Teutamus* [48] also included
165 asymmetry in the female genitalia as a diagnostic character for *T. politus*, and expanded
166 the sample of specimens examined; asymmetry in male pedipalp was not reported in
167 either of these cases.

168

169 Here we present a general review of genital asymmetries in spider literature, grouping
170 them in previously described categories of genital asymmetry and discussing the
171 existence of a new category of female genital asymmetry (here called Chaotic
172 Asymmetry). We also analyzed the specific case of the species *Teutamus politus* by
173 collecting new specimens in Thailand and documenting male and female genitalia using
174 diverse morphological methods. This gives evidence of the first cases of both
175 directional asymmetry in males and females, and developmental male genital
176 asymmetry in Entelegynae spiders.

177

178 **Material and Methods**

179 **Literature review-** We performed an informal search in taxonomic literature of several
180 Synspermiata and Entelegyne families. Selection of publications was initially based on
181 reported cases in literature [1,3,8,10,11] and then expanded depending on the
182 occurrences found within each family. We did not contemplate individual cases of clear
183 FA but this type of asymmetry is included in our discussion. We considered *T. politus*
184 as a good model for testing basic hypotheses on genital asymmetry because of the clear
185 external and internal morphology of female genitalia and Deeleman-Reinhold's [47]
186 note suggesting this could be a case of DA. Furthermore, we hypothesized that
187 morphological or behavioral compensation for female genital asymmetry could be
188 found in the male.

189

190 We considered male asymmetry as those cases that result in clear morphological
191 differences between right and left pedipalp regardless of having a developmental or
192 behavioral origin. Based on this, we also considered the pedipalp amputation that males
193 of *Echinoitheridion* and *Tidarren* perform on themselves in our review; especially since
194 the asymmetry has clear adaptive and evolutionary implications [14,41,50–52].

195

196

197 **Fieldwork-** We selected study sites and collecting dates based on the relative numbers
198 of collected adult specimens of *T. politus* mentioned in literature [47,48]. Fieldwork was
199 carried out in Thailand between July 29th and August 12th 2018; here we sampled 12
200 sites in total: eight in Phuket Island and four more in Krabi Province. We attempted to
201 cover a variety of vegetation types ranging from relatively well preserved mixed forests
202 to rubber and oil palm plantations. In each site we processed leaf litter using Winkler
203 extractors and direct collecting on ground, among leaf litter and under rocks and logs.
204 Hand collected specimens were kept alive in individual tubes. Winkler specimens were
205 collected in a mixture of propylene glycol and 96% ethanol. All the specimens have
206 been deposited in the collection of the Naturalis Biodiversity Center, Leiden, The
207 Netherlands.

208

209 **Behavioral observations-** Live specimens were kept individually in clean 15ml Falcon
210 tubes and fed with termites every two days. Seventeen males and 19 females were
211 selected and assigned unique numbers. Couples were formed preferably with specimens
212 from the same locality. Spiders were placed in a Petri dish (diameter 5 cm, height 1
213 cm); each dish was divided by a paper wall with a small opening so spiders could roam

214 freely but flee in case of aggression. Each couple was kept in the dish under constant
215 observation for a period of about three hours. After observations, all specimens were
216 sacrificed and stored in 96% ethanol.

217

218 **Morphological methods-** Somatic characters and male sexual structures were
219 photographed using a Leica MI6SC Stereomicroscope equipped with a Nikon DS-Ri2
220 camera. Female genitalia were dissected, digested using a pancreatine solution [53],
221 cleared with methyl salicylate. Observations were made using semi-permanent slide
222 preparations [54] in a Leica DM 2500 microscope with the same camera as above. Male
223 genitals were expanded using 10% KOH and distilled water in three 3 min. cycles
224 leaving the pedipalps in distilled water overnight to stabilize them for photography.
225 Female epigyna and male pedipalps were prepared for SEM and mounted following
226 Alvarez-Padilla and Hormiga [53] SEM images were obtained using a JEOL JSM-
227 6480LV electron microscope.

228

229 The following abbreviations are used in the text and figures: **Female genitalia:** A,
230 atrium; CD, copulatory ducts; CO, copulatory openings; Fd, fertilization ducts; Sa,
231 secretory ampullae (*sensu* Dankittipakul, Tavano, and Singtripop [48]); S, spermatheca.

232 **Male genitalia:** B, male pedipalp bulb; Cy, cymbium; C, pedipalp conductor; E,
233 embolus; Fe, femur; H, basal hematodocha; Pa, patella; RTA, tibia retro lateral
234 apophysis; Sd, sperm duct; sT, sub tegulum; T, tegulum; Ti, tibia.

235

236 **Results**

237 **Literature review.**—We reviewed publications that directly focus on genital
238 asymmetry as well as taxonomic literature that tangentially describe or illustrate

239 asymmetrical morphology. We found ca. 150 species across thirteen spider families
 240 with indications of asymmetric genitalia (Table 1) representing less than 0.3% of all
 241 spider species World Spider Catalog [55]; and about 13.5% of all the currently valid
 242 species in the genera reviewed for this study. Synspermiata has at least five families
 243 (Ochyroceratidae, Oonopidae, Pholcidae, Sicariidae and Telemidae) where some kind of
 244 asymmetry has evolved accounting for ca. 90 species (Table 1). Asymmetry was found
 245 in both female and male genitalia; female asymmetry is more frequent, being found in at
 246 least five oonopid, three sicariid, two pholcid and two ochyroceratid genera. In addition,
 247 most genera in the Telemidae have evolved a single sac-like seminal receptacle; some
 248 species show seemingly asymmetric modifications of this sac, leaning and sometimes
 249 spiraling to one side. However, intraspecific variation has not been documented. Male
 250 asymmetry is less common, being found in three oonopid and two pholcid genera, and
 251 ambiguously suggested for two ochyroceratid species [18,56]. Nevertheless, it is
 252 prevalent in *Escaphyella* and *Paradysderina*, where about 20 species show asymmetric
 253 male pedipalps (2 e).

254

255 **Table 1.- Spider taxa with genital asymmetry reports in literature.**

Family	Species	External (E) / Internal (I)	Female / Male	Type of asymmetry	Distribution	Source	
Synspermiata							
Oonopidae	<i>Aschnaonops marta</i>	E	M	DA/AS*	Neotropical	Platnick et al. [57]	
	<i>Aschnaonops meta</i>	I	F	AS*	Neotropical	“	
	<i>Escaphiella</i> (8 spp)	E	M	DA/AS*	Neotropical	Platnick and Dupérré [46]	
	<i>Lionneta</i> (2 spp)	I	F	AS/FA*	Seychelles	Saaristo [58]	
	<i>Ischnothyreus jivani</i>	I	F	AS/FA*	Seychelles	“	
	<i>Paradysderina</i> (12 spp)	E	M, F	AS/FA*	Neotropical	Platnick and Dupérré [19]	
	<i>Reductoonops</i> (2 spp)	I	F	AS*	Neotropical	Platnick and Berniker [59]	
	<i>Triaeris</i> (5 spp)	I	F	DA*	Pantropical	Platnick et al. [60]	
	Ochyroceratidae	<i>Althepus</i> (5 spp)	I	F	AS/FA*	South-East Asia	Deeleman-Reinhold [61]; Li et al. [16]
		<i>Speocera</i> (8spp)	I, E*	M*, F	AS*	Pantropical	Lin, et al. [20]; Tong and Li [56]; Tong et al.[18]
Pholcidae	<i>Mesavolivar yurani</i>	I	F	DA*	Venezuela	Huber [15]	
	<i>Metagonia</i> (9 spp)	I	F	AS*	Bolivia, Brazi, Peru	Ferreira et al. [62]; Huber [63]; Huber et	

						al. [64]; Machado, Ferreira and Brescovit [63]; Perez-Gonzalez and Huber [65]
	<i>Metagonia mariguitarensis</i>	E male/ I female	M, F	DA	Brazil	Huber [63]
	<i>Panjange lanthana</i> group (3 spp)	E	M	DA*	Philippines	Huber [10]
Sicariidae	<i>Hexophthalma</i> (3spp)	I	F	AS/FA *	South America	Magalhaes, Brescovit, and Santos [17]
	<i>Loxosceles</i> (4spp)	I	F	AS/FA *	North to South America; Africa	Gertsch and Ennik [66]; Lotz [67]
	<i>Sicraius</i> (7 spp)	I	F	AS/FA *	South America	Magalhaes, Brescovit, and Santos [17]
Telemidae	<i>Cangoderses christae</i>	I	F	AS/FA *	Côte d'Ivoire	Wang and Li [68]
	<i>Kinku turumanya</i>	I	F	AS/FA *	Ecuador	Dupérré and Tapia [69]
	<i>Telema</i> (14 spp)	I	F	AS/FA *	East and South-East Asia	Wang and Li [70]; Wang and Li [71]; Lin and Li [72]; Lin, Pham and Li [20]
Entelegynae						
<u>Araneoidea</u>						
Theridiidae	<i>Asygyna</i> (2 spp)	E, I	F	AS	Madagascar	Agnarsson [22]
	<i>Echinotheridion</i> (9 spp)	E	M	AS	Neotropical	Knoflach [41]
	<i>Tidarren</i> (24 spp)	E	M	AS	America, Tropical Africa	Knoflach and van Harten [14]
<u>RTA</u>						
Cithaeronidae	<i>Cithaeron</i> (2 spp)	I	F	CA	South America, South-East Asia, North Africa	Platnick [73]; Platnick and Gajbe [45]; Ruiz and Bonaldo [28]
Hahniidae	<i>Neoantistea</i> (2 spp)	I	F	FA/AS/CA*	Nearctic	Opell and Beatty [26]
Gnaphosidae	<i>Apopyllus</i> (9 spp)	I	F	FA/CA*	Neotropical	Azevedo, et al.[25]
Liocranidae	<i>Jacaena mihun</i>	I	F	CA	Thailand,	Deeleman-Reinhold, [47]
	<i>Teutamus politus</i>	E, I	M ^a , F	DA	Thailand, Malaysia	”
	<i>Teutamus</i> (4 spp)	E, I	F	AS	Sumatra	”
Phrurolithidae	<i>Scotinella</i> (2 spp)	E, I	F	AS	USA	Penniman [42]
Prodidomidae	<i>Moreno ramirezi</i>	I	F	CA	Argentina	Platnick, Shadab, and Sorkin [74]
Trachelidae	<i>Trachelas</i> (7 spp)	I	F	CA	North and Central America	Platnick and Shadab [44]; Platnick and Shadab [43]; Rivera-Quiroz and Alvarez-Padilla [27].

256 Summary of cases and types of spider genital asymmetry, mostly from taxonomic
 257 literature. (AS, antisymmetry; CA, chaotic asymmetry; DA, directional asymmetry; FA,
 258 fluctuating asymmetry). *indicates cases where information is scarce, ambiguous or few
 259 specimens were examined. ^a described in the present work.
 260

261 In Entelegynae, more than 60 species in eight families show genital asymmetry. Almost
 262 half of the cases were found in the Theridiidae with ca. 35 species in three genera
 263 (*Asygyna*, *Echinotheridion*, and *Tidarren*). The rest are scattered among seven families

264 in the RTA clade (Cithaeronidae, Hahniidae, Gnaphosidae, Liocranidae, Phrurolithidae,
265 Prodidiomidae, Trachelidae) (Table 1). Most genital asymmetry reports in Entelegynae
266 include only female genitalia. Female internal asymmetry was the most common,
267 showing a wide range of variation on spermathecae and copulatory ducts (Fig. 3d-h).
268 Female external asymmetry was only found in *Asygyna* (Fig. 3a), *Scotinella* (Fig. 3b)
269 and *Teutamus* (Fig. 7a,d). Male genital asymmetry in Entelegynae had only been
270 reported in the theridiid *Echinotheridion* and *Tidarren* (Fig. 3c); these two genera
271 exemplify a unique behavior that results in genital mutilation; however, normal
272 developmental asymmetry, rather than behaviorally induced, had never been described
273 in Entelegynae literature before this work.

274

275 Most male asymmetries in literature appear to be AS with the exception of the DA in
276 *Metagonia mariguitarensis*, two species of *Escaphiella* and the newly described
277 pedipalps of *Teutamus politus*. Three species of the *Panjange lanthana* group, and
278 several more of *Aschnaonops*, *Escaphiella*, and *Paradysderyna* might also be DA but
279 only a few specimens have been examined. Female genital asymmetry most of the times
280 involves only internal structures such as ducts, bursa, and spermatheca. Both AS (Fig.
281 3a-b) and CA (Fig. 3d-h) are relatively common. External asymmetry is not usual and
282 had only been described in *Asygyna*, Theridiidae (Fig. 3a) and *Scotinella*, Phrurolithidae
283 (Fig. 3b) (apparently AS); and *Teutamus*, Liocranidae (Fig. 7) (DA and apparent AS).

284

285

286 **Remarks on *Teutamus politus* Thorell, 1890**

287 (Figures 5-7)

288 A total of 60 female and 35 male specimens were collected as a result of our fieldwork
289 in Thailand. External female genitalia and male pedipalps were observed and compared
290 for all specimens. Four females and five males had their genitals dissected and prepared
291 for detailed examination.

292

293 **Male genital morphology-** All pedipalp segments with the exception of the bulb (B)
294 seem to be completely symmetrical. Bulbs show at least three clear asymmetries
295 between the right and left sides: i) left B is slightly wider than the right one (Fig. 5b,e; c,
296 f; 6a); ii), left side has a flatter and wider tegulum (T) (Fig. 5f) projected anteriorly in
297 retrolateral view (Fig 5e); and iii) the left conductor (C) is conical and straight (Fig. 6b),
298 slightly pointing towards the cymbium (Cy) in lateral view (Fig. 5d, f); while the right C
299 is flattened, hook-shaped (Fig. 6c) and pointing away of the Cy in lateral view (Fig. 5a ,
300 c). There is no apparent difference in the length and shape of the emboli (E) or the
301 spermatic ducts (Sd). This suggests that the asymmetry might not be linked to
302 functional distinction of left and right pedipalp.

303

304 **Figure 5.- Asymmetric male genitalia of *Teutamus politus*.** Right pedipalp: a)
305 prolateral view. b) retrolateral view. c) ventral view. Left pedipalp: d) prolateral
306 view. e) retrolateral view. f) ventral view. Scale bars: **a, b, d, e** = 0.5 mm. **c, f** =
307 0.25 mm.

308

309 **Figure 6.- Expanded asymmetric male genitalia of *Teutamus politus*.** a) comparative
310 retrolateral view. b) left pedipalp prolateral view. c) right pedipalp prolateral
311 view. Scale bars: **a** = 0.5 mm. **b, c** = 0.25 mm.

312

313

314 **Female genital morphology-** Externally, the epigynal plate is flattened and fused to the
315 ventral scutum (Fig. 7a). Copulatory openings (CO) are placed close together, forming
316 an atrium facing the left side of the venter and located anteriorly to the bean-shaped

317 spermatheca (Fig. 7a-c). Left spermatheca is slightly shorter than right one (Fig. 7c).
318 Copulatory ducts (CD) are equally long. Right CD anterior to the right spermatheca, left
319 CD located in between both spermathecae (Fig. 7c, e). Asymmetric attachment of CD to
320 spermathecae with the right being anterior to that of the left one (Fig. 7b, c). Both CD
321 have secretory ampullae (Sa) close to their middle portion (Fig. 7b, c). Fertilization
322 ducts (Fd) short and simple, originating from the posterior end of the spermatheca and
323 pointing in the same direction (Fig. 7 e). Despite the clear difference in shape, there is
324 no morphological evidence that suggests functional differentiation between right and
325 left structures.

326

327 **Figure 7.- Asymmetric female genitalia of *Teutamus politus*.** a) epigynum ventral
328 view. b) dissected and cleared vulva ventral view. c) same, dorsal view. d)
329 vulva, ventral view, SEM. e) same, dorsal view. Scale bars: **a, b, c** = 0.25 mm. **d**
330 = 150 μ m. **e** = 100 μ m

331

332 **Behavioral observations-** A total of 25 different couples were tested. Initially couples
333 were formed with males and females from the same collection site. Males were more
334 difficult to keep alive than females with most males dying within three days of
335 collection. Due to this, males and females from different sites were also coupled. There
336 were no successful observations of either courtship or mating. Spiders preferred to
337 explore the dish or stand still and, whenever they got too close, they usually avoided
338 each other. In general, interactions between females and males were brief and non-
339 aggressive. Four females laid egg sacs in the Falcon tubes.

340

341 **Discussion**

342

343 **Literature review.**—Taxonomic literature is the biggest repository of primary
344 descriptive data on the world’s biodiversity. However, illustrations and description are
345 difficult to interpret and might be influenced by the number of studied specimens, state
346 of preservation, preparation artifacts and even illustration techniques. As an example,
347 the species *Cithaeron indicus* shows clear asymmetric female genitalia in its original
348 description [45] but appears symmetrical in a later publication [75] (Fig. 8). Illustrators
349 sometimes avoid introducing variation by drawing one half of a given structure and then
350 tracing the other side based on it. This might simplify understanding and drawing some
351 structures but could also lead to overlooking important information in the illustration
352 process. Similar biases have been observed in some species of *Trachelas* [43,44] and
353 could be present elsewhere. As pointed out by Huber and Nuñez [10], preparation
354 artifacts might also play a role in the identification and interpretation of asymmetric
355 structures. Weakly sclerotized internal genitalia (as that typically found in non-
356 Entelegynae spiders) are often prone to create artifacts during specimen preparation and
357 an interpretation without sufficient knowledge of intraspecific variation might be
358 misleading. Entelegyne spiders tend to have more heavily sclerotized bodies being less
359 sensible during the preparation process and allowing a more robust interpretation of
360 their genital morphology.

361

362 **Figure 8.- Example of illustration bias.** Vulva, ventral view. a) *Cithaeron indicus*;
363 modified Platnick and Gajbe [45]. b) Same; modified from Gajbe [75].

364

365 Descriptions of male spider genitalia are also subject to preparation artifacts or
366 methodological biases. Male genitalia preparation and examination is usually done by
367 dissecting, studying and illustrating only one pedipalp. Although this is a very efficient
368 approach and does not represent a problem on most occasions, some cases of

369 asymmetric genitalia might go unnoticed. This has resulted in a more difficult
370 assessment of male asymmetry; as an example, *Metagonia mariguitarensis* was
371 believed to be the only species with male genital asymmetry [8]. However, DA in males
372 of *T. politus* had never been discovered before, apparently because the right male
373 pedipalp had simply been overlooked in previous descriptions. Similarly two *Speocera*
374 species [18,56] have their male pedipalps ambiguously described as “asymmetric” but
375 no more details were given.

376

377 In comparison, recent revisionary studies on the oonopid genera *Aschnaonops*,
378 *Escaphiella*, *Paradysderyna* and *Reductonops* [19,46,57,59] took special care in
379 comparing the right and left male pedipalps revealing many more cases of genital
380 asymmetry. In all of these, male pedipalps show clear differences in bulb development
381 and embolus shape between right and left (Fig. 2 e). In at least two species of
382 *Escaphiella* [46] enough specimens have been examined to suggest that asymmetry in
383 these species is directional.

384

385

386 **Evolutionary trends of genital asymmetry.**— We found evidence of ca. 150 cases of
387 asymmetry in spider genitals in thirteen different families. In previous broad-scope
388 reviews, only some examples in Pholcidae and Theridiidae had been taken in account.
389 Reports on insects suggest that genital asymmetry rarely appears isolated and is usually
390 a shared trait between closely related species [3,4,76]. Here, we found some similar
391 patterns with several species within a genus showing at least one type of genital
392 asymmetry. This pattern is more common in the Synespermiata, but was also observed
393 in Entelegynae (Table 1). Although the known number of cases and families with

394 asymmetrical genitalia has increased significantly, this still represents less than 0.3% of
395 all known spider species. The low incidence of genital asymmetry in spiders has been
396 mainly explained by the presence of two sperm transfer structures in the male [1,3].
397 Huber, Sinclair, and Schmitt [1] remark that in comparison to insects, most spider
398 asymmetry originates in females instead of males, and most insect asymmetry originates
399 as DA, while most or all spider asymmetry originates as AS. Many examples support
400 the first claim, which also fits a cryptic female choice hypothesis [9]. Nevertheless, we
401 found numerous “new” examples of male asymmetry hidden in taxonomic literature
402 (Table 1), highlighting the many cases in the Oonopidae where male asymmetry has
403 apparently not coincided with modified female genitalia. As for the second claim, we
404 found that DA might not be as rare as previously thought. Examples of DA include two
405 confirmed cases in *Metagonia* [8] and *Teutamus*, both involving male and female
406 genitalia; two more in *Escaphiella* [46], that include only male pedipalp; and some
407 more in *Panjange* [10], *Mesovolivar* [15], and three Oonopidae genera that suggest
408 asymmetry directionality but are not conclusive.

409

410 Many spider asymmetries seem to fit in the AS category, although only a handful have
411 been evaluated for the appearance of right or left-sided asymmetries within a sample as
412 in Phrurolithidae and Theridiidae [22]. Also, we found some cases in which female
413 copulatory ducts are long, coiled and entangled in a way that does not fit any of the
414 three known types of asymmetry. We called this chaotic asymmetry (CA) because the
415 great variation between individuals of the same species does not allow distinguishing
416 either a dextral or a sinistral form.

417

418 Other cases difficult to assess are: the reduction of spermathecae to a single receptacle,
419 as seen in some oonopids [35,51,52], pholcids [62,63,65], and telemids [20,68–72] (Fig.
420 2f, g); and the presence of odd numbered spermathecae in some sicariids [17,66,67,77]
421 and ochyroceratids [16,61,78] (Fig. 2 b, c). Both phenomena can sometimes generate a
422 seemingly asymmetric morphology. Although good illustrations and photographs of
423 these are available in literature (e.g. figs. 20: Magalhaes, Brescovit, and Santos [17];
424 figs 14 and 19: Li *et al.* [16]; fig. 8: Lin, Pham and Li [20]; fig. 7: Wang and Li [70])
425 only some cases in the Sicariidae [17,66] have reported intraspecific variation.

426
427 A correct interpretation of the type of asymmetry based only on the available literature
428 is complicated. Many cases describe single specimens or small samples and do not
429 include enough information to assess the character variation within the species. This is a
430 key piece of information since the proportions of forms within the population are crucial
431 to distinguish the type of asymmetry and the evolutionary mechanisms behind it. Here
432 we include examples that, to the best of our knowledge, fit the definition of each type of
433 genital asymmetry and give hypotheses that could explain their origin.

434
435 **Fluctuating Asymmetry (FA).**— This kind of asymmetry is defined by van Valen [5]
436 as “the inability of organisms to develop in precisely determined paths”. In other words,
437 FA describe random morphological fluctuations around a symmetric mean
438 [3,5,33,79,80]. FA incidence, relation to environmental factors, and its influence within
439 populations has been studied on some Lycosidae and Pholcidae [17–21]. Here we found
440 that some cases, like the hahniid *Neoanthistea*, some oonopid and telemid genera
441 (mentioned as FA* in Table 1), and other “malformed” specimens in literature might be
442 cases of FA. Similarly, the great intraspecific variation observed in the female genitalia

443 of some sicariids [17,66], range from asymmetries in number, size and shape of
444 spermathecae to almost symmetric structures. This suggests that asymmetries in this
445 family and similar cases in the ochyroceratid *Althepus* [16,61] might be fluctuating.
446 Nevertheless, most FA asymmetries require more specimens and careful examination to
447 determine better the nature of the observed phenotypes. A few species that show AS
448 (*Scotinella britcheri* and *S. fratella*) and all species with CA (*Cithaeron praedonius*,
449 *Jacaena mihun*, among others) show great morphological variation of female internal
450 genitalia within the population; however, these variations are never around a symmetric
451 mean and thus we do not consider them to be fluctuating.

452

453 **Antisymmetry (AS).**— We found this type of asymmetry in two different families and
454 at least four genera; notably, all known species of *Echinotheridion* and *Tidarren* share
455 this trait. This specific case of AS induced by an uncommon genital automutilation
456 behavior may also be the best studied and understood. In these theridiid genera, male
457 spiders show no preference for either left or right pedipalp self-emasculatation, and no
458 selection of right or left form by females has been observed. Likewise, females of
459 *Scotinella britcheri* and *S. fratella* show two basic forms with some range of variation
460 in-between but no significant predominance within the studied population [42]; *Asygina*
461 *huberi* and *A. coddingtoni* [22], and probably some *Teutamus* species like *T. brachiatus*,
462 *T. poggi*, and others (as illustrated by Dankittipakul, Tavano, and Singtripop [48]) also
463 fit this AS model. Likewise, some asymmetric species in the Ochyroceratidae,
464 Oonopidae, Pholcidae, and Telemidae could show AS. However, larger numbers of
465 specimens are needed to identify the proportion of forms and type of asymmetry.

466

467 Palmer (1996) divided asymmetry as genetic (larval) and external (post-larval)
468 depending on the developmental stage where it is originated. In spiders, genital
469 development is only apparent after the last molt. Therefore, the exact moment where AS
470 appears, especially in females, is difficult to interpret. Evidence on snails [81],
471 crustaceans [6], and insects [6,76,82] suggest genetic AS to be an evolutionarily
472 unstable or transitional state between symmetry and DA, or even a reversal phase from
473 DA [3,6]. Similarly, a genetic assimilation process of external AS could ultimately lead
474 to DA [3,6,83]. We consider the few confirmed spider AS to represent both types of
475 asymmetry: genetic in *Asygyna* and *Scotinella*, and external in *Echinotheridion* and
476 *Tidarren*.

477

478 Another interesting observation is the sex biased incidence of AS. This seems to be also
479 the case in some insect groups like Odonata, Orthoptera, Mantodea, and others [1,2,76].
480 In *Asygyna* and *Scotinella*, asymmetry has only been reported in females; while the
481 theridiids *Echinotheridion* and *Tidarren* only show asymmetry on male pedipalps.
482 Appearance of AS in *Asygyna* and *Scotinella* might be related to an intrasexual
483 competition between females; while, AS in *Echinotheridion* and *Tidarren* is considered
484 an example of antagonistic co-evolution derived from the extreme size dimorphism
485 between sexes [14,41,50–52]. Neither mechanical, behavioral nor functional
486 differentiation between chiral forms has been reported in the cases above.

487

488 **Chaotic asymmetry (CA).** — This new category of asymmetry does not fit the
489 definition of any of the three traditional types. Females usually develop long and
490 convoluted copulation ducts where the great variation between specimens does not
491 allow a clear distinction between a dextral and sinistral form. All known examples of

492 this type of asymmetry are found in the Entelegynae clade. Platnick [73] mentioned for
493 *Cithaeron praedonius* (Cithaeronidae): “No two females show identical patterns of
494 epigynal duct coiling; for that matter, no individual specimen shows identical coiling of
495 the ducts of the right and left sides”. Similar morphological variation (Fig. 3d-h) has
496 been observed in some species of the following genera: *Apopyllus* (Gnaphosidae) [25],
497 *Neoantistea* (Hahniidae) [26], *Moreno* (Prodidiomidae) [74], *Jacaena*, (Liocranidae)
498 [49] and *Trachelas* (Trachelidae) [27,43,44].

499

500 The origin of these internal genital modifications has not been investigated and its
501 relation to a functional differentiation between sides or packing of other internal organs
502 cannot be ruled out. We hypothesize that the development of this kind of asymmetry is
503 related to complexity in internal female genitalia and this could explain the absence of
504 examples in the genitally simple Synspermiata. The absence of a clear right/left
505 pattern and great variation between individuals suggest that copulatory duct shape is not
506 under a strict selection. This might be related to a simplification in pedipalp sclerite
507 complexity and embolus length (as seen in *Trachelas*, *Jacaena* and *Moreno*). In
508 contrast, some *Apopyllus* males have fairly complex male genitals with an extremely
509 long embolus that usually coils around the bulb. Female ducts show slight asymmetries
510 between right and left sides and authors mention internal variation between conspecific
511 females. This genus also shows intraspecific variation in the RTA and external genitalia
512 and it is hypothesized to be an instance of male-female coevolution [25]. The cases of
513 *Cithaeron indicus*, *Moreno ramirezi* and both *Neoantistea* species are doubtful; in the
514 former, the male is not known, and in *Moreno* and *Neoantistea*, species were described
515 based on just one female or variation was not documented; the observed asymmetry

516 could be fluctuating, antisymmetric, a developmental abnormality or even an artifact of
517 preparation.

518

519 If pedipalp bulb sclerite reduction is related to the appearance of CA, the question
520 would be why is it so rare? Within Entelegynae, several groups have reduced male
521 pedipalp complexity; however, CA has not evolved nearly as many times. This might be
522 explained by the evolution of long and convoluted CD prior to male sclerite reduction
523 which would, hypothetically, reduce selective pressure on the female copulatory ducts.

524

525 **Directional asymmetry (DA).**— In insects, DA is the most common type of
526 asymmetry [1,2]; however, in spiders, DA seems to be quite rare. So far, in
527 Synesperiata, only the pholcid *Metagonia mariguitarensis* [8] had been confirmed as
528 DA; however, there are reports of consistent one-sided asymmetries in other members
529 of this clade. In *Escaphiella gertschi* and *E. itys*, all examined males have
530 developmental differences between right and left pedipalp [46]. More asymmetric male
531 pedipalps have been described for three *Panjange* species of the *lanthana* group [10],
532 *Aschnaonops marta* [57], at least six species of *Paradysderina* [19], and several
533 species of *Escaphiella* [46]. Likewise, female internal genitalia of *Mesovolivar yurani*
534 [15]; and some species of *Paradysderina* [19], *Reductoonops* [59] and *Triaeris* [60]
535 show asymmetries that seem to be consistent within their samples; nevertheless, the
536 number of specimens examined in many cases is too small to confirm directionality.

537

538 The story seems to be different for Entelegynae spiders where more complex
539 development of genitals might inhibit the evolution of directional asymmetry. Although
540 implicit in the description of *Teutamus politus* female genitalia by Deeleman-Reinhold

541 (2001), the present study is the first report of DA in the entelegyne clade. *Teutamus*
542 *politus* is also the first example of developmental male genital asymmetry in the
543 Entelegynae. Previously, male asymmetry in this clade was only known from
544 teratogenic specimens and the unique AS phenotype created by self-emasculatation in
545 *Tidarren* and *Echinotheridion*.
546
547 Putative cases of male DA in *Escaphiella* and other oonopids may not be related to
548 modifications in female genitalia [46,57] but to functional segregation of the right and
549 left pedipalps. Similarly, the genus *Triaeris* has many cases of female genital
550 asymmetry that have not been linked to male pedipalp modifications. In fact, some
551 species of this genus are believed to be parthenogenetic [60]. In contrast, directional
552 genital asymmetries in *M. mariguitarensis* and *T. politus* have been found in both sexes,
553 which might indicate that selection by female choice is the underlying cause. In these
554 species, males would have to change morphologically or modify mating positions to be
555 able to have successful copulation. Morphological modifications in both sexes have
556 been confirmed for *M. mariguitarensis* [8] and *T. politus*; however, the implications for
557 mating behavior continue to be a mystery.
558
559 Changes in mating position have been suggested to be associated with many cases of
560 DA in insect genitalia [1,4,11]. Unfortunately we were not able to test this in the case of
561 *T. politus* using live specimens; nevertheless, observations in *Agroeca* Bristowe (1958)
562 and other RTA spiders [12,85] suggest that copulation is achieved by the male climbing
563 over the female and stretching over a side while the female slightly turns her abdomen;
564 this process is alternated between right and left side. In *T. politus*, female genital
565 opening location makes it virtually impossible to have successful mating attempt from a

566 right-side position. Instead, a male must insert both pedipalps always from the left side
567 in relation to the female body. Morphological modifications like: left bulb being slightly
568 bigger (Fig. 5c, f), having a ventrally flattened tegulum (Fig. 5f), and a straight conical
569 conductor (Fig. 5f) instead of the flattened, hook-shaped conductor of the right side
570 (Fig. 5c) are consistent with this hypothesis. In addition, this evidence seems to back the
571 hypothesis discussed by Schilthuisen (2013) and Huber, Sinclair, and Schmitt (2007)
572 stating that in spiders asymmetry is most likely female-initiated and male changes
573 appear as an evolutionary response.

574

575

576 **Conclusions**

577

578 Genital evolution is a complex and interesting topic. The appearance of asymmetric
579 morphologies is a puzzling phenomenon that has often been overlooked. Here we
580 reported *T. politus* as the first case of directional asymmetry, and the first
581 developmental asymmetry in male genitals in Entelegynae. We also searched for as
582 many cases as possible in taxonomic literature; however, many more might be waiting
583 to be (re)discovered. Our review showed that there have been multiple origins of genital
584 asymmetry in at least thirteen families, and in some cases (e.g. Oonopidae, Pholcidae,
585 Theridiidae, Liocranidae) two or more within the same family. A correct assessment of
586 genital asymmetry based on taxonomic legacy literature is difficult mainly due to the
587 lack of data, description and illustration biases, and number of specimens and variation
588 descriptions.

589

590 As has been shown by previous works on genital asymmetry in insects and spiders,
591 there is no single explanation for the evolution of this trait, but some generalizations can
592 be made. In contrast to insects and other arthropod groups, the low number of genital
593 asymmetric species in spiders might indicate that the appearance of these morphological
594 modifications might reduce subsequent speciation rates or even increase extinction
595 rates; specialized lineages tend to have a reduced capacity to diversify and therefore
596 might be considered evolutionary dead ends [86]. However, our observations indicate
597 that cases of sexual asymmetry in spiders, although rare, are more common than was
598 previously thought. Furthermore, they have evolved independently several times but
599 rarely appear isolated and most of the times seem to be clustered within a genus or
600 closely related genera, as in the cases of Oonopidae, Pholcidae, Theridiidae, and
601 probably Liocranidae. The evolution of genital asymmetries in spiders might be a good
602 candidate to be tested as a potential evolutionary dead end.

603

604 Several hypotheses for the appearance of asymmetry in spiders have been proposed and
605 include natural selection, sexual selection by female choice and antagonistic co-
606 evolution (not mutually exclusive). We considered *Echinotheridion* and *Tidarren* to be
607 examples of antagonistic co-evolution where the male has evolved self-emasculatation in
608 response to the extreme sexual dimorphism in size and aggressive behavior in the
609 female. No selection between left and right is apparent in these genera, thus no
610 directionality is observed. DA cases like *T. politus* seem to support the hypothesis that
611 correlates changes in mating position to genital asymmetry; however, other examples
612 still need to be studied. DA in *T. politus* and some pholcid examples, AS in *Scotinella*
613 and *Asygyna*, and CA cases in *Jacaena*, *Cithaeron* and *Trachelas* support the hypothesis
614 of female-initiated asymmetry in spiders; however, male DA in Oonopidae and AS in

615 some theridiids conflict with this explanation. Further and more detailed study on
616 internal genitalia and comparative study of male right and left pedipalps may yield new
617 and valuable information to explain the evolutionary pattern of genital asymmetry. We
618 hope that this review will aid in the study, development and testing of hypotheses on
619 sexual evolution. We specifically hope it sparks discussions on the complex interactions
620 between males and females, and appearance of interesting phenomena like genital
621 asymmetry.

622

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631

632

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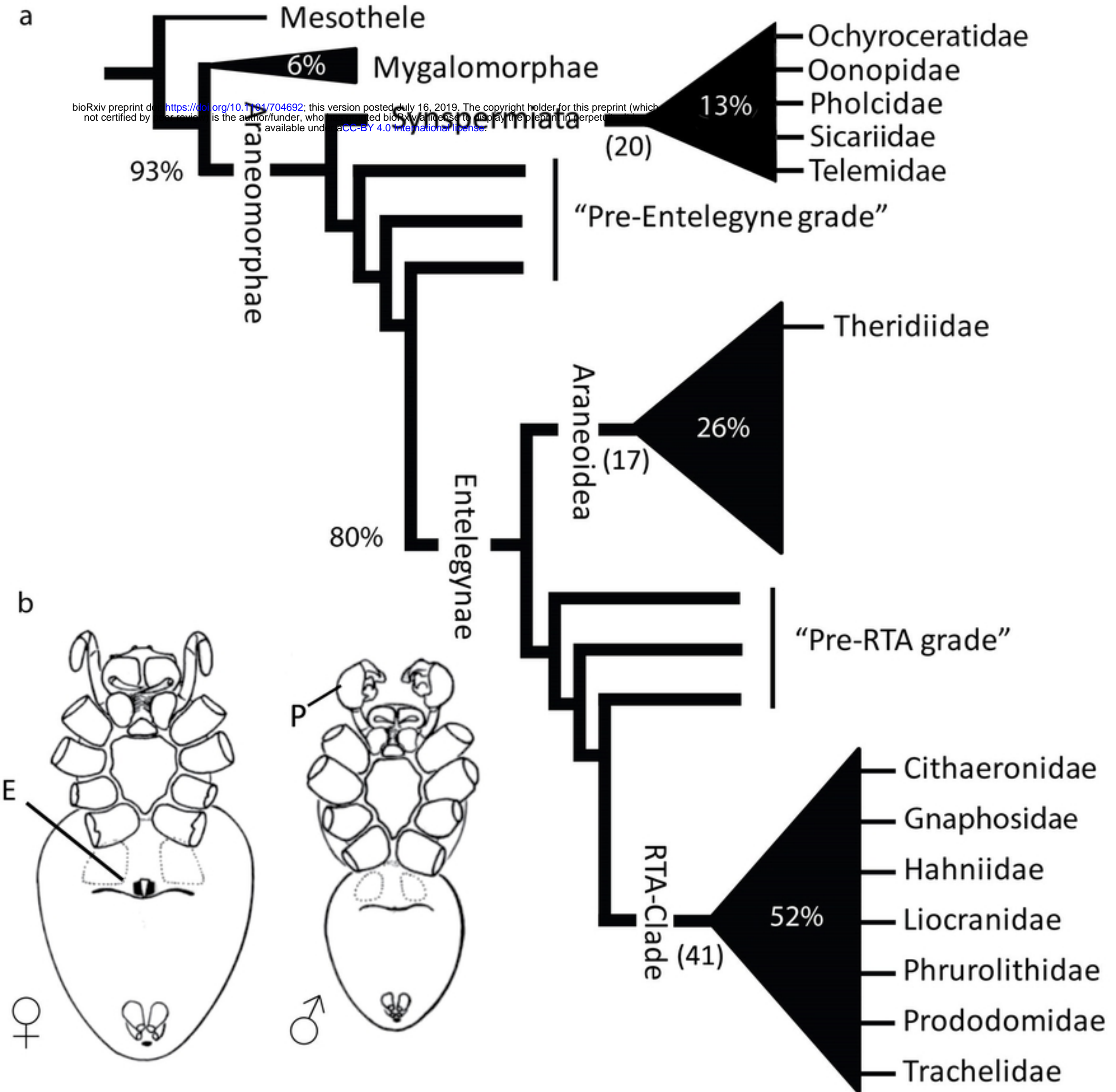
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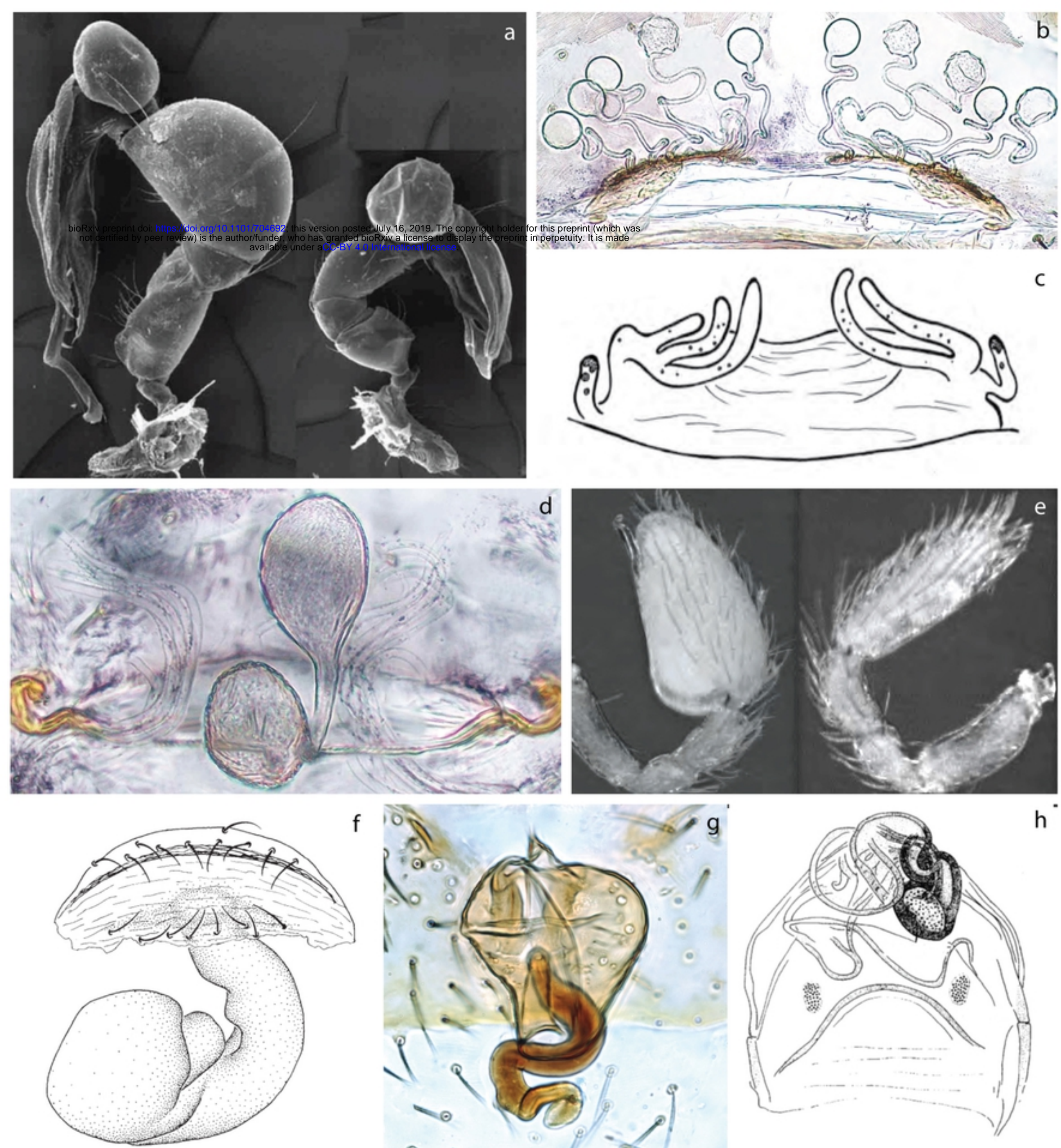


Figure 2

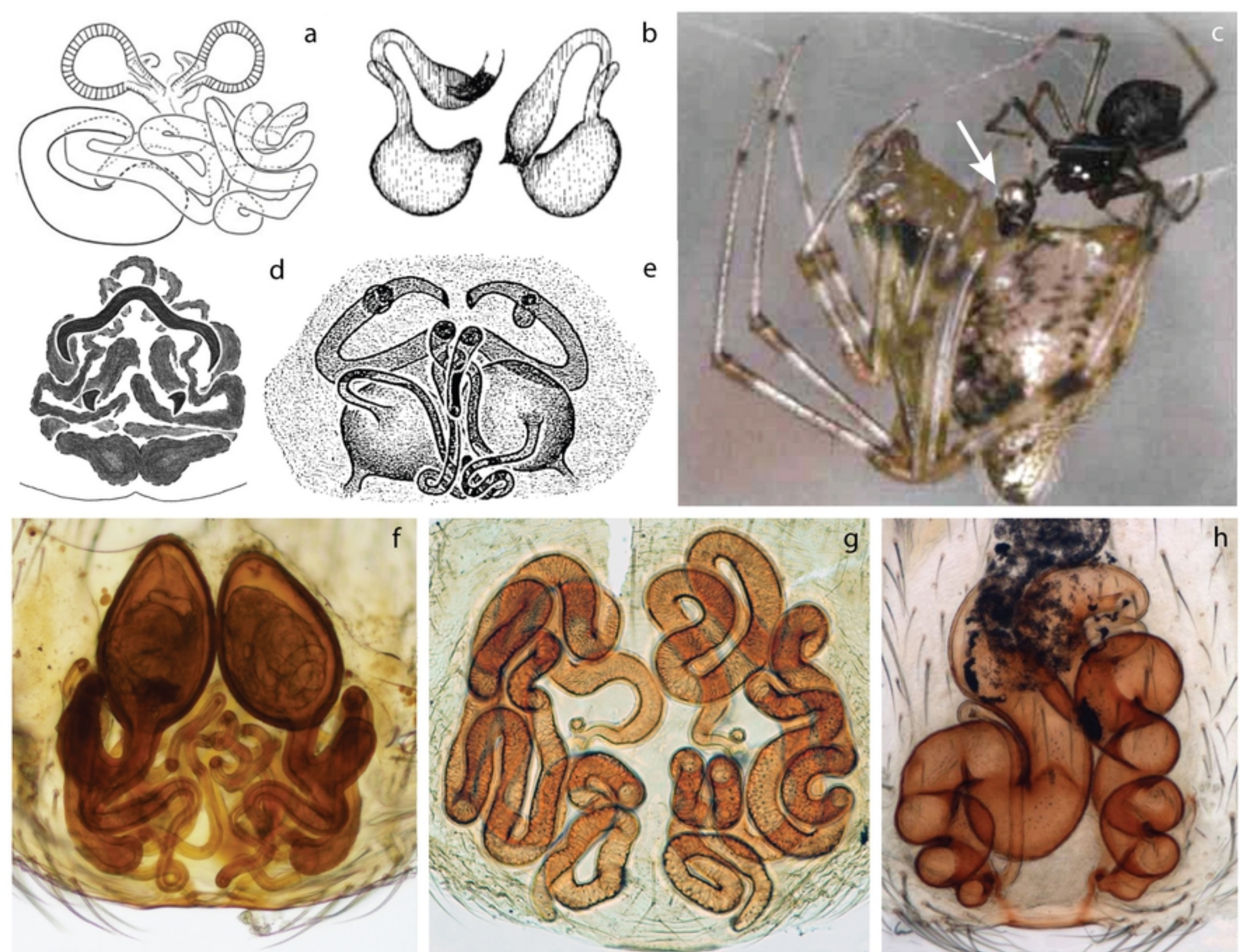


Figure 3

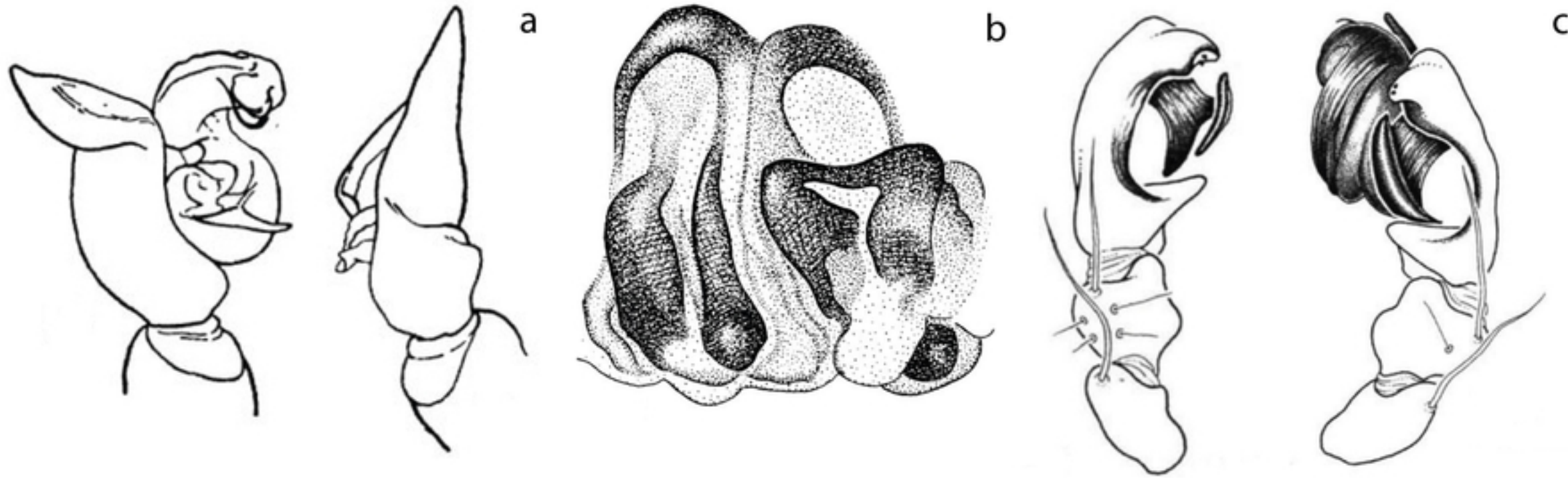
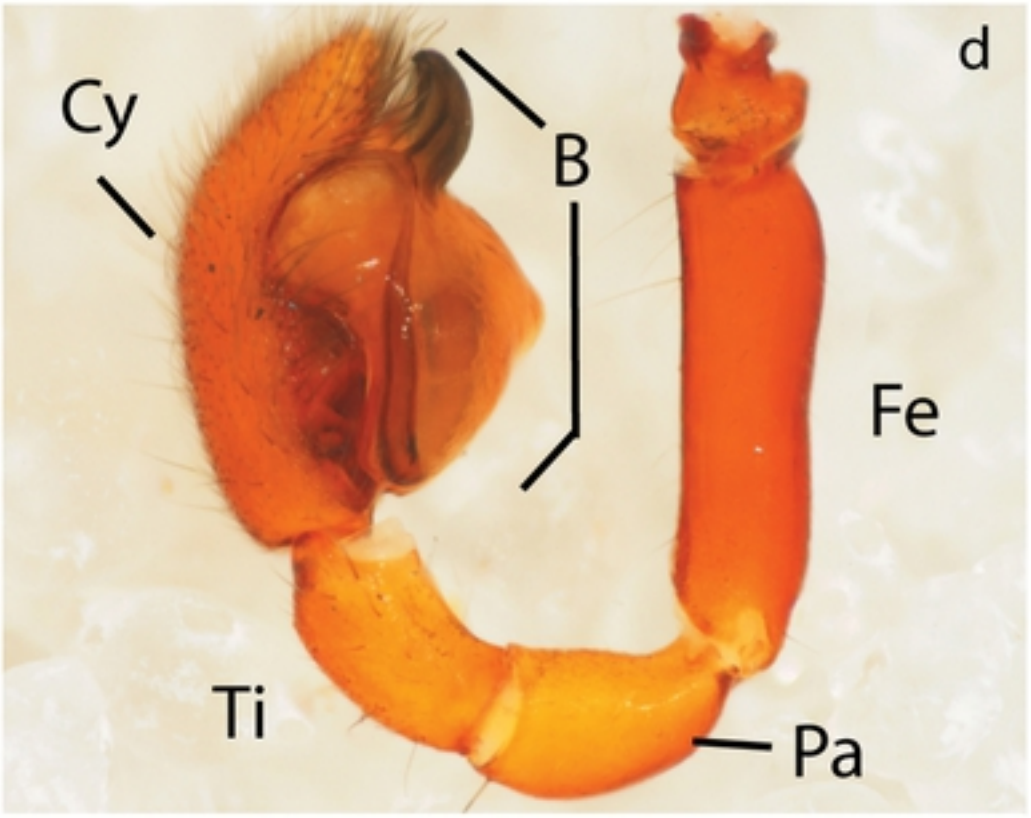


Figure 4



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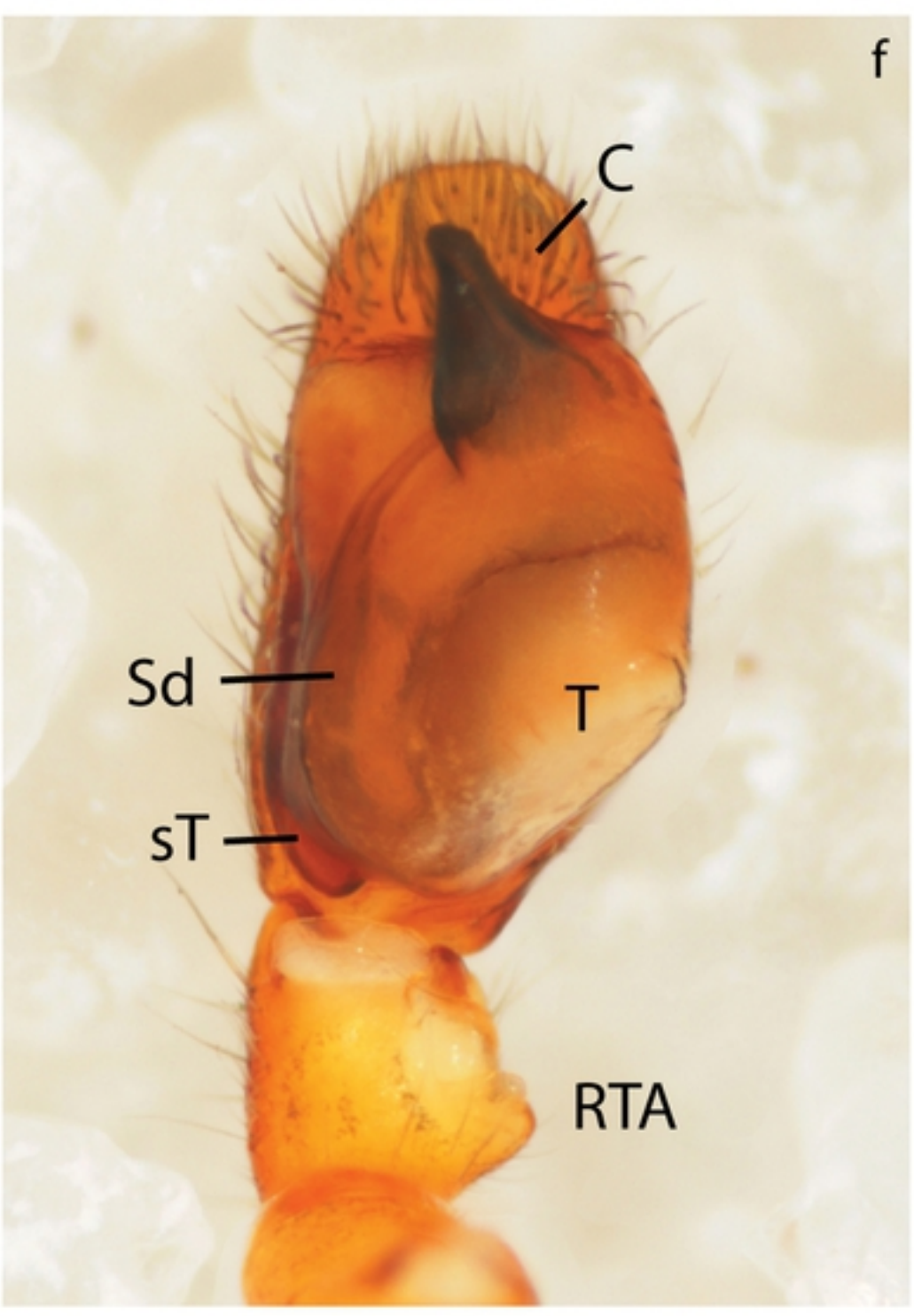
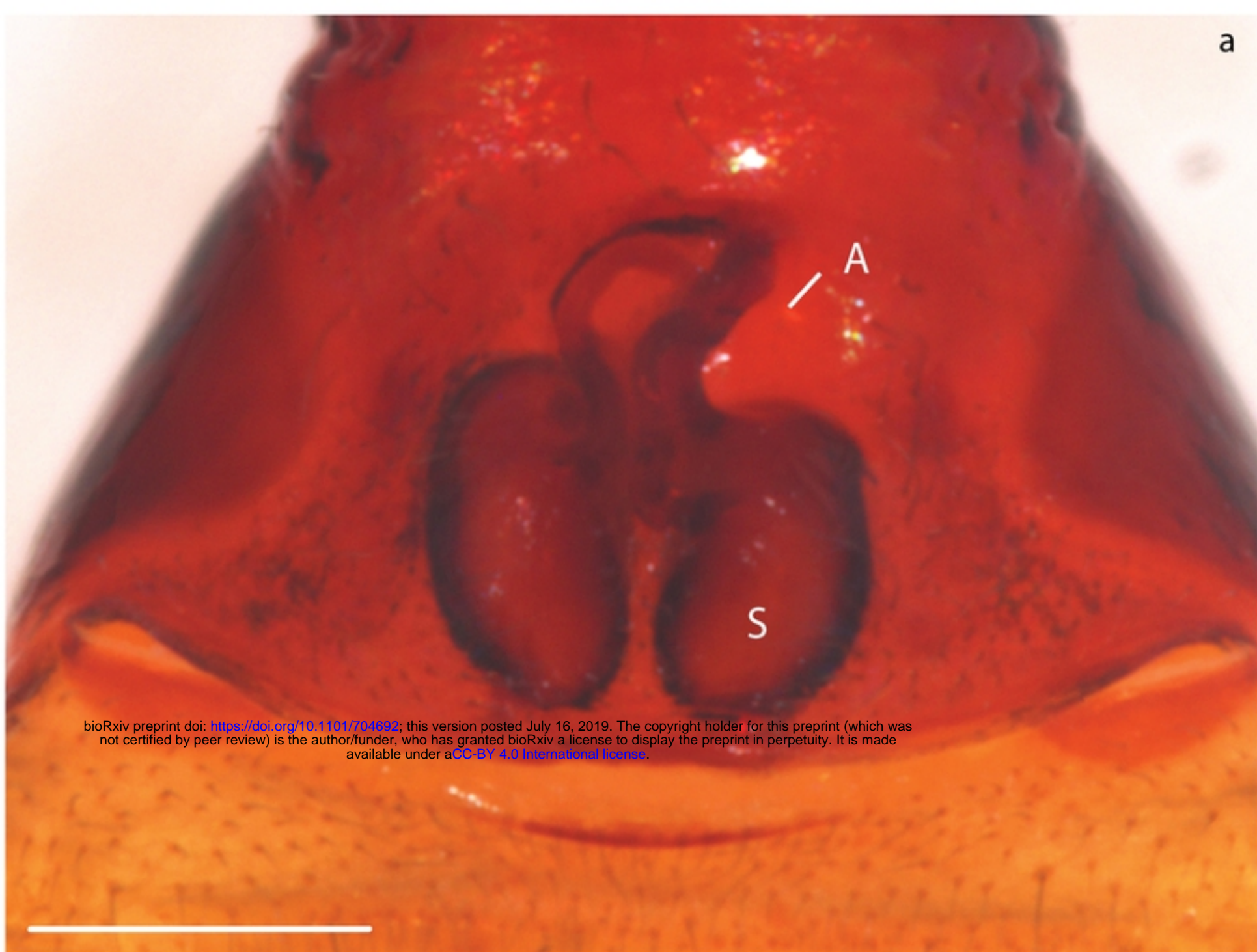


Figure 5

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Figure 6



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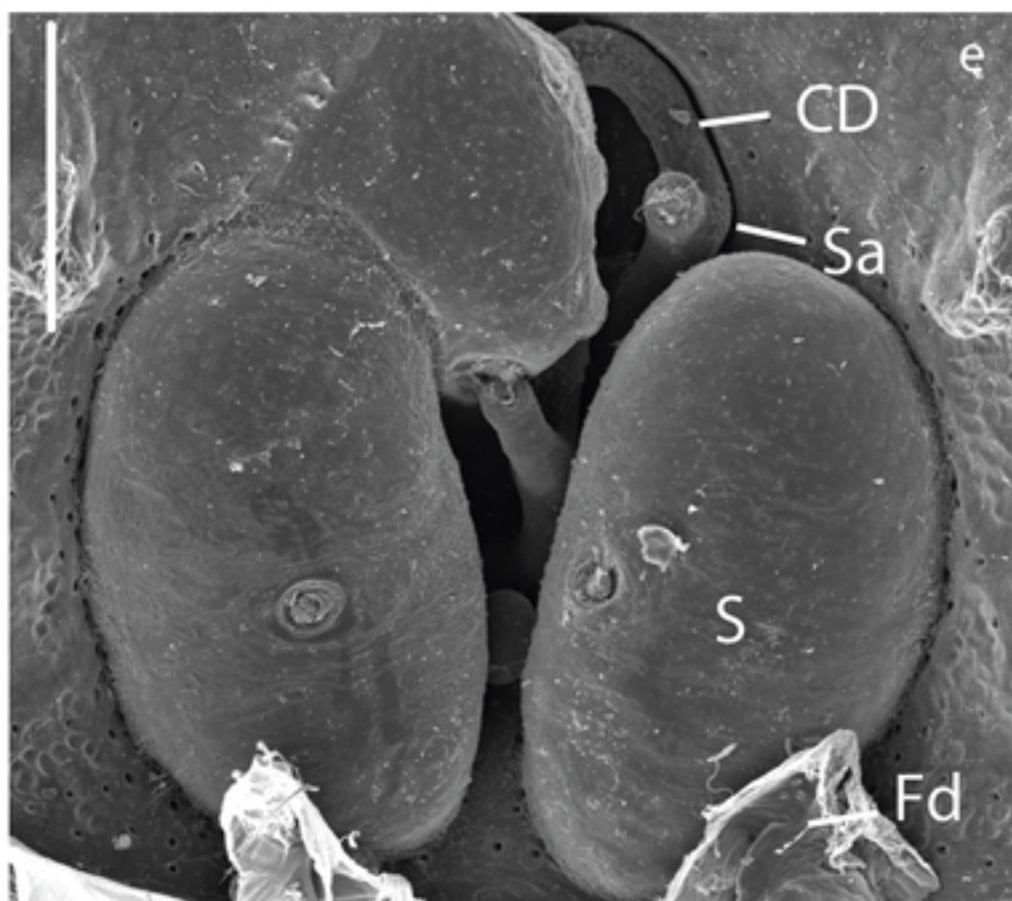
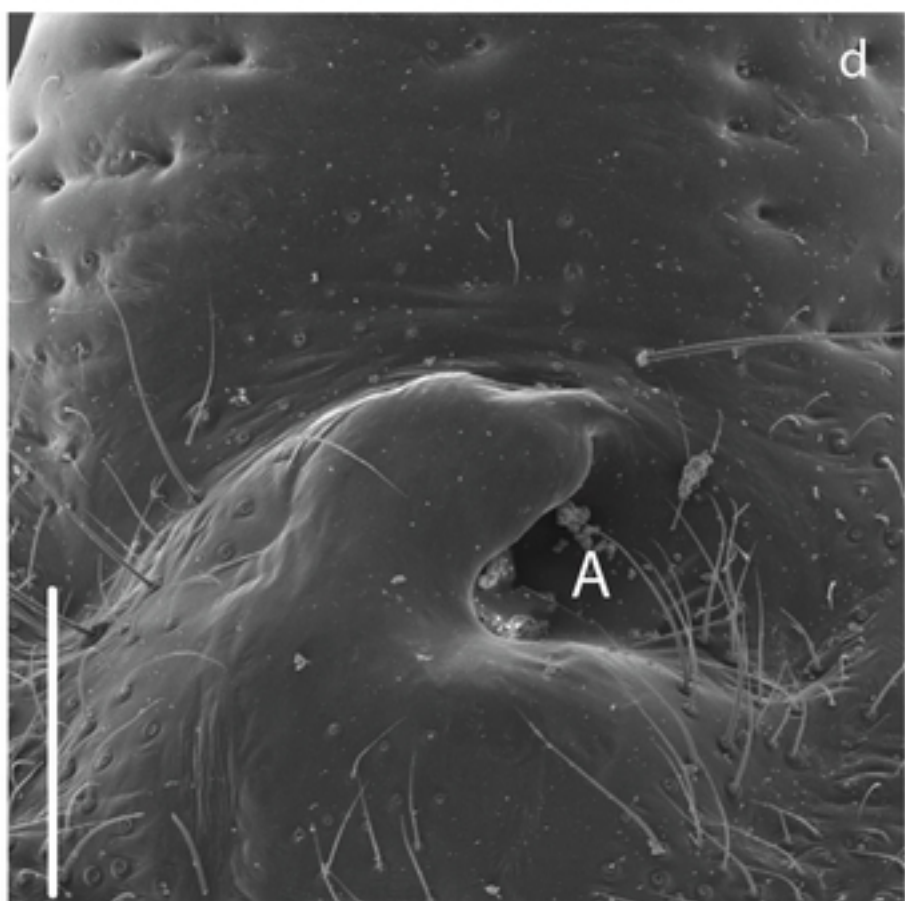
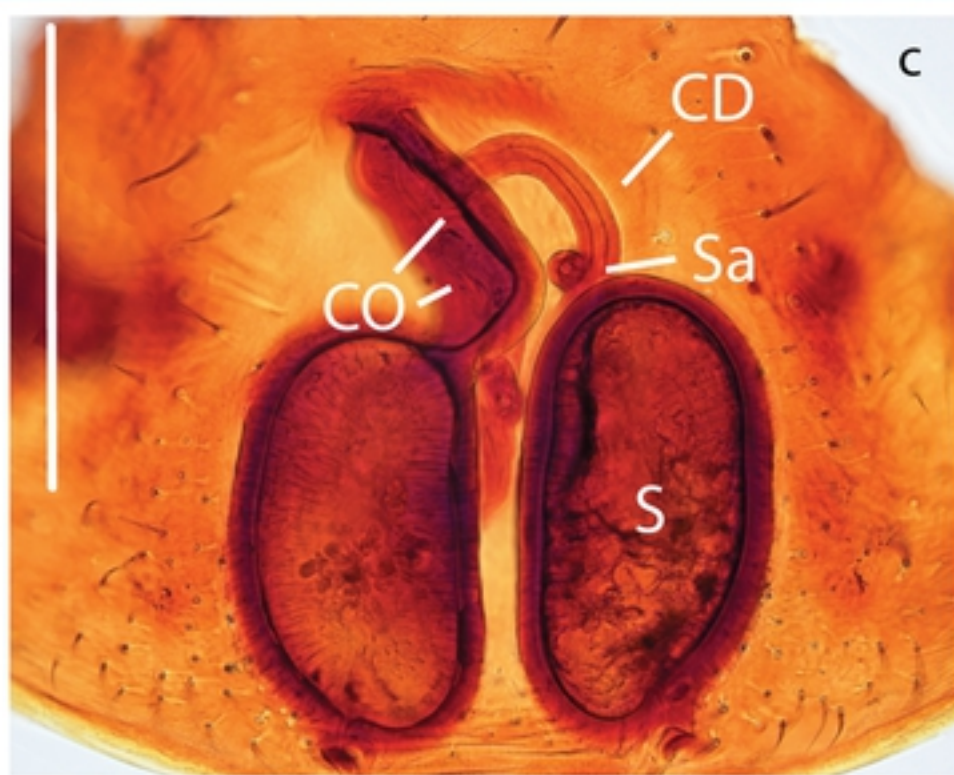
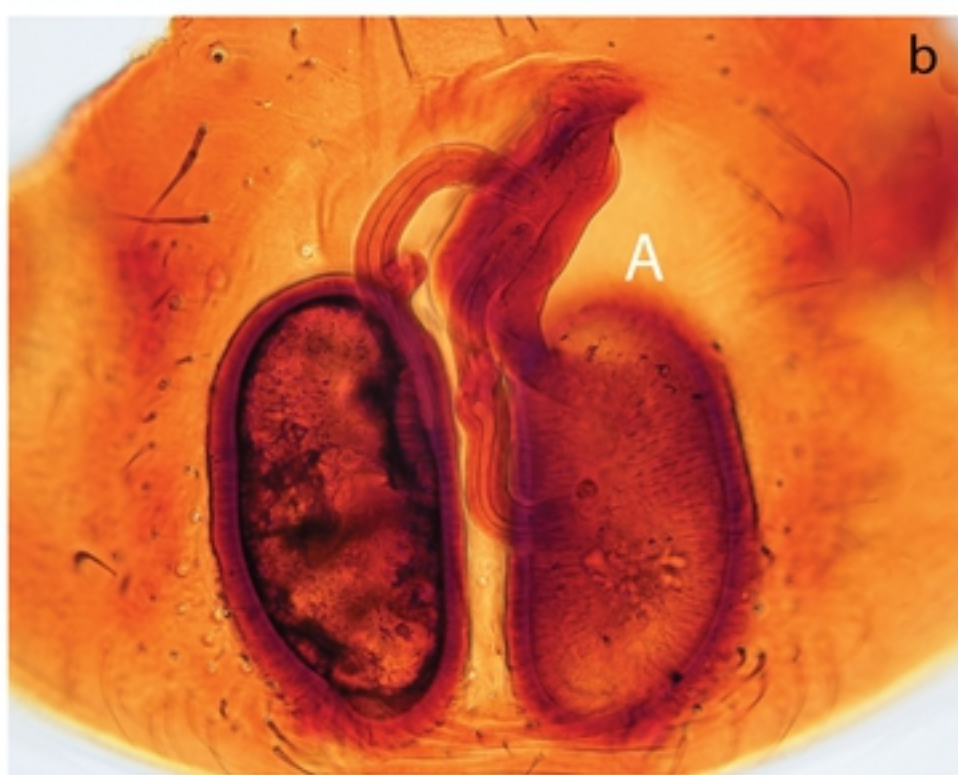


Figure 7

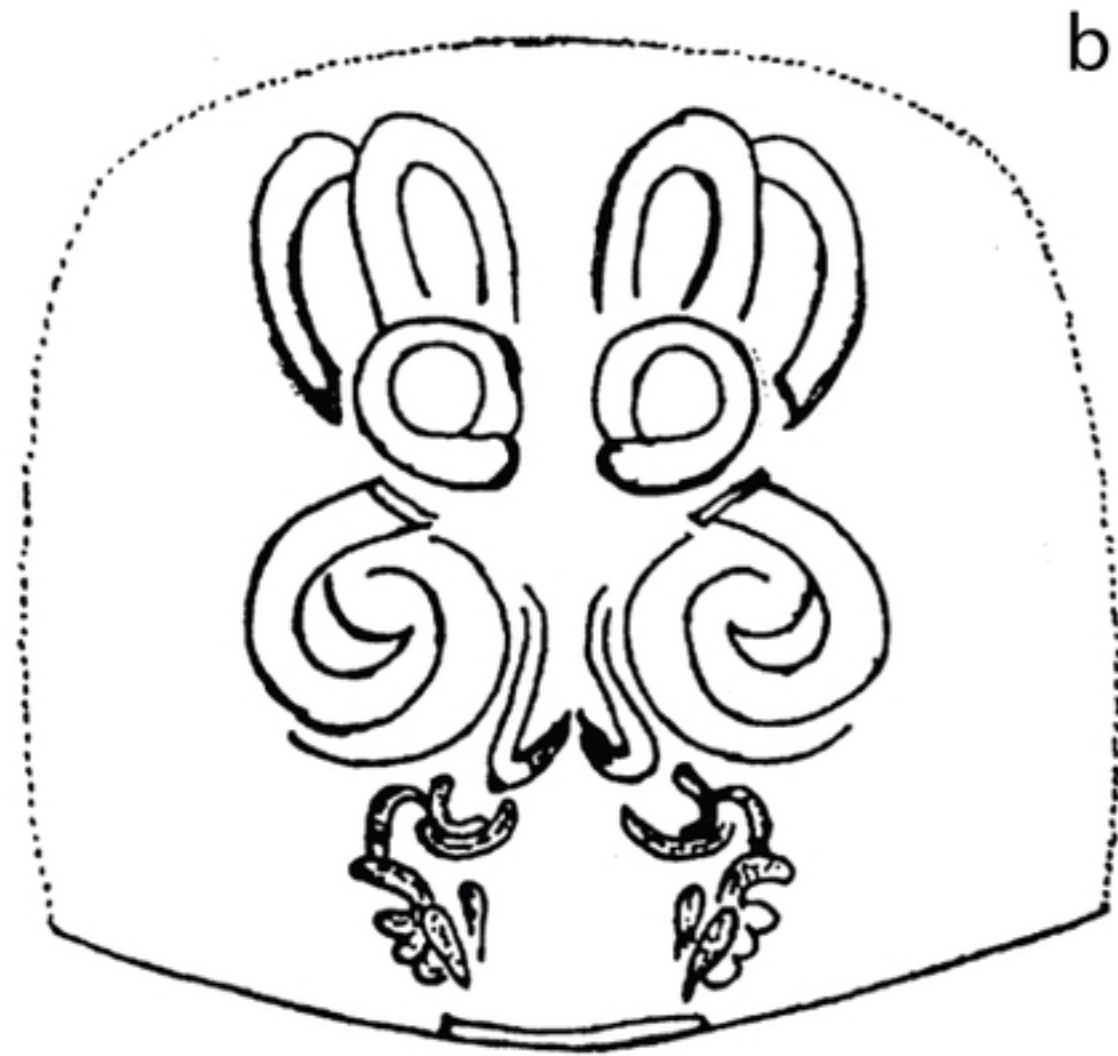
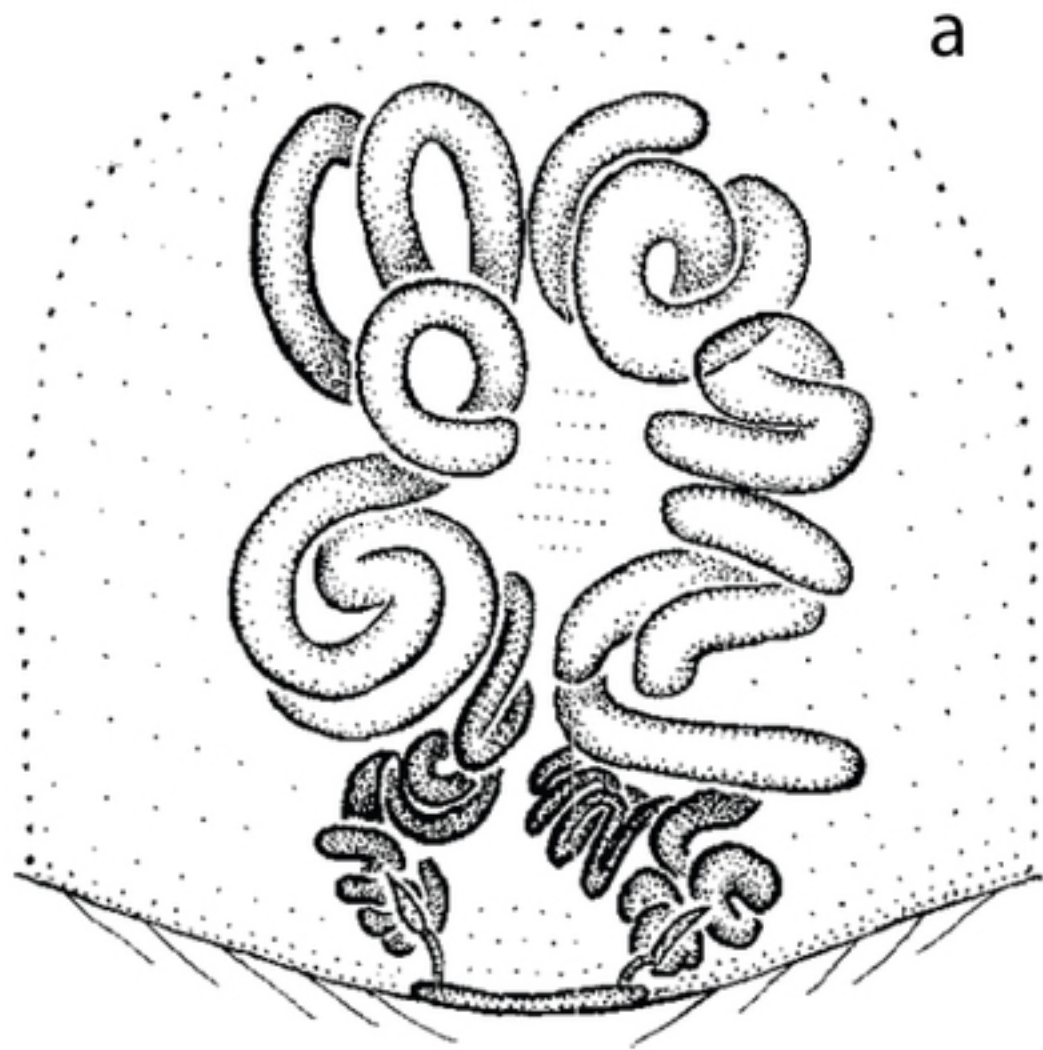


Figure 8