

1 A defensive body plan was pre-adaptive for termitophily in the rove beetle tribe
2 Termitohospitini (Staphylinidae: Aleocharinae).

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19

20 ABSTRACT

21

22 Termitophily—the symbiosis of organisms with termite societies—has evolved a
23 disproportionate number of times within the rove beetle subfamily Aleocharinae (Staphylinidae).
24 Among aleocharine termitophiles, defensive (limuloid) and mimetic (physogastric &
25 physothoracic) body forms have evolved convergently, but due to lack of a comprehensive
26 aleocharine phylogeny, the context in which termitophily and associated adaptations evolve is
27 unknown. We present the first example of a robust, morphology-based phylogenetic placement
28 of an exclusively termitophilous tribe, the Termitohospitini. Termitohospitini is recovered to be
29 nested within Myllaenini *sensu nov*, and sister to Myllaena (new synonymy). Furthermore, we
30 also recovered the small tribe Masuriini nested within Myllaenini *sensu nov* (new status).

31 Reconstructing ecological transitions within this clade, we present evidence that the stem lineage
32 of Myllaenini sensu nov invaded intertidal marine habitats, the common ancestor for Myllaena +
33 Termitohospitini then transitioned to freshwater riparian habitats, with Termitohospitini alone
34 subsequently shifting to termitophily. We conclude that: (1) Termitohospitini was ancestrally a
35 limuloid-bodied riparian inhabitant; (2) a limuloid form may have been pre-adaptive for defense
36 against host attack during the evolution of termitophily; (3) the strongly tapered abdomen of an
37 ancestral limuloid body was a constraint on the evolution of physogastry, leading to the
38 emergence of the unusual physothoracic body form observed in termitohospitines that likely
39 integrates these obligate termitophiles to life inside termite colonies.

40

41 KEYWORDS

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43 Intertidal, adaptation, coevolution, phylogeny, systematics, Myllaenini, Pronomaeini, Masuriini,
44 marine, Isoptera

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47 “one of the most astonishing spectacles in all natural history.” — Richard Dawkins

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50 INTRODUCTION

51

52 Some of the most ecologically complex and taxonomically diverse organismal
53 interactions center around the societies of social insects, in particular those of ants and termites
54 (Rettenmeyer et al. 2011, Kistner 1979, 1982, Hölldobler & Wilson 1990, Parker 2016).
55 Organisms that have evolved symbioses with ants and termites are termed myrmecophiles and
56 termitophiles, respectively, and as a group these symbionts display a bewildering array of
57 morphological and behavioral specializations. Among termitophiles, two body forms, mimetic
58 (Figs. 8–9B) and defensive (Figs. 1–7), have convergently evolved numerous times. Mimetic
59 species typically possess swollen “physogastric” or “physothoracic” (Figs. 8–9B) body forms—
60 the consequence of increased fat body growth that results in a grotesquely expanded body,
61 stretching out the intersegmental membrane between sclerites. Physogastry is thought to

62 transform the sclerotized body into one that resembles the relatively soft-bodied host termites
63 (Cunha et al. 2015), often to a striking degree (Dawkins 1996, Kistner 1969). In such species,
64 body segments begin to distend soon after the adults eclose (post-imaginal growth) (Kistner
65 1969), and in some instances elongation of leg segments even occurs, breaking the cardinal rule
66 that arthropods grow by moulting. In defensive, or limuloid (horseshoe crab shaped) groups, the
67 abdomen is posteriorly tapered, lateral margins of the body are typically expanded and head
68 deflexed, protecting vulnerable appendages dorsally from attack (Figs. 1–4, 6–7) (Kistner 1979,
69 Parker 2016).

70 Across the Arthropoda, termitophily has evolved only rarely, if at all (REF #####). One
71 exception is the rove beetle subfamily Aleocharinae, where this lifestyle has arisen at least 11
72 times, and over 650 described species are known to be symbiotic with termites (Kistner 1969,
73 Kanao et al. 2012). Such an exceptional predisposition to evolving this type of symbiosis raises
74 an immediate question over potential traits that might be pre-adaptive for the aleocharine
75 capacity to successfully invade termite nests. One approach to identifying putative pre-
76 adaptations is to explore the evolutionary interplay between morphological and ecological
77 specializations leading up to independent origins of termitophily across the Aleocharinae
78 phylogeny. However, a major impediment exists in the lack of phylogenetic resolution in
79 Aleocharinae, and in the social insect symbiont lineages in particular (Ashe 2007). Consequently,
80 we have little understanding of the ecological contexts within which termitophily evolves among
81 aleocharines, and the character transformations leading to the specialized morphologies seen in
82 many termitophilous clades. This phylogenetic impediment is particularly due to the taxonomic
83 challenges one confronts with Aleocharinae; their small size often obscure species level
84 differences, and vast taxonomic diversity impedes their study (there are 16,468 described species,
85 making them the largest animal subfamily [Newton unpublished data]) (Ashe 2007).
86 Compounding the situation, social insect symbionts are notoriously challenging to collect due to
87 their low abundances (similar to army ant symbionts [Kistner 1979]) and difficulty in extracting
88 them from inside nests (Kistner 1998, personal observations).

89 During a survey of aleocharine morphology, conducted for a comprehensive
90 morphological treatment of the subfamily (Eldredge in prep.), we discovered that the lacinia (a
91 food gathering structure of the mouthparts [Betz et al. 2003]) of one specialized termitophilous
92 tribe, Termitohospitini (Figs. 1–10B, S4), is strikingly similar to Myllaenini (Figs. 11A–11B, S4),

93 a tribe notable for genera that have specialized on marine-intertidal and freshwater-margin
94 habitats, but which are entirely free-living. Furthermore, a previous study has suggested that the
95 particular lacinial morphology of Myllaenini is in part characteristic for the tribe (Ahn & Ashe
96 2004). Despite the marked ecological disparity between termitohospitines and myllaenines,
97 further examination revealed the genus Myllaena (Myllaenini) and many termitohospitines
98 shared a unique sensory organ on the aboral surface of maxillary palpomere 3 (Figs. 12A–12B).
99 Therefore, we hypothesized a priori that a phylogenetic analysis would reveal the inclusion of
100 Termitohospitini within a broader Myllaenini, and a sister-group relationship between Myllaena
101 + Termitohospitini. Here, we present the first instance of a robust phylogenetic hypothesis
102 concerning the common ancestry of a termitophilous aleocharine lineage, Termitohospitini,
103 providing a unique opportunity to explore the sequence of morphological and ecological
104 character evolution leading to the origin of symbiosis in a termitophilous aleocharine lineage.

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106

107 Materials and Methods

108

109 Specimen Work

110

111 Specimens for this study were made available by the Snow Entomological Museum
112 Collection (University of Kansas) and numerous field expeditions conducted by Kanao and
113 Maruyama (also see Acknowledgements). We again emphasize that symbionts of social insects
114 are exceedingly rare and the difficult to collect. Symbionts number one per thousands of colony
115 members, and nests are often subterranean and inaccessible (Kistner 1979, 1998).

116 To examine the morphology, typical procedures were taken of KOH tissue digestion,
117 chlorazol black staining, examination in glycerin, and permanent mounting in Euparal (for
118 details see Eldredge 2012 Hanley & Ashe 2003, Maruyama 2004). Light- (Olympus SZX7
119 stereomicroscope and Olympus BX51 compound microscope) and scanning electron microscopy
120 was used for morphological examination. Whole body images were taken using a Visionary
121 Digital™ BK Plus Lab System with Cannon EOS 7D. Images were captured from multiple focal
122 planes and combined with CombineZM

123 (<http://hadleyweb.pwp.blueyonder.co.uk/CZM/News.htm>).

124

125 Dataset Construction

126

127 For this study, we modified the dataset of Ahn & Ashe (2004). Sixteen taxa and nine
128 additional characters were added for a total matrix dimension of 74 taxa by 108 characters (S1–
129 2). The dataset was constructed and handled in Mesquite v3.1.0 (Maddison & Maddison 2016).

130

131 Phylogenetic Analyses

132

133 Parsimony analyses were done with TNT v1.1 (Goloboff et al. 2000) implementing ratchet
134 and drift algorithms to increase exploration of tree space, and 1,000 bootstrap replicates were
135 performed. Analyses were done locally.

136 The Bayesian reconstructions were analyzed in MrBayes v3.2.6 (Ronquist & Hulsenbeck
137 2003) using the Mkv model (Lewis 2001). Several strategies to account for among site character
138 rate differences were implemented by modifying the Mkv model. Gamma rates with four and
139 eight rate categories were implemented in MrBayes v3.2.6 hosted by CIPRES Science Gateway
140 (Miller et al. 2010), and a log-normal distribution was implemented with a modified version of
141 MrBayes (Harrison & Larsson 2015) hosted by KINBRE (University of Kansas). Analyses were
142 run for at least 50 million generations and was monitored with Tracer v1.5 (Rambaut &
143 Drummond 2007) to confirm ample mixing. Sampled trees were summarized with DendroPy
144 v3.12 (Sukumaran & Holder 2010).

145 PAUP* v4.0b10 (Swofford 2002) and WinClada v1.00.08 (Nixon 2002) were used to plot
146 apomorphies. Ancestral state reconstructions were performed using a Mk likelihood
147 reconstruction available in Mesquite v3.1.0.

148 We initially began by reanalyzing the Ahn & Ashe (2004) dataset with the objective of
149 adding termitohospitine representatives for preliminary insight. During this course, we
150 discovered errors in the published dataset, preventing reconstruction of the presented results.
151 Through author correspondence the source of error was identified and corrections are reflected in
152 the dataset presented in this study (S2).

153

154 Results

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156 Phylogeny Reconstruction

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158 Parsimony

159 106 most parsimonious trees of 785 steps were recovered (majority rule consensus tree,
160 Fig. 14). Termitohospitini was recovered monophyletic with strong support (92% bootstrap),
161 notably supported by the anterior migration of the anterior tentorial pits (char. 99:1) and
162 antennae set in acetabullae (char. 100:1) (Figs. 13a–13b, S6–7). Further, Termitohospitini was
163 recovered sister to Myllaena, which in turn is sister to Dimonomera and nested deep within the
164 Myllaenini. The sister-group relationship of Termitohospitini and Myllaena was found to be
165 supported by the presence of a unique sensory structure on maxillary palpomere 3 (char. 103:1)
166 (Figs. 12a–12b).

167 A clade consisting of Myllaenini sensu Ahn & Ashe was recovered to share a most recent
168 common ancestor with Dysacryta and Masuria (Masuriini): Myllaenini sensu nov. Myllaenini
169 sensu nov is supported by, among others, the unique lacinial morphology of large interdigitating
170 setae (char. 26:2) (Figs. 11A–11B, S4). Pronomaeini was not recovered sister to Myllaenini
171 sensu nov.

172 The genus *Rothium* was rendered paraphyletic by *Lautaea murphyi*, which formed a clade
173 with *R. pallidus*, *R. giulianii* and *R. sonorensis*; *Brachypronomaea esakii* and *Polypea coralli*
174 formed a clade with *R. ashlocki* and *R. evansi*. The *Lautaea* clade is not supported by a clear
175 synapomorphy, but the *Brachypronomaea* clade is supported by the mentum being medially
176 squarely produced (char. 108:1).

177 A clade consisting of *Bryothinusa*, Genus1, Genus2, *Amazonopora*, *Dimonomera*,
178 *Myllaena*, and *Termitohospitini* was recovered in all analyses: *Myllaenini sensu stricto*, which is
179 supported by a four-articled mid tarsi (char. 105:1) among other characters, but no unambiguous
180 changes support this node.

181 The monophyly of *Bryothinusa* was unresolved in the Bayesian analyses, but was
182 recovered in the parsimony analysis. *Bryothinusa* monophyly is supported by an incomplete
183 infraorbital carina (char. 5:1) and quadrate pronotum (char. 57:3).

184 Very little support was recovered overall. Most of the recovered nodes failed to generate
185 any support, and those that did were concentrated at the deepest nodes and tips.

186

187 Bayesian

188 The general results were similar to those recovered from a parsimony analysis (Figs. 15–
189 16). Termitohospitini was recovered monophyletic and sharing a MRCA with Myllaena and
190 Dimonomera in all analyses with strong support. The general topology of Myllaenini sensu
191 stricto is similar with the parsimony results, but less resolved. In particular, the Bayesian
192 analyses failed to support the monophyly of Bryothinusa which remains unresolved. In all the
193 analyses, Lautaea was recovered sister to Genus 3, differing from the parsimony analysis.

194 In the Mkv+G4 analysis (Fig. 15), besides from Rothium ashlocki + R. evansi, Masuria and
195 Lautaea + Genus3, a significant proportion of Myllaenini sensu nov members were unresolved in
196 their placement.

197 In the Mkv+G8 analysis (Fig. 16), Myllaenini sensu nov was recovered but with little
198 support. Besides from Rothium ashlocki + R. evansi, the placement of Rothium, Polypea and
199 Brachypronomaea was unresolved.

200

201 Character Evolution

202

203 Ground/leaf litter habitat was recovered ancestral for Myllaenini sensu nov (Fig. 18). The
204 group subsequently transitioned from riparian to marine, and reverted back to riparian habitat
205 before infiltrating termite nests with Termitohospitini. Myllaenini sensu nov is ancestrally
206 parallel-sided. Limuloid form evolved twice, once in the mangrove swamp inhabiting Lautaea
207 murphyi and the common ancestor of Dimonomera + Myllaena + Termitohospitini, transitioning
208 to physogastry and physothoracy within the termitohospitines. The ancestral state for the MRCA
209 of Coptoxenus + Coptophysa + Coptophysella is ambiguous.

210

211 Taxonomy

212

213 Taxonomy proposed by Ahn & Ashe (2004):

214

215 Myllaenini sensu Ahn & Ashe

216 = Dimonomerini (Ashe 1999)

217 Amazonopora
218 Brachypronomaea
219 Bryothinusa
220 Dimonomera
221 Dysacrita
222 Lautaea
223 Myllaena
224 Philomina
225 Polypea
226 Rothium

227

228 The following updated taxonomic scheme is proposed following the results of this study:

229 Myllaenini *sensu nov*

230 = Dimonomerini (Ashe 1999)

231 = Masuriini (new status)

232 = Termitohospitini (new synonymy)

233 *Amazonopora*

234 *Brachypronomaea*

235 *Bryothinusa*

236 *Dimonomera*

237 *Dysacrita*

238 *Lautaea*

239 *Masuria*

240 *Myllaena*

241 *Philomina*

242 *Polypea*

243 *Rothium*

244 *Blapticoxenus*

245 Termitohospitina

246 *Coptophysa*

247 *Coptophysella*

248 *Coptotermocola*
249 *Coptoxenus*
250 *Hetairotermes*
251 *Japanophilus*
252 *Neotermitosocius*
253 *Paratermitosocius*
254 *Sinophilus*
255 *Termitobra*
256 *Termitohospes*
257 *Termitosodalis*

258

259

260 DISCUSSION

261

262 Ecological Origins of Termitophily

263

264 Termites are generally ground inhabiting social insects. By extension of the ground
265 habitat, some inhabit dead wood, but the majority build subterranean nests that may be
266 accompanied by above-ground architecture. Therefore, we logically expected that termitophilous
267 lineages would have a similar ancestral ecology, living on the ground/leaf litter or in dead and
268 decaying wood. Counter to this notion, we recovered a freshwater-riparian habitat-type for the
269 common ancestor of Myllaena + Termitohospitini. This is surprising, since riparian habitats are
270 notoriously underutilized by termites (as well as by ants). A fraction of Myllaena inhabit
271 ground/leaf litter in the tropics, and it is possible that these ground environments are quite
272 saturated, acting as transitional habitats between riparian zones and more strict terrestriality. We
273 hypothesize that termitohospitines evolved from ancestors that ventured into soaked ground/leaf
274 litter habitats in a tropical environmental setting. Furthermore, we posit that certain extant
275 Myllaena lineages are currently experimenting with this riparian-to-ground habitat transition.

276

277 Pre-adaptation and Subsequent Constraint of the Limuloid Body Plan

278

279 Although we observe a parallel-sided body as ancestral for Myllaenini sensu nov,
280 reconstruction shows that Termitohospitini evolved from a limuloid ancestor. Dimonomera and
281 Myllaena are very similar in form overall, which suggests that Termitohospitini evolved from a
282 Myllaena-like, limuloid-bodied ancestor. For riparian limuloid aleocharines like Myllaena, the
283 limuloid body plan is used for pushing, wedging and weaving through substrate. Analogous
284 examples are *Gymnusa* and *Deinopsis*, early diverging (Ashe 2005) free-living riparian
285 aleocharines that have similar body forms and live in similar habitats. There appears to be a
286 negative correlation between “limuloid-ness” and abdominal flexibility. The more tapered the
287 abdomen, the less flexible it becomes. Again, *Gymnusa* and *Deinopsis* represent analogues of
288 this phenomenon.

289 In contrast, limuloid bodies in social insect symbionts are adaptive for defense against
290 host attack. The numerous examples of symbiotic aleocharines converging on limuloid
291 ecomorphology are strong evidence of its adaptive nature (Parker 2016). In many examples, the
292 dorsum of the body is broadly expanded laterally, shielding vulnerable appendages underneath.
293 Termitohospitini were ancestrally limuloid but the mimetic physogastric or physothoracic shape,
294 evolved from this limuloid ancestral condition. It is likely that the ancestral limuloid body plan
295 was pre-adaptive for termitophily in the termitohospitines, catalyzing diversification in this niche.
296 Such a scenario parallels a recent hypothesis for the evolution of termitophily elsewhere in
297 Aleocharinae: in the tribes Trichopseniini and Mesoporini, quasi-limuloid morphology of a
298 *Gymnusa-Deinopsis*-like ancestor is posited to have been preadaptive for specialization inside
299 termite colonies (Yamamoto et al. 2016).

300 Interestingly, termitohospitines represent the sole example of physothoracy in the
301 Aleocharinae (Figs. 8–9B). There may be several explanations for this uniqueness. Physothoracy
302 may be a strategy to achieve mimicry when the abdomen is too inflexible to easily evolve
303 physogastry. Indeed, a relatively inflexible abdomen appears to be correlated with the limuloid
304 body plan that termitohospitines inherited from a Myllaena-like ancestor. Alternatively,
305 physothoracy may represent an intermediate state toward full physogastry, or perhaps functions
306 as a wholly different function inside colonies, unrelated to social integration. Due to the limited
307 number of species of mimetic termitohospitines, it is presently difficult to establish the polarity
308 of this character, which might help distinguish between these alternatives. Consequently,
309 reconstruction of the MRCA of the mimetic termitohospitine clade remains unresolved (Fig. 18).

310

311 Mouthpart Degradation: A Result of Adaptation to Trophallaxis

312

313 A conspicuous characteristic of Myllaenini sensu nov is their extremely stylate
314 mouthparts that greatly extend past the apex of their head (i.e. clypeus). Although all of the
315 mouthpart components are to some extent elongated, the most noticeable are the maxilla and
316 labium. Observations of Myllaena in captivity revealed that the labium, which protrudes past all
317 other mouthparts, is a highly extrusible structure. At rest, the labium is typically retracted into
318 the recesses of the oral cavity, and does not visibly protrude, which is made possible by the
319 elongate premental tendons (Eldredge pers. obs.).

320 The maxilla is the primary appendage used in food gathering, and the labium is primarily
321 used for sensing. Observations of Bryothinusa have shown these beetles are carnivorous and also
322 scavenge on small arthropods between sand particles in marine-intertidal habitats (Wong & Chan
323 1977). Similarly elongate mouthparts are convergently found in the riparian limuloid genera
324 *Gymnusa* and *Deinopsis*. Observations of *Gymnusa* showed these beetles are carnivorous, and
325 when feeding, probed the food item with the labium and gathered food with the maxilla
326 (Eldredge pers. obs.). Using these examples of correlated form and function as evidence for a
327 dietary type, Myllaenini appears to be a predaceous tribe; the flexible and elongate labium is
328 probably used to probe substrate interspaces to search for food, and the stylate maxilla used for
329 extracting interstitial food items.

330 The large interdigitated teeth are a synapomorphy for Myllaenini sensu nov (S5), but many
331 of the termitohospitines have much shorter and curved maxillae (Figs. 11a–11b, S5).

332 Degradation of the mouthparts is a common theme among social insect symbionts, and is thought
333 to be correlated to a simplified diet: feeding on soft-bodied brood or directly fed by workers.

334 Until recently, the diet of Termitohospitini was unknown, but observations of beetles in culture
335 have revealed that Termitohospitini are fed trophallactically by host termite workers, and also
336 groom and are groomed by the workers (Figs. 10a–10b). Grooming host termites may also allow
337 the beetles to collect colony-specific odors. We suggest that degradation of mouthpart
338 morphology is adaptive for this transition from a predatory diet to one based around trophallaxis
339 and grooming. Notably, the Termitohospitini genus *Termitosocius* possesses the elongate, more
340 stylate mouthparts characteristic of free-living Myllaenini sensu nov. We therefore speculate that

341 *Termitosocius* is early-diverging among Termitohospitini, retaining the ancestral predatory diet
342 within termite nests.

343

344 Putting It All Together: Evolutionary Origins of Termitophily in Termitohospitini

345

346 Our reconstructions show that termitohospitines evolved from a riparian limuloid
347 ancestor with stylate mouthparts. We hypothesize that transitioning to a more terrestrial
348 environment caused an increase in the frequency of interactions with termites, and the limuloid
349 defensive form was pre-adaptive for protection against termite aggression. Termitohospitini
350 further modified the ancestral limuloid form by laterally expanding dorsal sclerites to shield
351 vulnerable appendages underneath (Figs. 1–4, 6–7). Subsequently, some lineages evolved
352 trophallactic behavior Fig. 10b), and the mouthparts degraded and shortened in length. Given this
353 hypothesis, *Termitosocius* appears most primitive in overall morphology, and may embody what
354 a stem termitohospitine might have looked like during the lineage's ecological transition to life
355 inside termite colonies.

356

357

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364

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436

437 FIGURE CAPTIONS

438

439 Figures 1–8. Habitus of representative Termitohospitini. (1) *Termitohospes* undesc. sp. (2)
440 *Paratermitosocius* undesc. sp. (3) *Termitosodalis* undesc. sp. (4) *Termitosocius microps*. (5)
441 *Neotermitosocius bolivianus*. (6) *Blapticoxenus brunneus*. (7) *Coptotermocola clavicornis*. (8)
442 *Coptoxenus* sp.

443

444 Figures 9–10. Termitohospitini in situ et in vitro. (9) *Coptoxenus* sp. in situ with host. (10a)
445 *Sinophilus* sp. in vitro being groomed by host. (10b) *Sinophilus* sp. in vitro engaging in
446 trophalaxis food exchange.

447

448 Figures 11–13. *Myllaena* sp. and Termitohospitini scanning electron micrograph images of
449 various body parts. (11a) *Myllaena* sp. acinia. (11b) *Sinophilus* sp. lacinia. (12a) *Myllaena* sp.
450 maxillary palpomere 3. (12b) *Termitohospes* sp. maxillary palpomere 3. (13a) *Myllaena* sp.
451 anterior tentorial pit. (13b) *Termitohospes* sp. anterior tentorial pit.

452

453 Figure 14. Parsimony majority rule consensus tree with bootstrap values.

454

455 Figure 15. Bayesian Mk_v + $\Gamma(4)$ majority rule consensus tree with posterior probabilities.

456 Habitus illustrations from top to bottom: *Masuria* sp., *Myllaena* sp. and *Termitohospes* sp..

457

458 Figure 16. Bayesian Mk_v + $\Gamma(8)$ majority rule consensus tree with posterior probabilities.

459 Habitus illustrations from top to bottom: *Masuria* sp., *Myllaena* sp. and *Termitohospes* sp..

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461 Figure 17. Parsimony majority rule consensus tree of Myllaenini *sensu nov* with body form and
462 ecology Mk reconstructions.

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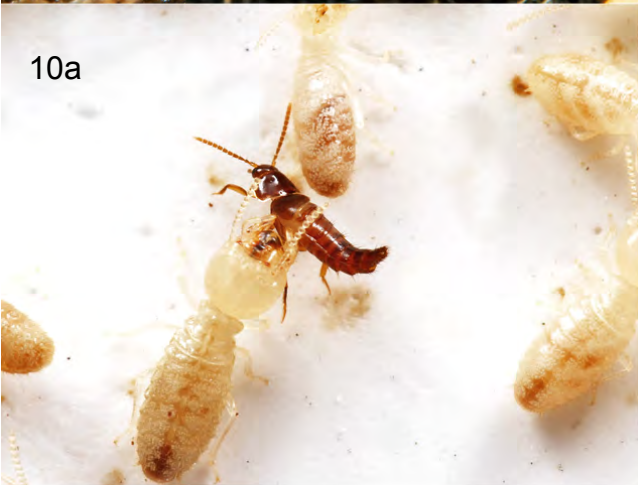
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10a

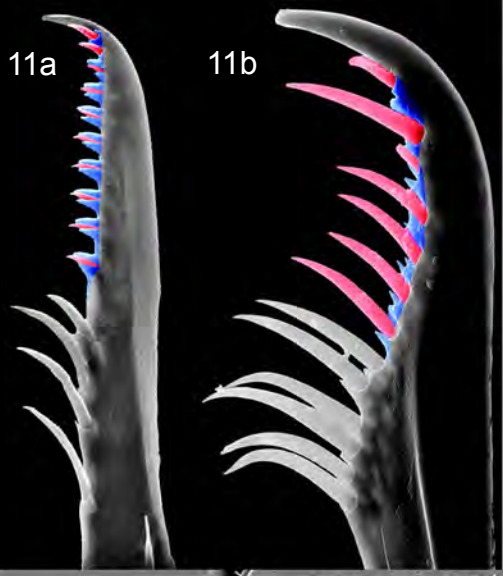


10b



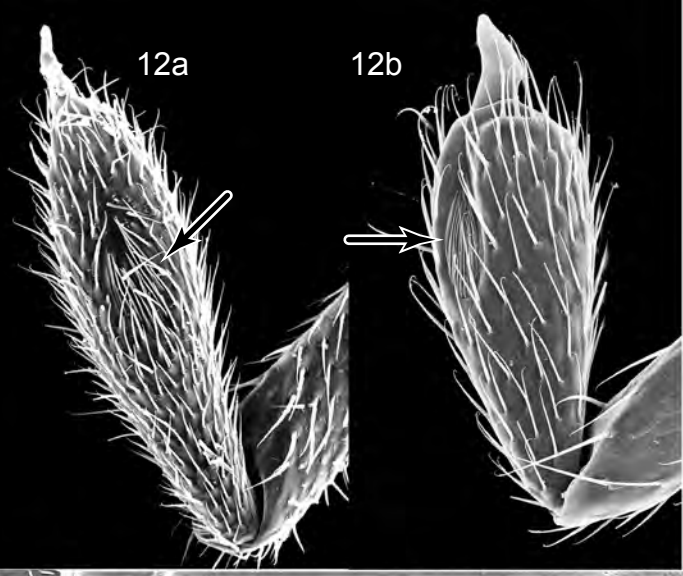
11a

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12a

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13a

13b

