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**Scanning Electron Microscopy Investigations of Third Instar Larva of *Cordylobia rodhaini*
(Diptera: Calliphoridae), Agent of Furuncular Myiasis**

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1 Abstract

2 A scanning electron microscopy study of the 3rd larval instar of *Cordylobia rodhaini* Gedoelst
3 (Diptera: Calliphoridae), causing obligatory furuncular myiasis, is presented here for the first time.
4 The larvae were collected from a patient exposed to them in the tropical rainforest of Kibale
5 National Park (Uganda). Distinctive features are described in sequence from the anterior region to
6 the posterior region, highlighting the morphological features of antennae, maxillary palps, structures
7 related to mouth opening, sensory structures, thoracic and abdominal spines, anterior and posterior
8 spiracles. The results are compared with those of other Calyptrata flies, mainly from the family
9 Calliphoridae and, when possible, with *C. anthropophaga* Blanchard (Diptera: Calliphoridae), the
10 only other species of genus *Cordylobia* investigated by scanning electron microscopy.

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15 Keywords

16 *Cordylobia rodhaini*, SEM, 3rd instar larva, morphology, furuncular myiasis

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Introduction

28 *Cordylobia rodhaini* Gedoelst (Diptera: Calliphoridae) is a fly species causing an obligatory
29 furuncular myiasis. *C. rodhaini* is distributed in tropical Africa, mostly in rainforest. The species
30 typically parasitizes mammals, especially monkeys, rodents and small antelopes. Humans are
31 occasionally infested but the myiasis by *C. rodhaini* is far less common than that caused by *C.*
32 *anthropophaga* Blanchard (Diptera: Calliphoridae) (Zumpt 1965). These two species, with *C.*
33 *ruandae* Fain (Diptera: Calliphoridae) are the only three species of the genus *Cordylobia* Gruenberg
34 (Veraldi et al. 2014). Since 1902 only 25 cases of human furuncular myiasis by *C. rodhaini* have
35 been reported (Pezzi et al. 2014). Studies on morphology of 3rd instar *C. rodhaini* were previously
36 performed by stereomicroscopy and light microscopy (Gedoelst 1909, Rodhain 1915, Bertram
37 1938, Fain 1953, Zumpt 1965, Kremer et al. 1970, Pampiglione et al. 1991): however,
38 ultrastructural analyses of body surface by scanning electron microscopy (SEM) have never been
39 performed. Here we describe by SEM the 3rd instar larva of *C. rodhaini* to provide a more detailed
40 description of this dipteran of medical and veterinary importance.

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Materials and Methods

43 Five larvae identified as 3rd instar *C. rodhaini* based on previous stereomicroscopic observations
44 were collected from a patient who was likely exposed to them in June 2014 while traveling in the
45 tropical rainforest of Kibale National Park (Uganda) (Pezzi et al. 2014). The larvae were prepared
46 according to the following procedure: they were fixed in 3% glutaraldehyde in sodium cacodylate
47 buffer 0.125 M, pH 7.3, for 24 hours, rinsed three times for 15 min in the same buffer and postfixed
48 in 2% osmium in sodium cacodylate buffer for 24h. After postfixation the samples were rinsed three
49 times for 15 min in the buffer, then dehydrated in a graded ethanol series (12 h for each grade) and
50 critical point dried in a Balzers CPD 030 dryer (Leica Microsystems, Wetzlar, Germany). The
51 samples were then mounted on stubs and coated with gold-palladium in a Sputter Coated S150
52 Edwards (HHV Ltd, Crawley, UK). All SEM observations were conducted at the Electronic

53 Microscopy Centre of University of Ferrara on a Zeiss EVO 40 SEM (Zeiss, Milan, Italy). For each
54 morphological aspect such as thoracic and abdominal spines at least 20 samples were examined in
55 all five specimens. Two of the larvae were dissected for detailed observations of the anterior and
56 posterior ends. The area of posterior spiracles and cuticular depressions was measured in the
57 posterior end.

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Results

60 The 3rd instar larva of *C. rodhaini* is barrel-shaped with a tapering anterior end, a cylindrical
61 pseudocephalon and truncated posterior end with two posterior spiracles (Fig. 1A-D), in agreement
62 with previous light and stereomicroscopy data (Gedoelst 1909, Rodhain 1915, Bertram 1938, Fain
63 1953, Zumpt 1965, Kremer et al. 1970, Pampiglione et al. 1991). The pseudocephalon shows two
64 prominent lobes (Fig. 1B, C), the pseudocephalic lobes, each one bearing an antero-lateral
65 maxillary palpus and a dorsal antenna (Fig. 2A). The maxillary palpus contains a cluster of sensilla
66 located at the top of a conic structure: each cluster has a knob sensillum, a pit sensillum, four
67 papillary sensilla and a bilobed protrusion. Behind the cluster of sensilla the maxillary palpus has a
68 basiconic sensillum and a coeloconic sensillum in dorsal position (Fig. 2B). The antenna has a blunt
69 conic shape composed of two structures, a conic basal ring and a distal dome (Fig. 2C). Two mouth
70 hooks (“denticles”) with broad tips are visible in the anterior ventral part of the pseudocephalon
71 (Fig. 1B, C). On both sides of the mouth hooks there is a group of oral ridges with 9-10 lobes, each
72 one covered by tiny conic denticles with 1-3 tips (Fig. 1B and 2D, E). In the centre of the oral
73 ridges there is the ventral organ composed of a circular pit sensillum, a small pit sensillum with an
74 opening partially surrounded by a ridge, and a button-shaped papillary sensillum (Fig. 2F). Between
75 the mouth hooks, along the dorsal-ventral axis there is a cluster of conic preoral denticles with
76 down curved tips (Fig. 2G). The mouth opening has a crescent shape with a triangular labium (Fig.
77 2H), showing in the lower part two trichoid sensilla with one seta and 3-4 dome-shaped papillary
78 sensilla (Fig. 2H-J).

79 The first segment of the thoracic region is divided into two bands: the anterior one is covered by
80 cuticular spines, while the posterior one is thinly folded (Fig. 3A). The spines, pointing towards the
81 posterior end of the body, are drop-shaped and $60.8 \pm 11.0 \mu\text{m}$ long in the dorsal part (Fig. 3B) and
82 sharply conic and $40.1 \pm 12.6 \mu\text{m}$ long in the ventral part (Fig. 3C). In the posterior band devoid of
83 spines there are several sensilla, either coeloconic (Fig. 3D) or campaniform (Fig. 3E). In the
84 second thoracic segment there are some campaniform sensilla similar to those of the first thoracic
85 segment (Fig. 3F). On both sides of the first thoracic segment, near the border with the second
86 segment, there is an anterior spiracle composed of a ridge with 5-6 domes. (Fig. 3G). As for the first
87 thoracic segment, the second and third thoracic segments are also covered by cuticle spines pointing
88 towards the posterior end. However, these spines are all conic and $150.3 \pm 18.5 \mu\text{m}$ long (Fig. 1A,
89 B). Concerning the abdominal segments, they are all completely covered by conic cuticle spines,
90 $167.2 \pm 12.7 \mu\text{m}$ long (Fig. 3H). However, the spines of the last two abdominal segments are fewer
91 and smaller in size: those of the second to last abdominal segment are $69.4 \pm 9.5 \mu\text{m}$, while those of
92 the last segment are $36.6 \pm 11.3 \mu\text{m}$ long (Fig. 4A). The last two segments have coeloconic sensilla,
93 either isolated or in pairs (Fig. 4B, C). There are two fan-shaped posterior spiracles (Fig. 1D and
94 4D), with area $1.1 \pm 0.3 \times 10^5 \mu\text{m}^2$. Each one of them is slightly raised, with three sinuous slits
95 branching off from a respiratory button partially covered by an operculum (Fig. 4E). Between the
96 respiratory button and the slits there are 7-8 papillae arranged in a dorsal-ventral row (Fig. 4E and
97 inlay). Above and below the posterior spiracles there are several cuticular depressions, with area 6.6
98 $\pm 1.3 \times 10^3 \mu\text{m}^2$ (Fig. 4F) with deeply corrugated and perforated bottoms (Fig. 4G).

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Discussion

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The morphological studies by SEM of the larvae of the genus *Cordylobia* are very limited (Gall et al. 1987, Bettoli et al. 1993) and the present one is the first on *C. rodhaini*. The only two studies available deal with *C. anthropophaga*: the first one describes the cephalic region, cuticle spines and posterior region of a 2nd instar (Bettoli et al. 1993) and the second one the cephalic region and the

105 first thoracic segment of an unidentified instar (probably the third one) (Gall et al. 1987).
106 Comparing the results of SEM observations on *C. rodhaini* 3rd instar to those obtained in *C.*
107 *anthropophaga*, it is possible to detect several differences. For example, in *C. rodhaini* the mouth
108 hooks are more rounded and no cluster of conic denticles or oral grooves are found in *C.*
109 *anthropophaga*, which in place of the oral grooves exhibits triangular spines similar to those of the
110 thoracic segments (Gall et al. 1987). The cluster of conic denticles appears typical of *C. rodhaini*,
111 although all three larval instars of *Cochlyomyia macellaria* Fabricius (Diptera: Calliphoridae) have
112 dental sclerites in the same position (Mendonça et al. 2014a). The grouping of oral grooves in *C.*
113 *rodhaini* resembles that of the 3rd instar of *Wohlfahrtia magnifica* (Schiner) (Diptera:
114 Sarcophagidae) (Ruíz-Martínez et al. 1990). In *C. rodhaini* these accessory structures of the mouth
115 hooks, together with thoracic and abdominal spines, probably help the larva to feed and later exit
116 the host and burrow in the soil to pupate. Moreover, the morphology of antennae of 3rd instar *C.*
117 *rodhaini* appears similar to that of the 2nd and 3rd instar of *C. anthropophaga* (Gall et al. 1987) and
118 of 3rd instars of other species of the family Calliphoridae (Sandeman et al. 1987; Leite et al. 1993;
119 Sukontason et al. 2005, 2006, 2008; Mendonça et al. 2010, 2014a, 2014b; Ubero-Pascal et al. 2012;
120 Jang et al. 2013). The cluster of maxillary palp sensilla on the top of a conic structure found in *C.*
121 *rodhaini* is also present in *C. anthropophaga* (Gall et al. 1987). Concerning the ventral organ, no
122 mention or description of this structure is found in previous works describing the ultrastructural
123 morphology of *C. anthropophaga*, therefore the presence of this organ and its morphology is
124 described for the first time in the genus *Cordylobia*. In *C. rodhaini* the ventral organ is different in
125 comparison to 3rd instars of other Calliphoridae, characterized by short spines (Sukontason et al.
126 2003a, Ubero-Pascal et al. 2012). No description of the labium is available in *C. anthropophaga* but
127 the labium of *C. rodhaini* is not divided in lobes as in 3rd instar *Chrysomya rufifacies* (Macquart)
128 (Diptera: Calliphoridae) (Sukontason et al. 2003b).
129 The SEM investigation confirms previous data on 3rd instar *C. rodhaini* showing a different
130 morphology of cuticle spines according to their position (Bertram 1938, Fain 1953, Pampiglione et

131 al. 1991). However, the SEM images show two types of cuticle spines in the first thoracic segment,
132 one drop-shaped in the dorsal region and the other sharply conic in the ventral region. Moreover,
133 comparing the cuticle spines of the first thoracic segment of 3rd instar *C. rodhaini* with those of the
134 same segment in *C. anthropophaga*, the *C. anthropophaga* spines are all conic with a wider base
135 (Gall et al. 1987).

136 The cuticular depressions found in the last two abdominal segments of 3rd instar *C. rodhaini* have
137 not been described in *C. anthropophaga* but similar structures have been described in the second
138 thoracic segment of 3rd instar *Gasterophilus nasalis* (L.) (Diptera: Oestridae) (Leite et al. 1999).
139 Similar structures have also been found in the last abdominal segment of 2nd instar *Calliphora*
140 *vicina* Robineau-Desvoidy (Diptera: Calliphoridae) and, less evident, in the 3rd instar of the same
141 species (Ubero-Pascal et al. 2012). Concerning the sensilla, the campaniform ones found in the first
142 and second thoracic segment of 3rd instar *C. rodhaini* are similar to the “papillae” of 3rd instar
143 *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) (Shaheen et al. 2004) while the coeloconic ones
144 are similar to those described in 2nd instar *C. anthropophaga* (Bettoli et al. 1993). Besides
145 confirming the data obtained during previous observations on 3rd instar *C. rodhaini* (Gedoelst 1909,
146 Bertram 1938, Fain 1953, Zumpt 1965, Kremer et al. 1970, Pampiglione et al. 1991), the SEM
147 analyses of posteriors spiracles shows the presence of an “operculum” in proximity of the button
148 spiracles of the respiratory plates and of a row of basiconic sensilla between the respiratory button
149 and the sinous slits. No ultrastructural description of posterior spiracles is available for 3rd instar *C.*
150 *anthropophaga*. This study provides ultrastructural information useful to discriminate 3rd instar *C.*
151 *rodhaini* from the same instar of *C. anthropophaga*. No data by SEM are presently available on the
152 other species of the genus *Cordylobia*, *C. ruandae*. More ultrastructural investigations are required
153 on larval instars of this genus in order to extend the morphological details able to discriminate
154 among its three species responsible of furuncular myiasis.

155

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For Review Only

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232

233

Figure Legends

234 **Fig. 1.** Scanning electron micrographs of 3rd instar *Cordylobia rodhaini*. (A) Entire body in ventral
235 view, an asterisk indicates the second to last segment and the diamond indicates the last segment.

236 (B) Anterior region in lateral-ventral view. (C) Anterior region in dorsal view. (D) Posterior end of
237 body. AD: abdomen; AO: anal opening; AS: anterior spiracle; CAS: campaniform sensillum; CCD:
238 conic preoral denticles; CS: coeloconic sensillum; LA: labium; MH: mouth hooks; OR: oral ridges;
239 PL: pseudocephalic lobe; POS: posterior spiracles; PSE: pseudocephalon; TR: thorax; TR1: first
240 thoracic segment; TR2: second thoracic segment.

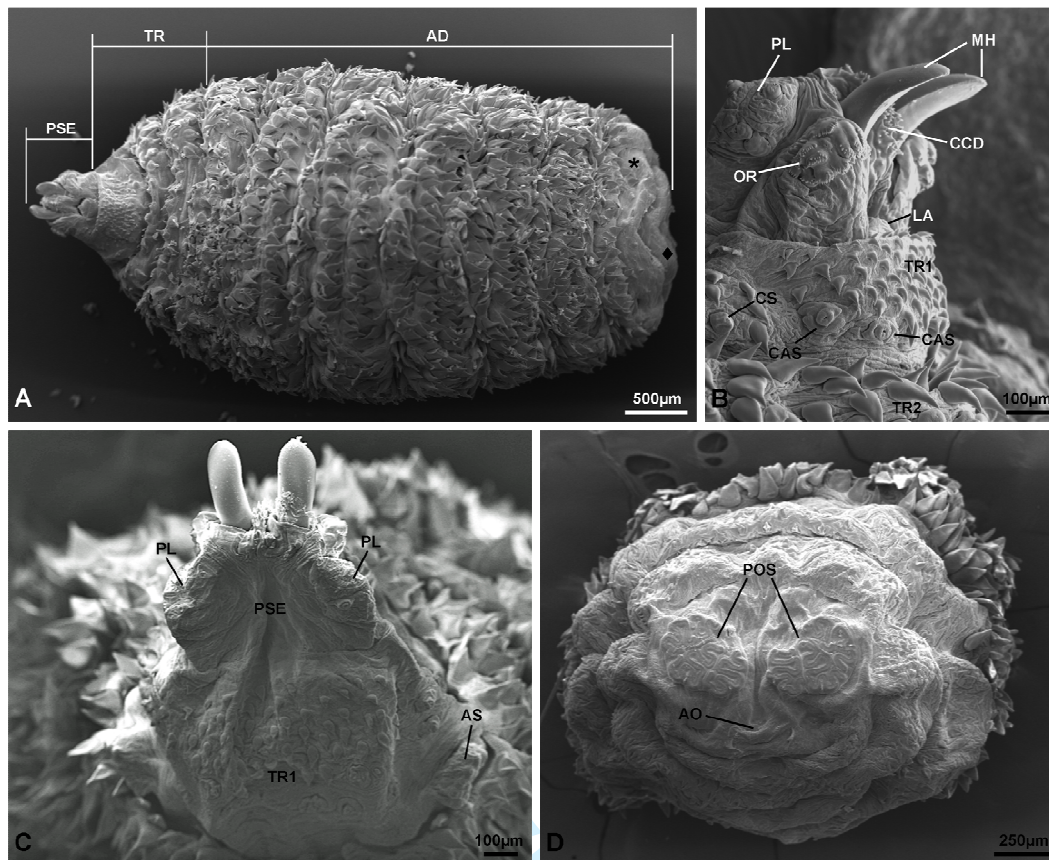
241 **Fig. 2.** Scanning electron micrographs of 3rd instar *C. rodhaini*. (A) Pseudocephalic lobe: the dashed
242 line outlines the maxillary palpus. (B) Maxillary palpus: the dashed line outlines the cluster of
243 sensilla at the top of the conic structure. (C) Antenna: the asterisk indicates the distal dome and the
244 diamond indicates the conic basal ring. (D) Oral ridges and ventral organ. (E) Details of the oral
245 ridges with tiny conic denticles. (F) Details of the ventral organ. (G) Details of the conic preoral
246 denticles. (H) Mouth opening: the arrowheads indicate the dome-shaped papillary sensilla and the
247 arrows indicate the trichoid sensilla. (I) Trichoid sensillum in the lower part of the labial lobe. (J)
248 Dome-shaped papillary sensilla of the lower labial lobe. AN: antenna; BP: bilobed protrusion; BS:
249 basiconic sensillum; KS: knob sensillum; PIS: pit sensillum; PIS1: circular pit sensillum; PIS2: pit
250 sensillum with ridge; PS: papillary sensillum; PSC: papillary sensilla within a cluster; VO: ventral
251 organ. Other abbreviations as in Fig. 1.

252 **Fig. 3.** Scanning electron micrographs of 3rd instar *C. rodhaini*. (A) First thoracic segment, dorsal
253 view: the dashed line outlines the two bands of the first thoracic segment, and the arrowheads
254 indicate the campaniform sensilla. (B) Details of cuticular spines of the first thoracic segment,
255 dorsal region. (C) Details of cuticular spines of the first thoracic segment, ventral region. (D)
256 Coeloconic sensillum of the first thoracic segment. (E) Campaniform sensillum of the first thoracic
257 segment. (F) Campaniform sensillum of the second thoracic segment. (G) Anterior spiracle: the

258 asterisk indicates the dome line. (H) Conic cuticular spines covering the abdomen with exception of
259 the second to last and last segments. AB: anterior band; PB: posterior band.

260 **Fig. 4.** Scanning electron micrographs of 3rd instar *C. rodhaini*. (A) Details of the lateral region of
261 the second to last abdominal segment (asterisk) and of the last abdominal segment (diamond). (B)
262 Coeloconic sensillum of the last abdominal segment. (C) A pair of coeloconic sensilla. (D) Posterior
263 region of the last abdominal segment: the rectangle outlines the region enlarged in F and the
264 arrowheads indicate the cuticular depressions. (E) Respiratory button: the arrowheads indicate the
265 papillary sensilla, the inlay shows an enlarged papillary sensillum. (F) Detail of Fig. 4D showing
266 the cuticular depressions. (G) Details of the bottom of a cuticular depression. CD: cuticular
267 depression; OP: operculum; RB: respiratory button; SS: sinuous slit. Other abbreviations as in Fig.
268 1.

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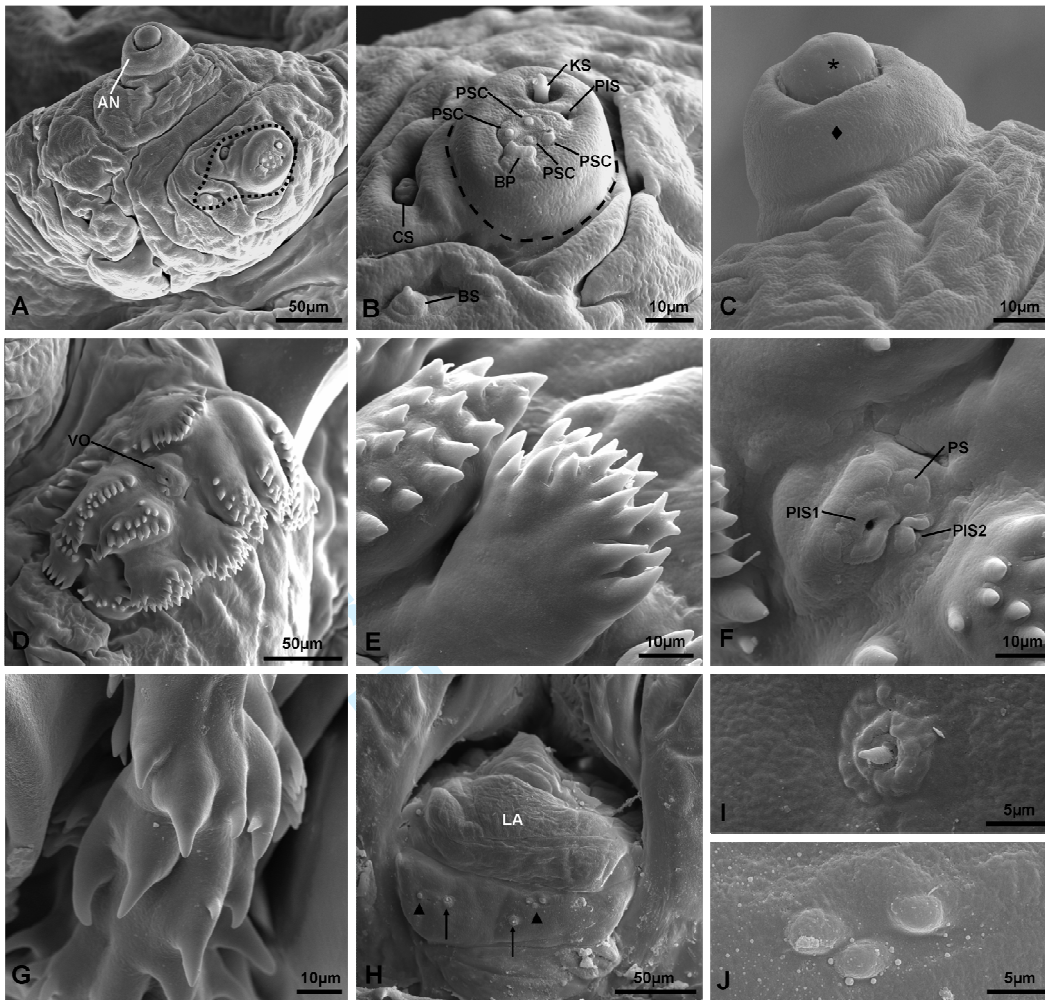


270

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 272 view, an asterisk indicates the second to last segment and the diamond indicates the last segment.

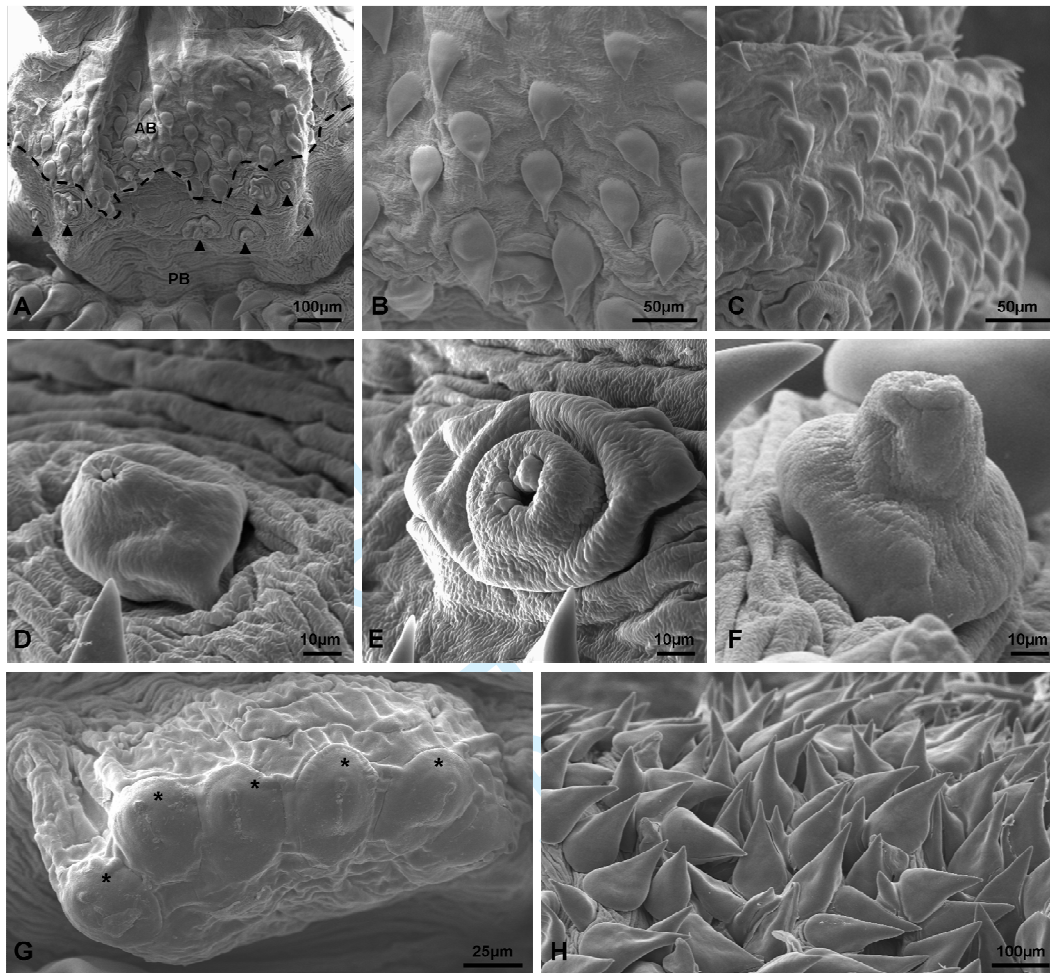
273 (B) Anterior region in lateral-ventral view. (C) Anterior region in dorsal view. (D) Posterior end of
 274 body. AD: abdomen; AO: anal opening; AS: anterior spiracle; CAS: campaniform sensillum; CCD:
 275 conic preoral denticles; CS: coeloconic sensillum; LA: labium; MH: mouth hooks; OR: oral ridges;
 276 PL: pseudocephalic lobe; POS: posterior spiracles; PSE: pseudocephalon; TR: thorax; TR1: first
 277 thoracic segment; TR2: second thoracic segment.

278



279

280 **Fig. 2.** Scanning electron micrographs of 3rd instar *C. rodhaini*. (A) Pseudocephalic lobe: the dashed
 281 line outlines the maxillary palpus. (B) Maxillary palpus: the dashed line outlines the cluster of
 282 sensilla at the top of the conic structure. (C) Antenna: the asterisk indicates the distal dome and the
 283 diamond indicates the conic basal ring. (D) Oral ridges and ventral organ. (E) Details of the oral
 284 ridges with tiny conic denticles. (F) Details of the ventral organ. (G) Details of the conic preoral
 285 denticles. (H) Mouth opening: the arrowheads indicate the dome-shaped papillary sensilla and the
 286 arrows indicate the trichoid sensilla. (I) Trichoid sensillum in the lower part of the labial lobe. (J)
 287 Dome-shaped papillary sensilla of the lower labial lobe. AN: antenna; BP: bilobed protrusion; BS:
 288 basiconic sensillum; KS: knob sensillum; PIS: pit sensillum; PIS1: circular pit sensillum; PIS2: pit
 289 sensillum with ridge; PS: papillary sensillum; PSC: papillary sensilla within a cluster; VO: ventral
 290 organ. Other abbreviations as in Fig. 1.



291

292 **Fig. 3.** Scanning electron micrographs of 3rd instar *C. rodhaini*. (A) First thoracic segment, dorsal

293 view: the dashed line outlines the two bands of the first thoracic segment, and the arrowheads

294 indicate the campaniform sensilla. (B) Details of cuticular spines of the first thoracic segment,

295 dorsal region. (C) Details of cuticular spines of the first thoracic segment, ventral region. (D)

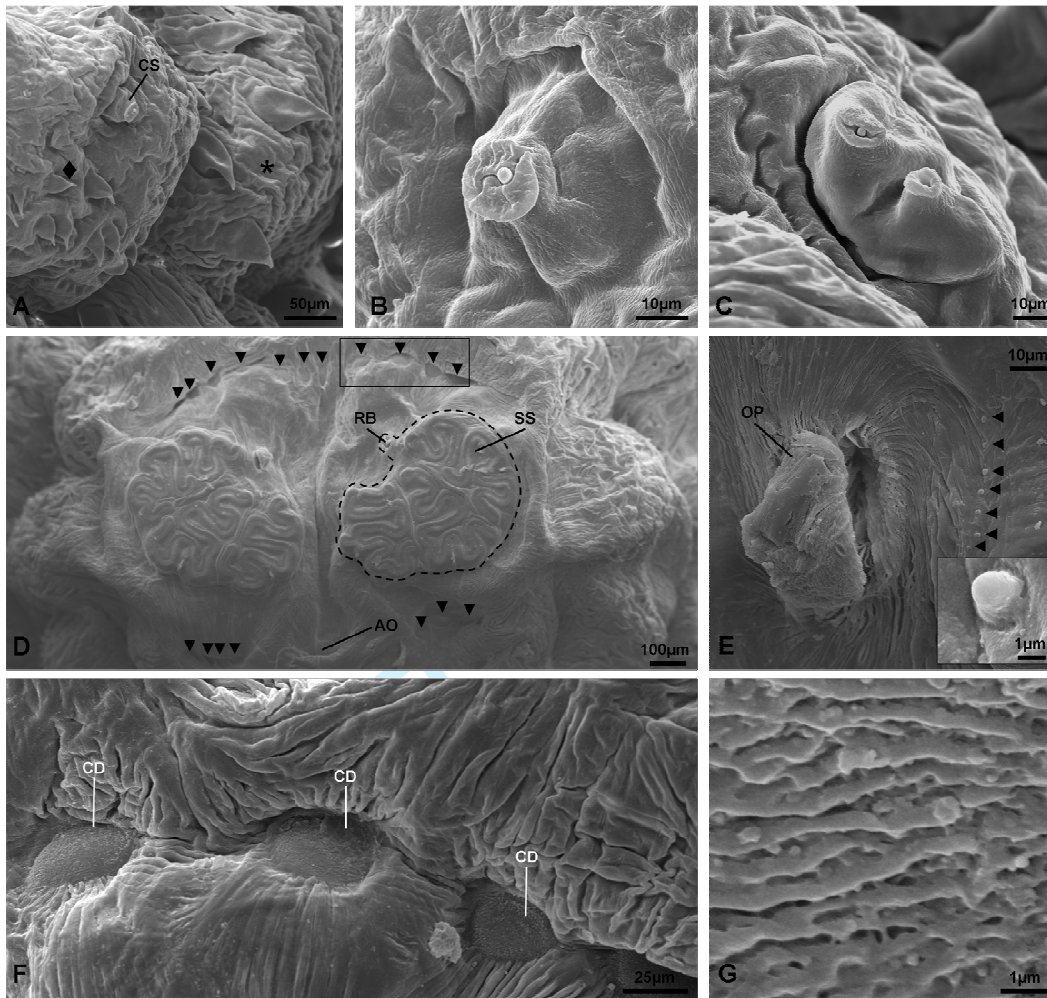
296 Coeloconic sensillum of the first thoracic segment. (E) Campaniform sensillum of the first thoracic

297 segment. (F) Campaniform sensillum of the second thoracic segment. (G) Anterior spiracle: the

298 asterisk indicates the dome line. (H) Conic cuticular spines covering the abdomen with exception of

299 the second to last and last segments. AB: anterior band; PB: posterior band.

300



301

302 **Fig. 4.** Scanning electron micrographs of 3rd instar *C. rodhaini*. (A) Details of the lateral region of
 303 the second to last abdominal segment (asterisk) and of the last abdominal segment (diamond). (B)
 304 Coeloconic sensillum of the last abdominal segment. (C) A pair of coeloconic sensilla. (D) Posterior
 305 region of the last abdominal segment: the rectangle outlines the region enlarged in F and the
 306 arrowheads indicate the cuticular depressions. (E) Respiratory button: the arrowheads indicate the
 307 papillary sensilla, the inlay shows an enlarged papillary sensillum. (F) Detail of Fig. 4D showing
 308 the cuticular depressions. (G) Details of the bottom of a cuticular depression. CD: cuticular
 309 depression; OP: operculum; RB: respiratory button; SS: sinuous slit. Other abbreviations as in Fig.
 310 1.