

1 **For:** *Journal of Economic Entomology*
2 (Household and Structural Insects section)

Corresponding author:

3
4 Chow-Yang Lee
5 Urban Entomology Laboratory
6 Vector Control Research Unit
7 School of Biological Sciences
8 Universiti Sains Malaysia
9 11800 Penang, Malaysia
10 Email: chowyang@usm.my

11
12
13 **Differences in climbing ability of *Cimex lectularius* and *Cimex***
14 ***hemipterus* (Hemiptera: Cimicidae)**

15
16
17 DAE-YUN KIM¹, JOHAN BILLEN², STEPHEN L. DOGGETT³ AND CHOW-YANG
18 LEE^{1,4}

19
20 ¹Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences,
21 Universiti Sains Malaysia, 11800 Penang, Malaysia.

22
23 ²KU Leuven, Zoological Institute, Naamsestraat 59, box 2466, B-3000 Leuven, Belgium.

24
25 ³Department of Medical Entomology, Westmead Hospital, Westmead NSW 2145, Australia.

26 ⁴Corresponding author. Email: chowyang@usm.my
27

28
29 **Running title:** Kim et al. Climbing ability of *Cimex lectularius* and *C. hemipterus*

30

31

32 ABSTRACT The climbing abilities of two bed bug species, *Cimex lectularius* L. and *Cimex*
33 *hemipterus* (F.) were determined by evaluating their escape rates from smooth surface pitfall
34 traps using four commercial bed bug monitors (Verifi™ Bed Bug Detector, ClimbUp® Insect
35 Interceptor, BlackOut™ Bed Bug Detector, and SenSci Volcano™ Bed Bug Detector). All
36 detectors were used in the absence of lures or attractants. Unlike *C. lectularius*, adult *C.*
37 *hemipterus* were able to escape from all traps. On the other hand, no or low number nymphs
38 of both species escaped, depending on the evaluated traps. Examination on the vertical
39 friction force of adults of both species revealed a higher vertical friction force in *C.*
40 *hemipterus* than in *C. lectularius*. Scanning electron micrograph observation on the tibial pad
41 of adult bed bugs of *C. hemipterus* showed the presence of a greater number of tenent hairs on
42 the tibial pad than on that of adult *C. lectularius*. No tibial pad was found in the fourth and
43 fifth instars of both species. Near the base of the hollow tenent hairs is a glandular epithelium
44 that is better developed in adult *C. hemipterus* than in adult *C. lectularius*. This study
45 highlights significant morphological differences between *C. lectularius* and *C. hemipterus*,
46 which has profound implications in the monitoring and potential management of bed bug
47 infestations.

48 **Key Words** bed bug, tenent hair, vertical friction force, climbing, pitfall trap, monitoring,
49 management.

50 **Introduction**

51 The current global resurgence of bed bugs involves two species, namely the common bed bug,
52 *Cimex lectularius* L. and the tropical bed bug, *Cimex hemipterus* (F.). *Cimex lectularius* is
53 mainly found in the temperate and subtropical regions, while the latter species is distributed in
54 the subtropical and tropical regions (Omori 1939, 1941, Usinger 1966, Doggett et al. 2003,
55 How and Lee 2010, Zulaikha et al. 2016). In some regions such as in Africa (Newberry 1988,

56 1989), Australia (Doggett et al. 2003), Florida (Hixson 1943, Campbell et al. 2016), and
57 Taiwan (Lee 2013), both species can co-exist sympatrically.

58

59 The detection and monitoring of bed bugs is one of the most important aspects of integrated
60 pest management (IPM) against this insect (Wang & Copper 2011). Detection and monitoring
61 of bed bugs can be undertaken via visual inspections, through various traps and monitoring
62 devices, via canine detection (Pinto et al. 2007, Wang & Copper 2011, Boase & Naylor 2014),
63 or any combination of these. Amongst the monitors available in the market are pitfall and
64 sticky traps, some with the addition of lures such as heat, carbon dioxide, and/or insect/host
65 odors (active monitors), while others contain no lure (passive monitors) and provide a
66 harborage for bed bugs to enter. While many such products exist on the market, few have
67 been tested for efficacy via independent scientific tests.

68

69 Pitfall style traps of different designs have been found effective in detecting and monitoring *C.*
70 *lectularius* (Singh et al. 2013). These traps rely on a smooth inner wall surface to prevent
71 trapped bed bugs from escaping. Such traps with smooth surfaces can also be used as a barrier
72 to prevent bed bugs from reaching the sleeping hosts (Doggett 2013). However, if the trapped
73 bugs were able to climb up the smooth surfaces and escape, this would seriously compromise
74 monitoring efforts or their use as barriers. It is known that a buildup of dust and debris can
75 compromise the efficacy of pitfall traps against *C. lectularius* and hence maintenance
76 recommendations are usually included such as regular cleaning or the addition of talc
77 (Doggett 2013).

78

79 Most of the trap products were originally developed and tested in the U.S. where *C.*
80 *lectularius* is the major bed bug species. Many of these products have subsequently been

81 marketed in other parts of the world on the assumption that they also would work against the
82 species that were found there, such as *C. hemipterus*. In a preliminary laboratory investigation
83 though, it was observed that adult *C. hemipterus* could escape from a new pitfall trap, which
84 otherwise effectively contained *C. lectularius*. This raised an important question: are all pitfall
85 traps that are effective in containing *C. lectularius* also effective against *C. hemipterus*? If
86 differences are found, this would have profound implications for the monitoring of the latter
87 species and for the potential use of barriers against *C. hemipterus*. Furthermore, it would raise
88 the question why such differences exist. The results of the initial investigation prompted the
89 following study. The escape rate was compared between *C. lectularius* and *C. hemipterus* in
90 four commercially available monitors that were previously or presently marketed for bed bug
91 detection and monitoring in the US. Following this, the vertical friction force was determined
92 for both species on two types of surfaces (smooth and rough). Lastly, the leg morphology was
93 investigated to determine the possible reasons behind the differences in climbing ability of
94 both species.

95

96 **Materials and Methods**

97 **Insects.** Two strains for each species were used; *C. lectularius* (Monheim [MH] and Sydney
98 [SYD] strains), and *C. hemipterus* (Kuala Lumpur [KL] and Green lane [GL] strains) (Table
99 1). All strains were reared in the laboratory in glass jars (7 cm diameter x 9 cm height) and
100 provided with folded brown paper as harborage under environmental conditions of $26 \pm 2^\circ\text{C}$,
101 $70 \pm 5\%$ RH, and a 12-h photo period. All the insects used in this study were blood-fed on a
102 human volunteer once a week. An alcohol-preserved sample of adult *C. hemipterus* collected
103 from Papua New Guinea in 1932 [PNG strain] was used in part of the scanning electron
104 microscope (SEM) observation to examine if any morphological change could have been a
105 recent evolutionary adaptation due to human management interventions.

106

107 **Trap escape test.** The insects were evaluated in their ability to escape from four bed bug
108 pitfall traps namely, Verifi™ bed bug detector (FMC Professional Solutions, Philadelphia,
109 PA), ClimbUp® insect interceptor (Susan McKnight Inc, Memphis, TN) (without the talc),
110 BlackOut™ bed bug detector (Protect-A-Bed, Wheeling, IL), and SenSci Volcano™ bed bug
111 detector (Bedbug Central, Lawrenceville, NJ). All traps were cleaned with 70% ethanol and
112 dried in a 60°C incubator overnight before the test. While ClimbUp® traps are normally used
113 with the talc (as per manufacturer recommendation), they were evaluated in the absence of
114 talc in this study. Lures are available for use in conjunction with the Verifi and Volcano traps
115 to enhance their collection efficacy but they were not used in this study. One strain of each
116 species was tested: *C. lectularius* (SYD strain) and *C. hemipterus* (KL strain). The evaluation
117 was carried out by introducing 5 adult males, 5 adult females and 5 fourth to fifth instars into
118 the trap. The tested insects were placed in the pitfall for both Verifi and Volcano traps, and in
119 the outer pitfall for the ClimbUp and Blackout traps. The traps were placed inside test arenas
120 (50 length x 30 cm width x 10 cm height) and kept in an environmental chamber (Binder
121 Model KBF 240, Binder GmbH, Tuttlingen, Germany) under conditions of $26 \pm 1^\circ\text{C}$, $70 \pm 1\%$
122 RH, and 24-h scotophase. All these insects were blood-fed 5 days prior to the test. The
123 experiments began at 2000 hour, and at 4, 24, 48 and 72 hour post-treatment, the number of
124 insects that remained in the trap, and the number that escaped were counted. Observations
125 were made up to 72 hours for all traps except for the Volcano trap which was observed daily
126 for up to 10 days post-treatment. All experiments were replicated three times.

127

128 **Vertical friction force measurement.** To confirm that the differences in climbing ability of
129 the insects within the pitfall traps was due to better holding ability on the smooth surface of

130 one species over the other, the vertical friction forces of both *C. lectularius* and *C. hemipterus*
131 were measured with a Sartorius ED224S (Sartorius, Göttingen, Germany) electronic balance
132 using modified methods described by Betz (2002) and Hottel et al. (2015). A data recording
133 program, Sartorius Weight Anchor Software (Sartorius, Göttingen, Germany) installed on a
134 computer, was connected to the electronic balance using a Sartorius RS232 cable (Sartorius,
135 Göttingen, Germany). A bed bug was tethered to a polyethylene bristle by gluing (UHU
136 Power Glue®, UHU GmbH & Co. KG, Bühl, Germany) its dorsal side to one tip of the bristle.
137 The other tip of the bristle was inserted into a 30-g ball of modelling clay (Tack-it, A.W.
138 Faber-Castell (India) Ltd, Mumbai, India). The clay ball, which was attached to the bristle,
139 was placed on the balance with the tethered bed bug over the other end, and tared to zero.
140
141 Two surfaces were tested, namely a smooth surface (glass microscope cover slide), and a
142 rough surface (filter paper). The test surface was moved to the tethered bed bug to permit the
143 insect's legs to come into contact. Once the insect had contacted and gripped onto the test
144 surface, the software started recording the changes in the mass of the clay ball for 5 min. The
145 data was then converted from mass (g) to force (μN) using the formula $F = ma$, where $m =$
146 mass (g), and $a =$ acceleration = $-9.81 \text{ m}^2/\text{s}$. The mean vertical friction force (MVFF) was
147 calculated using the top five maximum readings. One strain of each species was tested: *C.*
148 *lectularius* (SYD strain), *C. hemipterus* (KL strain). Five adult males and five adult females
149 were used for each species. All these insects were blood-fed 5 days prior to the test. The
150 weight of the insects (g) was individually measured using the balance, and converted to μN
151 using the formula as stated above. The MVFF: insect weight ratio was calculated and used to
152 compare between both species, instead of using MVFF to equalize the effect of weight
153 differences between the species.

154

155 **SEM observation of the tibial pad (fossula spongiosa).** Five adult males and five adult
156 females of the two species (*C. lectularius* [MH and SYD strains], and *C. hemipterus* [KL, GL,
157 and PNG strains]) were used for SEM observation of the tibial pad. Two each of 4th instars
158 and 5th instar of *C. lectularius* (MH strain) were examined for the presence of the tibial pad.
159 Bed bugs were preserved in 70% ethanol, dehydrated and cleaned using acetone and a
160 Sonicator SC-52H (Sonicor Instrument Corp, New York, US). The fore-, mid- and hind-legs
161 of the specimens were excised and mounted on an aluminium stub. The specimens were
162 coated with gold and observed under a scanning electron microscope (SEM) at 15 kV (Leo
163 Supra 50VP field emission SEM, Carl Zeiss SMT, Oberkochen, Germany). The number of
164 tenent hairs (setae) on the tibial pad of each leg was counted from the SEM images taken
165 from different angles.

166

167 **Histological examination of the tibial pad in *C. lectularius* and *C. hemipterus*.** The tibiae
168 with attached tarsomeres of the six legs of three male and three female adults of both *C.*
169 *lectularius* (MH strain) and *C. hemipterus* (QSL strain) were cut off with microsurgery
170 scissors, and fixed in cold 2% glutaraldehyde, buffered with 50 mM Na-cacodylate and 150
171 mM saccharose. Tissues were postfixed in 2% osmium tetroxide in the same buffer,
172 dehydrated through a graded acetone series and embedded in Araldite resin. Serial semithin
173 sections with a thickness of 1 µm were made with a Leica EM UC6 ultramicrotome (Leica
174 Biosystems, Nussloch, Germany), stained with methylene blue and thionin, and examined
175 with an Olympus BX-51 light microscope (Olympus Corporation, Tokyo, Japan).

176

177 **Statistical Analysis.** Data in percentage of escaped bed bugs from the traps were converted
178 to arc-sine values before the analyses. *T*-test was used to compare between the trap escape
179 rates of *C. lectularius* and *C. hemipterus*. Data on vertical friction force, and the number of
180 tenent hairs for adult males and females of both species were subjected to one-way Analysis
181 of Variance (ANOVA) and means were separated using Tukey's HSD at 95% confidence
182 level. All analyses were performed using the statistical program SPSS version 20 (IBM Corp.,
183 Armonk, NY).

184 **Results**

185 **Trap escape test.** Results showed that all stages tested of *C. hemipterus* were able to escape
186 from each of the traps, with mean combined escape rates (%) ranging from 24.4 ± 4.4 to 75.6
187 ± 4.4 (Figure 1) per trap, at 72 hours post-treatment. In contrast, most traps were effective in
188 containing *C. lectularius* with only 0 to 2.2% escape rates, except for the ClimbUp insect
189 interceptor which registered a $26.7 \pm 10.2\%$ mean escape rate. The escape rates from all traps
190 were significantly different ($P < 0.05$) between *C. lectularius* and *C. hemipterus*. For the
191 Volcano trap, an observation of up to 10 days revealed an escape rate of $55.0 \pm 27.5\%$ for *C.*
192 *hemipterus*, while no *C. lectularius* escaped during the corresponding period.

193
194 When the monitors were tested against adults of *C. hemipterus*, the mean cumulative
195 percentage of escaped insects in Verifi and ClimbUp traps were significantly higher ($P < 0.05$)
196 than the BlackOut and the Volcano traps at 4 hours post-treatment (Figure 2). Regarding *C.*
197 *lectularius*, significantly ($P < 0.05$) more adults escaped from the ClimbUp trap than from the
198 other three evaluated traps. No nymphs of either species were able to escape from the
199 Blackout and Volcano traps (Figure 2). For *C. lectularius*, no nymphs escaped from Verifi
200 trap, but $13.3 \pm 6.7\%$ nymphs escaped from ClimbUp trap at 72 hour post-treatment. When

201 tested against adult *C. lectularius*, 3.3 ± 3.3 % and 33.3 ± 12.0 % adults escaped from Verifi
202 and ClimbUp traps, respectively. Despite some nymph escapees, there were no significant
203 differences ($P > 0.05$) in the mean cumulative percentage of escaped nymphal bed bugs of all
204 four evaluated traps. Both Blackout and Volcano traps were effective in containing the *C.*
205 *lectularius* adults with no escapees throughout the 72 hours evaluation period (as well as at 10
206 days post-treatment for Volcano trap).

207

208 Nymphs of *C. hemipterus* managed to escape from Verifi and ClimbUp traps at the rates of
209 26.7 ± 13.3 % and 20.0 ± 11.5 %, respectively at 72 hours. There were no nymph escapees for
210 the Blackout and Volcano traps. Verifi was the least efficient trap against the adults of *C.*
211 *hemipterus* with 100% escapees within 4 hours after the bed bugs were introduced into the
212 trap. ClimbUp trap also showed a high escape rate (>90 %) for *C. hemipterus* adults at 72 hour
213 post-treatment. In contrast, 60.0 ± 15.3 and 36.7 ± 6.7 % of adult *C. hemipterus* escaped from
214 Blackout and Volcano traps, respectively, during the same 72-hour evaluation period.

215

216 **Vertical friction force.** Visual observation revealed that when bed bugs were climbing on the
217 rough surface, the tarsal claws were used, while tibial pads only were used while climbing on
218 smooth surfaces (Figure 3). In this study, *C. hemipterus* demonstrated a significantly higher
219 vertical friction force ($P < 0.05$) of approximately 3 – 4x that of *C. lectularius* when tested on
220 smooth glass microscope cover slides (Table 2). However, there appeared to be no differences
221 in vertical friction force in both species when the insects were tested on filter paper as a rough
222 substrate. No differences in vertical friction force were found between adult males and
223 females of each species.

224

225 **SEM observation on tibial pad.** Tibial pads were present in the adults of both species, but
226 they were not present in the fourth instar (Figure 4) and fifth instar. The pads appear as an
227 oval disk of approximately 120 x 40 μm at the ventrodistal portion of the tibia of the 6 legs
228 (Figure 5). The ventral side of the pad is covered with parallel tenent hairs that have a golf
229 club-like appearance. The hairs have a length between 30 and 40 μm and a diameter around 2
230 μm , the club-like distal portion having a length of approximately 10 μm (Figure 5). The
231 number of tenent hairs on the tibial pad of *C. hemipterus* was significantly higher ($P < 0.05$)
232 than in *C. lectularius* (Table 3, Figure 5). The number of tenent hairs appeared to be similar
233 among the different strains of each species (Table 3), however, the fore- and mid-legs showed
234 more tenent hairs than the hind-legs. The three strains of *C. hemipterus* have almost twice the
235 number of tenent hairs ($P < 0.05$) on the hind-legs, compared to *C. lectularius*. The adult male
236 of *C. lectularius* had significantly more ($P < 0.05$) tenent hairs than the adult female of the
237 same species on their fore- and mid-legs, but this observation was not recorded for *C.*
238 *hemipterus*. The rank in total tenent hairs on the fore-, mid- and hind-legs for one side of the
239 body is as follows: *C. hemipterus* males = *C. hemipterus* females $>$ *C. lectularius* males $>$ *C.*
240 *lectularius* females.

241

242 **Histological examination of tibial pad in *C. lectularius* and *C. hemipterus*.** Serial
243 longitudinal sections through the distal tibial part revealed details of the internal anatomy of
244 the tibial pad region (Figure 6A – 6D). The tenent hairs are hollow with an internal diameter
245 around 1 μm , and penetrate the cuticular plate that forms the base of the tibial pad. In all legs
246 of both males and females of *C. lectularius* and *C. hemipterus*, the tegumental epithelium of
247 the tibia is differentiated into a glandular epithelium. This epithelium in all legs is more
248 pronounced in *C. hemipterus*, where it reaches a thickness of 30 μm , while in *C. lectularius* it
249 is approximately half as thick (Figure 6A – 6D). At its apical side, the epithelium shows a

250 subcuticular space in which its secretory products can be stored, and which is continuous with
251 the central space of the hollow tenent hairs. The epithelium is not associated with any
252 muscular tissue. The tibia contains two tendons, however, of which the ventral one passes in
253 the near vicinity of the glandular epithelium. Both tendons are connected with muscles in the
254 proximal part of the tibia and distally to the basitarsus, of which they direct the movements.

255

256 **Discussion**

257 Unlike *C. lectularius*, adult *C. hemipterus* were found to escape from all pitfall traps
258 evaluated. Examination of the vertical friction force of both species revealed higher vertical
259 friction force in *C. hemipterus*, compared to *C. lectularius*. As bed bugs climb on rough
260 surface using their tarsal claws, and on smooth surfaces using the tibial pads, it was suspected
261 that the differences in climbing ability between the two species must be related to variations
262 in the tibial pad. Although Wigglesworth (1938) previously reported that the tibial pad did not
263 assist in the climbing of smooth surfaces by the bed bug, he was experimenting using only *C.*
264 *lectularius*. Should he have worked on *C. hemipterus*, his conclusion would have been very
265 different. In the reduviid kissing bug (*Triatoma* spp.), it has been proposed that the tibial pads
266 enable the adult bugs to climb smooth surfaces (Gillett & Wigglesworth 1932, Weirauch
267 2007). Usinger (1966) described the presence of the tibial pad in *C. lectularius*, but did not
268 indicate its presence in *C. hemipterus*.

269

270 To date, the tibial pad had been reported as “fossula spongiosa” by Haridass &
271 Ananthkrishnan (1980), “apex of tibia” by Walpole (1987), and “tibial brush” by several
272 authors (Ferris & Usinger 1957, Baker et al. 2016). The tenent hairs (Haridass &
273 Ananthkrishnan 1980) were described as a “turf of hair” (Usinger 1966), “tibial brush setae”
274 (Baker et al. 2016), or “setae of tibial apex” (Walpole 1987).

275
276 SEM observation on the tibial pad revealed a greater number of tenent hairs in *C. hemipterus*
277 than in *C. lectularius*. By incapacitating the function of tenent hairs on the tibial pad using
278 glue, it was found that this affected the mating process of *C. hemipterus*, which led to a
279 reduction in reproductive success (Kim & Lee, unpublished data). It is speculated that the
280 tibial pad may play an important role in the reproduction of the bed bug, beyond that of
281 providing grip in the climbing of smooth surfaces. This argument is further supported by the
282 absence of tibial pads in the nymphal stages, which explains their poor climbing ability as
283 documented in this study. Using SEM, Walpole (1987) too reported that the tibial pad could
284 only be found in the adults of *C. lectularius* and *C. hemipterus*, but failed to determine its
285 function. In other insect orders such as Blattodea (Clemente & Federle 2008), Diptera
286 (Bauchhenss 1979, Gorb 1998, Gorb & Beutel 2001), Hymenoptera (Dirks & Federle 2011),
287 Coleoptera (Ishii 1987, Geiselhardt et al. 2011, Hosoda & Gorb 2011), as well as other
288 families in the order Hemiptera (Gillett & Wigglesworth 1932, Edwards & Tarkanian 1970,
289 Haridass & Ananthakrishnan 1980), the use of the tenent hairs for surface adhesion has been
290 documented. Baker et al. (2016) described the tenent hairs on the tibia pad as “tibia brush
291 setae” and also suggested that these setae may be functional during the mating process and for
292 climbing of various surfaces.

293
294 Although the precise mechanism of how the tibial pad allows climbing on smooth vertical
295 surfaces remains still unknown, it seems obvious that the hollow tenent hairs play a role in the
296 ability to do so. Their central space is continuous with the subcuticular space of the glandular
297 epithelium that is associated with the tibial pad (Baker et al. 2016), suggesting that a
298 glandular secretion can be released through the hairs. With an internal diameter around 1 μm ,
299 mere capillary action may be sufficient for this. In addition to capillarity, secretion may also

300 be pumped through the hollow hairs, although there is no direct muscular tissue associated
301 with the glandular epithelium. However, there is the ventral tibial tendon, that is situated in
302 close proximity to the epithelium, and which may cause a pressure onto the epithelium and
303 hence also on the fluid in the subcuticular space. Such pulling action of the tendon may occur
304 during climbing when the tarsomeres need to be moved in order to bring the tibial pad in the
305 right configuration with respect to the substrate. Increased pressure from the tendon onto the
306 glandular epithelium at the same time may then result in fluid being pumped into the tenent
307 hairs. A somewhat comparable situation is known in ants, in which adhesion to smooth
308 surfaces is possible through the arolium that acts as an adhesive pad, which is situated at the
309 tip of the pretarsus of each leg. The arolium is associated with a sac-like epithelial gland, that
310 works as a hydraulic system by pumping liquid into the arolium upon contraction of the leg
311 tendon (Federle et al. 2001). In a recent study comparing two ant species with very opposite
312 climbing ability, it was found that both species possessed an arolium gland, although it was
313 only poorly developed in the non-climbing species (Billen et al. 2016). These reports are in
314 line with the observations herein that both *Cimex* species possess a tibial pad, but that the
315 higher number of tenent hairs and the better developed glandular epithelium in *C. hemipterus*
316 allow this species to climb smooth vertical surfaces.

317

318 In the experiments described herein, the ClimbUp trap was evaluated without the addition of
319 talc, and *C. hemipterus* was able to easily escape from the monitor. When talc was applied (as
320 per manufacturer recommendations), the monitor was able to contain insects of both *C.*
321 *lectularius* and *C. hemipterus* with minimal escapees (< 5%) over a 72 hour period (Kim &
322 Lee, unpublished data). Wang & Cooper (2011) reported that regular talc powder
323 reapplications on pitfall-type monitors are required to prevent bed bugs from escaping. Hottel
324 et al. (2015) found that talc powder reduced the vertical pulling force of *C. lectularius* on

325 glass surfaces and the ClimbUp inner wall. In addition, from our experience, the number of
326 trapped escapees could be minimized by placing a small piece of folded corrugated cardboard
327 inside the pitfall trap. However, this would require further efforts as the harbourage would
328 need to be examined during monitoring programs. It is anecdotally known that the
329 performance of pitfall traps declines in use over time due to the build-up of dust and debris,
330 and can even overcome the addition of talc (Singh et al. 2013, Wang et al. 2013, Cooper et al.
331 2016). A trial examining variably aged traps could provide insights into the required
332 maintenance intervals to prevent escape of the respective species.

333
334 The present study demonstrated the need for the design of a more efficient bed bug trap (and
335 the need to reconsider trap maintenance intervals) that could be used against both *C.*
336 *lectularius* and *C. hemipterus*. New low friction surfaces for pitfall traps could also be
337 explored, as long as the cost per unit item for the trap would not financially compromise any
338 monitoring program. It remains unknown at this stage why these sympatric species of bed
339 bugs could have these differences in leg morphology, and the possible biological trade-offs it
340 may have on *C. hemipterus*.

341

342 **Acknowledgments**

343 We thank Jamilah Afandi and Johari Othman (Electron Microscopy Unit, Universiti Sains
344 Malaysia) for assistance with scanning microscopy, An Vandoren (KU Leuven) for making
345 the histological sections, and Annie Eow for helping with the field collection of *C. hemipterus*.
346 This study was supported by Bayer Environmental Science, Singapore.

347

348 **References Cited**

349 **Baker, G.T., J. Goddard, and A. Lawrence. 2016.** Structure of the tibial brush setae of the
350 bedbug, *Cimex lectularius* L. (Hemiptera: Cimicidae). Poster presentation. Microscopy and
351 Microanalysis 2016. July 24 - 28, 2016. Columbus, Ohio, USA.
352 <http://www.microscopy.org/MandM/2016/program/abstracts/PDP-24.pdf> (retrieved on
353 August 8, 2016).

354 **Bauchhenss, E. 1979.** Die Pulvillen von *Calliphora erythrocephala* (Diptera, Brachycera) als
355 Adhäsionsorgane. Zoomorphologie 93: 99-123.

356 **Betz, O. 2002.** Performance and adaptive value of tarsal morphology in rove beetles of the
357 genus *Stenus* (Coleoptera, Staphylinidae). J. Exp. Biol. 205: 1097–1113.

358 **Billen, J., M.S. Al-Khalifa and R.R. Silva. 2016.** Pretarsus structure in relation to climbing
359 ability in the ants *Brachyponera sennaarensis* and *Daceton armigerum*. Saudi J. Biol. Sci.
360 (in press) <http://dx.doi.org/10.1016/j.sjbs.2016.06.007>

361 **Boase, C., and R. Naylor. 2014.** Bed bug management. pp. 8-22. In: Urban insect pests:
362 sustainable management strategies (P. Dhang, ed.), CABI Press, London, UK.

363 **Campbell, B.E., P.G. Koehler, L.J. Buss & R.W. Baldwin. 2016.** Recent documentation of
364 the tropical bed bug (Hemiptera: Cimicidae) in Florida since the common bed bug
365 resurgence. Fla. Entomol. 99: 549-551.

366 **Clemente, C.J., and W. Federle. 2008.** Pushing versus pulling: Division of labour between
367 tarsal attachment pads in cockroaches. Proc. R. Soc. B: Biol. Sci. 275:1329-1236.

368 **Cooper, R.A., C. Wang, and N. Singh. 2016.** Evaluation of a model community-wide bed
369 bug management program in affordable housing. Pest Manag. Sci. 72: 45 - 56.

370 **Doggett, S.L. 2013.** A Code of Practice for the Control of Bed Bug Infestations in Australia,
371 4th ed. Department of Medical Entomology, Westmead Hospital, Sydney, and The
372 Australian Environmental Pest Managers Association.

373 **Doggett, S.L., M.J. Geary, W.J. Crowe, P. Wilson, and R.C. Russell. 2003.** Has the
374 tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae), invaded Australia? J. Environ.
375 Health 3: 80-82.

376 **Edwards, J.S., and M. Tarkanian. 1970.** The adhesive pads of Heteroptera: a reexamination.
377 Physiol. Entomol. 45: 1-5.

378 **Dirks, J.-H., and W. Federle. 2011.** Fluid based adhesion in insects - principles and
379 challenges. Soft Mat. 7: 11047-11053.

380 **Federle, W., E.L. Brainerd, T.A. McMahon and B. Hölldobler. 2001.** Biomechanics of the
381 movable pretarsal adhesive organ in ants and bees. Proc. Natl Acad. Sci. USA 98: 6215-
382 6220.

383 **Ferris, G.F., and R.L. Usinger. 1957.** Notes on and descriptions of Cimicidae (Hemiptera).
384 Microentomol. 22: 1-37.

385 **Geiselhardt, S.F., S. Geiselhardt, and K. Peschke. 2011.** Congruence of epicuticular
386 hydrocarbons and tarsal secretions as a principle in beetles. Chemoecol. 21: 181-186.

387 **Gillett, J.D., and V.B. Wigglesworth. 1932.** The climbing organs of an insect, *Rhodnius*
388 *prolixus* Stal (Heteroptera-Reduviidae). Proc. R. Entomol. Soc. London B 111: 364-376.

389 **Gorb, S., and R. Beutel. 2001.** Evolution of locomotory attachment pads of hexapods.
390 Naturwissenschaften 88: 530-534.

391 **Gorb, S.N. 1998.** The design of the fly adhesive pad: distal tenent setae are adapted to the
392 delivery of an adhesive secretion. Proc. R. Soc. B: Biol. Sci. 265: 747-752.

393 **Haridass, E.T., and T.N. Ananthkrishnan. 1980.** Functional morphology of the fossula
394 spongiosa in some reduviids (Insecta - Heteroptera - Reduviidae). Proc. Ind. Acad. Sci. 89:
395 457-466.

396 **Hixson, H. 1943.** The tropical bedbug established in Florida. Records of *Cimex hemiptera*, F.,
397 in houses in several localities in 1938-42. Fla. Entomol. 26: 47.

398 **Hosoda, N., and S.N. Gorb. 2011.** Friction force reduction triggers feet grooming behaviour
399 in beetles. Proc. R. Soc. B: Biol. Sci. 278: 1748-1752.

400 **Hottel, B., R. Pereira, S. Gezan, R. Qing, W. Sigmund, and P. Koehler. 2015.** Climbing
401 ability of the common bed bug (Hemiptera: Cimicidae). J. Med. Entomol. 52: 289-295.

402 **How, Y.F., and C.Y. Lee. 2010.** Survey of bed bugs in infested premises in Malaysia and
403 Singapore. J. Vect. Ecol. 35: 89-94.

404 **Ishii, S. 1987.** Adhesion of a leaf feeding ladybird *Epilachna vigintioctomaculata*
405 (Coleoptera: Coccinellidae) on a vertically smooth surface. Appl. Entomol. Zool. 22: 222–
406 228.

407 **Lee, C.Y. 2013.** Bed bugs in Asia - Perspective from Southeast Asia, Global Bed Bug
408 Summit, 5–6 December 2013, Denver, Colorado.

409 **Newberry, K. 1988.** Production of a hybrid between the bedbugs *Cimex hemipterus* and
410 *Cimex lectularius*. Med. Vet. Entomol. 2: 297-300.

411 **Newberry, K. 1989.** The effects on domestic infestations of *Cimex lectularius* bedbugs of
412 interspecific mating with *Cimex hemipterus*. Med. Vet. Entomol. 3: 407-414.

413 **Omori, N. 1939.** Experimental studies on the cohabitation and crossing of two species of bed-
414 bugs (*Cimex lectularius* L. and *C. hemipterus* F.) and on the effects of interchanging of
415 males of one species for the other, every alternate days, upon the fecundity and longevity
416 of females of each species. Acta Jap. Medic. Tropic. 1: 127-154.

417 **Omori, N. 1941.** Comparative studies on the ecology and physiology of common and tropical
418 bed bugs, with special reference to the reactions to temperature and moisture. J. Med.
419 Assoc. Formosa 60: 555-729.

420 **Pinto, L.J., R.A. Cooper, and S.K. Kraft. 2007.** Bed bug handbook: The complete guide to
421 bed bugs and their control. Pinto & Associates, Inc. Mechanicsville, MD, USA.

422 **Singh, N., C. Wang, and R.A. Cooper. 2013.** Effect of trap design, chemical lure, carbon
423 dioxide release rate, and source of carbon dioxide on efficacy of bed bug monitors. *J. Econ.*
424 *Entomol.* 106: 1802-1811.

425 **Usinger, R.L. 1966.** Monograph of Cimicidae (Hemiptera - Heteroptera). Entomological
426 Society of America, College Park, Maryland.

427 **Walpole, D. 1987.** External morphology of the legs of two species of bed bugs (Hemiptera:
428 Cimicidae). *J. Entomol. Soc. South. Afr.* 50: 193-201.

429 **Wang, C. and R.A. Cooper. 2011.** Environmentally sound bed bug management solutions.
430 pp. 44 - 63. In: P. Dhang (ed.), *Urban Pest Management: An Environmental Perspective.*
431 CABI, Oxon, UK.

432 **Wang, C., N. Singh, R.A. Cooper, C.F. Liu, and G. Buczkowski. 2013.** Evaluation of an
433 insecticide dust band treatment method for controlling bed bugs. *J. Econ. Entomol.* 106:
434 347 - 352.

435 **Weirauch, C. 2007.** Hairy attachment structures in Reduviidae (Cimicomorpha, Heteroptera),
436 with observations on the fossula spongiosa in some other Cimicomorpha. *Zool. Anz.* 246:
437 155-175.

438 **Wigglesworth, V. 1938.** Climbing organs in insects. *Nature* 141: 974-975.

439 **Zulaikha, Z., A.M.A. Hafiz, A.R.A. Hafis, and A.A. Hassan. 2016.** A survey on the
440 infestation levels of tropical bed bugs in Peninsular Malaysia: Current updates and status
441 on resurgence of *Cimex hemipterus* (Hemiptera: Cimicidae). *Asian Pac. J. Trop. Dis.* 6:
442 40-45.

443

444 **Table 1: The bed bug strains used in this study.**

Species	Strain	Year established	Remarks
<i>C. hemipterus</i>	Greenlane (GL)	2015	field collected
	Kuala Lumpur (KL)	2005	lab colony
	Queensland (QSL)	~2004	lab colony
	Papua New Guinea (PNG)	1932	curated specimens in 70% ethanol
<i>C. lectularius</i>	Monheim (MH)	~1970	lab colony
	Sydney (SYD)	2004	lab colony

445

446

447 **Table 2. Mean vertical friction forces (MVFF) on smooth and rough surfaces, weights**
 448 **and MVFF: weight ratios of the adult male and female of *C. lectularius* and *C.***
 449 ***hemipterus*.**
 450

Species	Sex	MVFF \pm SE (μ N) ¹		weight (μ N)	MVFF: weight ratio	
		Cover glass	Filter paper		Cover glass	Filter paper
<i>C. hemipterus</i>	M	156.8 \pm 9.2a	2476 \pm 140.5a	25.5 \pm 2.5c	6.3 \pm 0.6a	102 \pm 13.4a
	F	184.8 \pm 37.8a	3203 \pm 579.5a	30.6 \pm 2.0bc	6.5 \pm 1.7a	110 \pm 25.0a
<i>C. lectularius</i>	M	48.7 \pm 10.2b	2338 \pm 307.3a	37.9 \pm 2.4ab	1.3 \pm 0.3b	62 \pm 6.7a
	F	42.2 \pm 11.8b	3181 \pm 516.0a	43.6 \pm 4.1a	1.0 \pm 0.2b	77 \pm 17.9a

451 ¹Means followed by different letters within the same column are significantly different (P < 0.05;
 452 Tukey's HSD).

453
 454

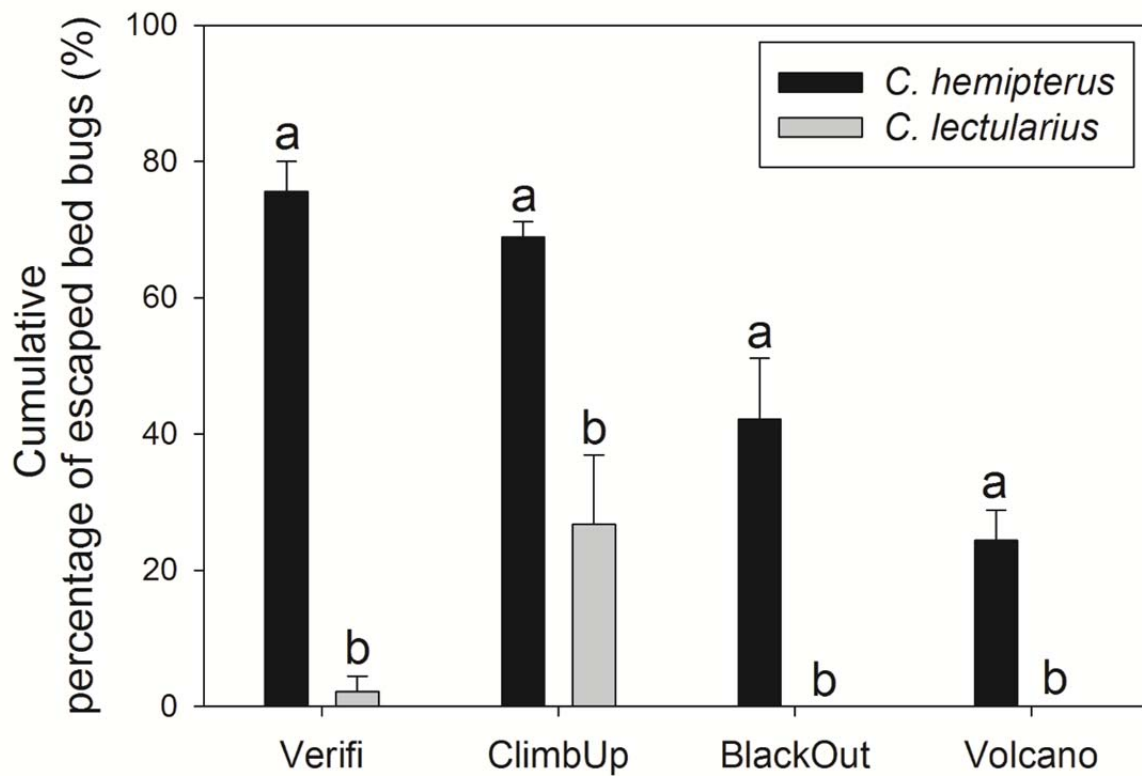
455 **Table 3. Mean number of tenent hairs on tibial pad of the fore-, mid- and hind-legs of adult *C.***
 456 ***hemipterus* and *C. lectularius*.**
 457

Species	Sex	Strains	Fore leg ¹	Mid leg ¹	Hind leg ¹	Total ¹
<i>C. hemipterus</i>	M	KL	117.9 ± 5.7a	107.1 ± 6.0a	86.2 ± 2.5a	335.7 ± 13.6a
		GL	101.8 ± 2.6ab	97.6 ± 3.4a	78.8 ± 2.5a	292.2 ± 9.2ab
		PNG	113.1 ± 2.9a	106.4 ± 2.7a	79.3 ± 2.4a	318.2 ± 8.1a
	F	KL	100.0 ± 8.1ab	112.7 ± 3.1a	88.1 ± 1.9a	309.7 ± 11.8a
		GL	105.7 ± 4.3a	110.3 ± 3.4a	87.7 ± 2.9a	328.6 ± 9.9a
		PNG	115.9 ± 3.6a	115.7 ± 4.2a	89.8 ± 1.7a	340.6 ± 10.0a
<i>C. lectularius</i>	M	MH	119.8 ± 7.3a	99.3 ± 5.4a	41.8 ± 2.2b	247.6 ± 10.4bc
		SYD	114.2 ± 5.3a	102.7 ± 3.9a	49.2 ± 2.0b	239.4 ± 12.2c
	F	MH	66.8 ± 0.7c	71.6 ± 1.4b	43.2 ± 2.4b	176.8 ± 4.0d
		SYD	79.6 ± 1.7bc	78.0 ± 3.9b	47.8 ± 1.9b	200.4 ± 7.0cd

458 ¹Means followed by different letters within the same column are significantly different (P<0.05,
 459 Tukey's HSD).
 460

461

Figure captions



463

464 **Fig. 1.** Cumulative percentage of combined escaped adults and nymphs of bed bugs (Mean \pm
465 SEM) from the four traps after 72-h post treatment. Letters above each bar are assigned for
466 comparison between the same trap and different letters denote significant difference (P<0.05,
467 t-test).

468

469

470

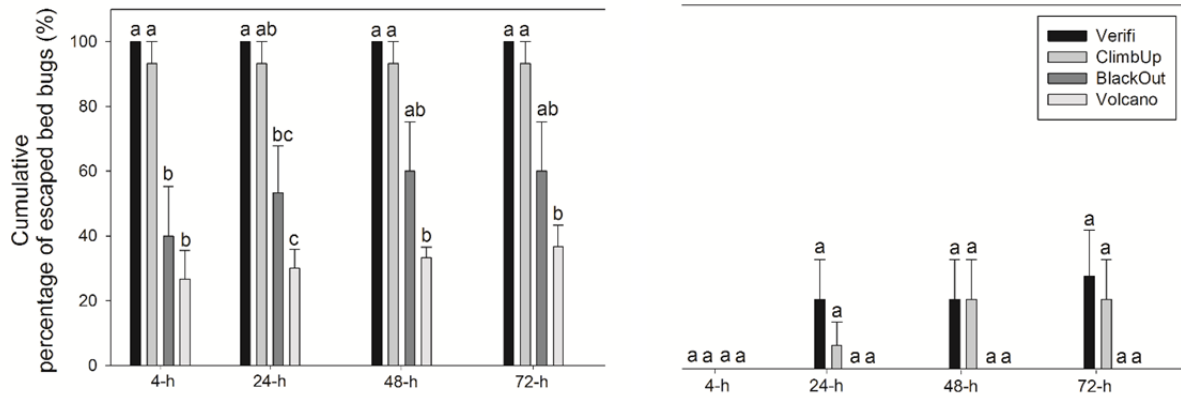
471

472

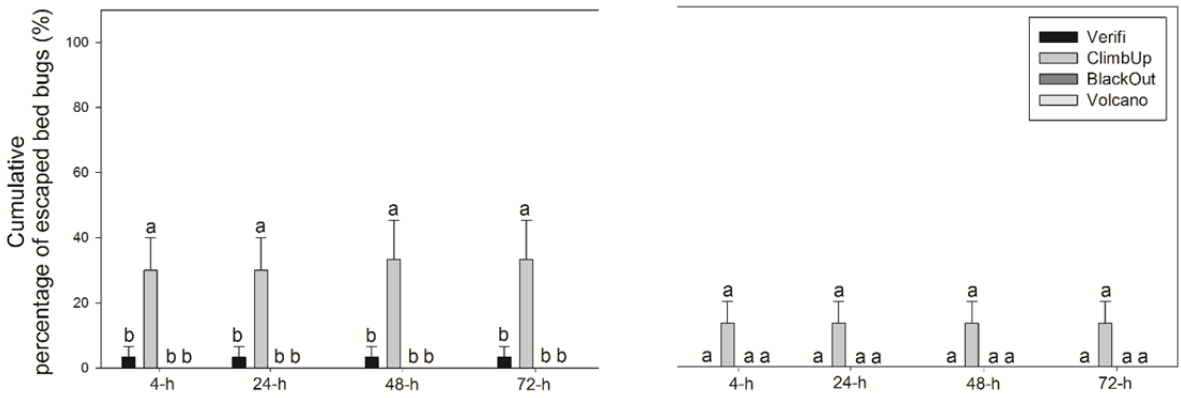
473

474

475



476

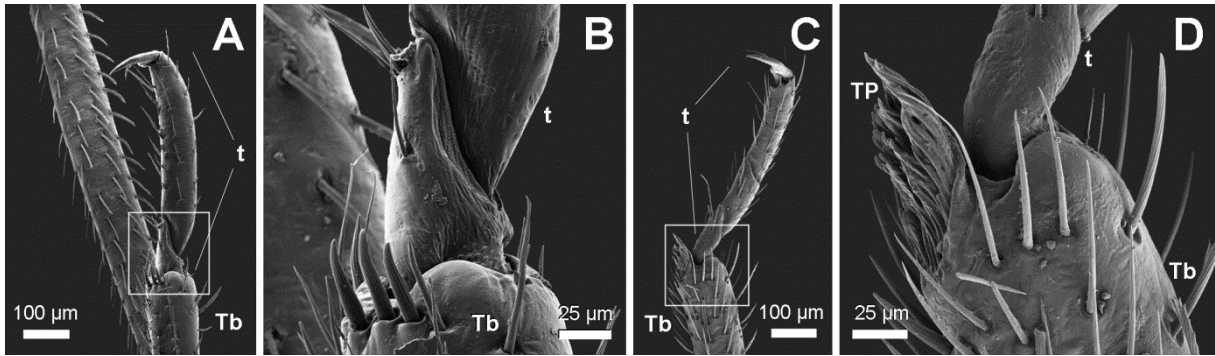


477 **Fig. 2.** Mean cumulative percentage of escaped bed bugs \pm SE at 4, 24, 48 and 72-h post
 478 treatment (Top left: *C. hemipterus* adults; Top right: *C. hemipterus* nymphs; Bottom left: *C.*
 479 *lectularius* adults; Bottom right: *C. lectularius* nymphs). Letters above each bar are assigned
 480 for comparison between the traps and different letters denote significant difference ($P = 0.05$,
 481 Tukey's HSD).

482

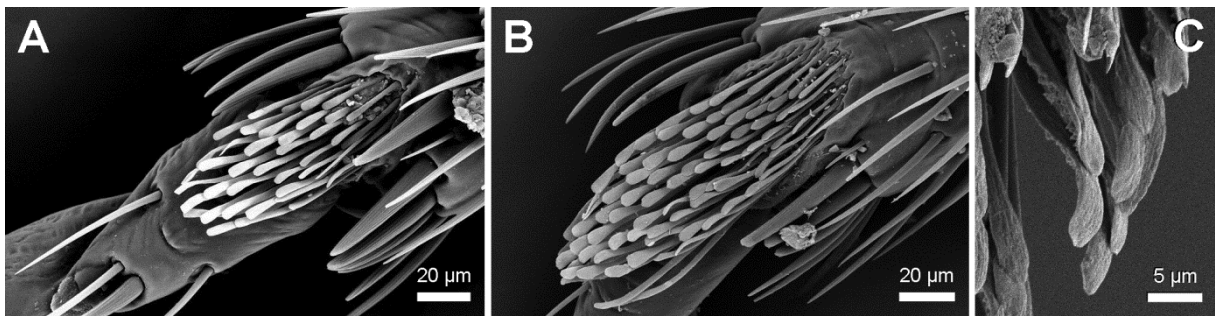


483 **Fig. 3.** Detail of foreleg of *C. hemipterus* male climbing on plastic petri dish (smooth surface)
 484 (A) and filter paper (rough surface) (B). Note that whitish tibial pad (arrow) is in contact with
 485 substrate only on smooth surface. Observation was made using live bed bugs under an SZ61
 486 stereomicroscope (Olympus, Tokyo, Japan) connected to a CCD camera.



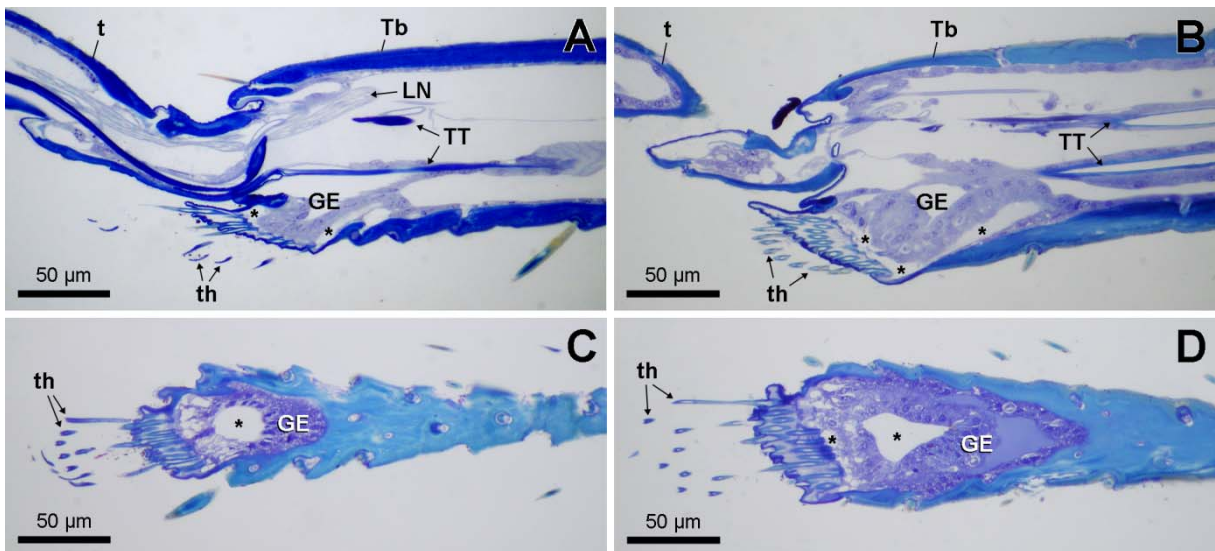
487

488 **Fig. 4.** SEM micrographs showing the absence of tibial pad in 4th instar *C. lectularius* (A, B),
 489 and presence of tibial pad in adult female *C. lectularius* (C, D). The framed area in A and C
 490 indicate the parts that are enlarged in B and D, respectively. t: tarsomeres, Tb: tibia, TP: tibial
 491 pad.



492

493 **Fig. 5.** SEM micrographs showing the tibial pad of the hind legs of an adult female in *C.*
 494 *lectularius* (A) and *C. hemipterus* (B). C. Detail of tenent hairs of male *C. hemipterus* mid leg.



495

496 **Fig. 6.** Longitudinal sections through the tibial pad of *C. lectularius* (A, male mid leg) and *C.*
 497 *hemipterus* (B, male fore leg), showing the glandular epithelium (GE), the subcuticular space
 498 (asterisks) and the hollow tenent hairs (th). Note the tibia contains two tibial tendons (TT), of
 499 which the ventral is close to the glandular epithelium. The sectioning plane in C (*C.*
 500 *lectularius* female hind leg) and D (*C. hemipterus* male mid leg) is perpendicular to that in A
 501 and B, and is more or less parallel to the ventral surface of the tibia. LN: leg nerve, t:
 502 tarsomeres, Tb: tibia. The histological organization in all legs is similar, the photographs
 503 shown in this figure were selected as they were the best available images.