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1 **Developmental morphology of a lyriform organ in the Western**
2 **black widow (*Latrodectus hesperus*)**

3
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13
14 Keywords: *Latrodectus hesperus*, lyriform organ, slit sensilla, development, allometry, vibration
15 detection.

16 Abstract

17 For most spiders their sensory world is dominated by their ability to detect vibrational stimuli. The
18 organs responsible for detecting substrate vibrations are located on the animals' extremities and
19 known as lyriform organs; close aggregations of membrane-covered slits in the cuticular exoskeleton.
20 The morphology and geometry of the lyriform organ is an important determinant of how it functions
21 and the range of stimuli it can detect. Most work on the morphology, mechanics, and physiology of
22 lyriform organs has been conducted on adult wandering spiders, *Cupiennius salei*, and little is known
23 about the morphology in other species or juveniles. We examine the morphology of the HS10 lyriform
24 organ in both adult and juvenile Western black widows (*Latrodectus hesperus*). We find
25 hypoallometric scaling of the lyriform organ, and the size of individual slits when compared to body
26 size. However, the cuticular pad distal to HS10 scales isometrically across successive instars. We also
27 find an increase in the number of slits within the lyriform organ with each moult. Future work should
28 address physiological responses of the organ across development, which could lead to a better
29 understanding of the function of the cuticular pad and stimuli pertinent to the survival of little studied
30 juvenile spiders.

31

32 Introduction

33 Spiders are exquisitely sensitive to vibration. From species that dwell and hunt on plants and the
34 ground to those that use silk webs and traps, vibration is commonly the stimulus modality used to
35 detect prey (Masters and Markl 1981; Klärner and Barth 1982; Landolfi and Barth 1996), predators
36 (Uetz et al. 2002; Lohrey et al. 2009; Uma and Weiss 2012) and potential mates (Schüch and Barth
37 1985; Maklakov et al. 2003; Elias 2003; Elias et al. 2005; Vibert et al. 2014). Vibrational signals are
38 detected with mechanoreceptive strain sensors embedded within the cuticular exoskeleton (see Barth
39 2004; Barth 2012 for reviews). Known as slit sensilla, these sensors detect strain in the cuticle, and can
40 be found singly, in loose clusters, or as distinct groupings known as lyriform organs. Their function
41 spans proprioception of self-generated strains from muscle contraction and haemolymph pressure
42 changes, to detection of externally generated vibrations (Barth 2012). However, it is the lyriform
43 organs that are primarily concerned with detecting vibrational displacement from the external
44 environment; stimuli that can be in the order of mere nanometres (Barth and Geethabali 1982; Hössl
45 et al. 2009).

46 Spiders have many lyriform organs which are exclusively found on their extremities, most frequently
47 at joints on distal sections of the legs (Pringle 1955; Barth 1971; Barth and Stagl 1976). The organs are

48 composed of an array of membrane-covered slits in the cuticular exoskeleton. When a vibrational
49 stimulus is transmitted to the lyriform organ, the compliant membranes are compressed by the
50 surrounding hard cuticle which stimulates neural dendrites (Walcott 1969; Barth et al. 1984; Molina
51 et al. 2009). Each slit within an organ has its own sensory cells and responds independently to a
52 stimulus (Barth and Geethabali 1982). The attachment of the dendrite to the outer membrane is
53 visible externally and is known as the coupling cylinder, the position of which does not necessarily
54 correspond to the position of maximal compression of the slit (Hösssl et al. 2007).

55 The main lyriform organ involved in detecting substrate vibrations is the HS10 organ, found dorsally
56 on the distal end of the metatarsus (MT), just proximal of the metatarsal-tarsal (MT-T) joint (Barth and
57 Libera 1970). Substrate vibrations are transmitted up the tarsus (T) causing it to strike the MT, at the
58 MT-T joint. At the distal end of the metatarsus, between the HS10 organ and MT-T joint, resides a
59 cuticular pad. In the wandering spider, *Cupiennius salei*, this pad is heterogeneous in composition,
60 being soft and compliant at its distal end, but hard and sclerotized at the proximal end, adjacent to
61 the organ (Young et al. 2014; Erko et al. 2015). The pad acts as a high pass filter, reducing the
62 transmission of biologically irrelevant stimuli below 40Hz, but also protecting the organ from high
63 amplitude stimuli that are potentially damaging (McConney et al. 2007; Young et al. 2014). The
64 location of the slit within the lyriform organ, its length and its aspect ratio are all important factors in
65 determining sensitivity to stimuli (Hösssl et al. 2006; Hösssl et al. 2007; Hösssl et al. 2009), and with its
66 own filter, the spider has evolved to detect a specific range of stimuli relevant to its survival and
67 reproduction. Morphological aspects of the lyriform organ are crucial to its function, raising the
68 question of how lyriform organs retain their function throughout development where the size of the
69 animal changes significantly.

70 Much of what has been learned about the physiology, mechanics and morphology of lyriform organs
71 has come from work on the wandering spider, *Cupiennius salei*, (see Barth 2012 for review) although
72 some work has also been conducted on web dwelling species (Finck 1981; Klärner and Barth 1982;
73 Barth 2002). The slit arrangement in HS10 in 4 spider species share a 'basic pattern' but variable
74 numbers of slits; from 21 slits in the *C. salei* to just 8-10 in the American house spider *Achaearanea*
75 *tepidariorum*. In addition to this the length of slits 2 and 11 in the HS10 organ have been measured
76 for 9 spider species (Barth 2002). Although there are comparisons of slit numbers in lyriform organs
77 between different instars in *C. salei* (Barth and Libera 1970), little is known about the development of
78 this organ throughout a spider's life span, or indeed to vibrational senses in juveniles in general.

79

80 We examine the HS10 metatarsal lyriform organ of the Western black widow, *Latrodectus hesperus*,
81 throughout developing instars to adult spiders (Figure 1). *L. hesperus* is a web-dwelling spider that
82 builds cob webs in arid environments (Kaston 1970). After emerging from the egg sac, spiderlings
83 spend up to 14 days in the natal web, before dispersing and remaining on solitary webs for the
84 majority of their lives (Kaston 1970). Mature males abandon their solitary webs to seek out female
85 mates where they perform courtship displays with a vibratory component (Ross and Smith 1979;
86 Kasumovic and Andrade 2004; Vibert et al. 2014). These courtship vibrations are not a stimulus found
87 in juvenile environments. However, the need to detect prey and predators will persist throughout all
88 life stages. Because the sensitivity of lyriform organs depends on their morphology, it is imperative to
89 understand how they change throughout spider development. Developmental changes in morphology
90 are likely to impact the range of environmental stimuli each instar is able to perceive; we would
91 therefore expect minimal changes to overall organ morphology if the biologically relevant stimuli
92 within the environment remain consistent throughout different stages of growth.

93

94 Materials and Methods

95 *L. hesperus* egg sacs were collected and incubated in plastic containers (length x width x height cm³ =
96 8.73 x 8.73 x 11.27; Amac Plastics) at 25°C until spiderlings emerged. In keeping with previous
97 terminology, the first instar was defined as the instar that emerged from the egg sac (Kaston 1970).
98 Shortly after emergence, spiderlings were transferred to individual containers (length x width x height
99 (cm) = 4.13 x 4.13 x 5.56) where they were allowed to moult into successive instars at 24°C. Up to 20
100 spiderlings were taken from each instar group, and euthanised in 70% ethanol where they were kept
101 until imaging.

102 Images of the HS10 metatarsal lyriform organ were taken using scanning electron microscopy (SEM).
103 To prepare samples for imaging spiders were removed from ethanol and affixed to an SEM stub using
104 double sided tape. For early instars the entire animal was positioned on the stub, but for adults and
105 later instars the first pair of legs were removed using fine scissors and positioned on the stub.
106 Photographs were taken with a Nikon DXM 1200 camera mounted on a Zeiss Stemi 2000-C dissecting
107 microscope. Images of the first pair of legs were taken, using Act-1 (Nikon Corp. 2000; Figure 1C), to
108 allow measurement of dimensions of the MT. Where necessary, hairs were shaved from around the
109 HS10 organ, however this was often not possible on smaller animals without causing damage to the
110 surrounding tissue or leaving behind significant debris. Samples were subsequently sputter coated
111 with gold (PS3, Polaron, Watford, UK) before SEM imaging (Hitachi S530 SEM, Hitachi, Tokyo, Japan).
112 Images were acquired and digitised using Quartz PCI imaging software (Quartz PCI, Quartz Imaging

113 Corporation, Vancouver, Canada) and morphological measurements made using Corel Draw (Corel
114 Corporation, Ottawa, Canada) and the JH CurveLength macro.

115

116 Results

117 The HS10 organ is present from the first instar through to adulthood in *L. hesperus*. There is a basic
118 set of 13 slits that are present across all instars examined (Figure 2 and 3). At each moult between
119 instars 1-5, HS10 gains an additional slit, added to the proximal end of the HS10 organ. The maximum
120 number of slits seen in any metatarsal HS10 organ was 23, and this number was only seen on the
121 largest spiders, the adult females. Males had a maximum of 20 slits. It was only possible to measure
122 the first 5 instars due to mortality so it is not possible to tell whether the addition of a single slit at
123 each moult remains the case after the 5th instar into the final moult to adulthood. The positions of the
124 slits are largely conserved between individuals, although there is a small degree of variation (Figure
125 3).

126 The coupling cylinder was visible in the larger HS10's of the adults due to the greater slit width. It was
127 also possible to see it in the SEM images of at least some juveniles in instars 1-4 (Figure 4). The position
128 of the coupling cylinder in adults of both sexes is somewhat offset from the centre of the longitudinal
129 length of the slit, corresponding with previous findings in the wandering spider *C. salei* (Barth and
130 Pickelmann 1975).

131 Relative to the growth of the MT, the HS10 organ shows little change in size across development and
132 has a hypoallometric relationship with MT length. The allometric coefficient (α), or slope of a straight
133 line fit to the log data is less than 1 ($\alpha = 0.45$, figure 5a). Slit length is also hypoallometric throughout
134 development, with a slightly smaller allometric coefficient ($\alpha = 0.31-0.36$, figure 5b). To reach mean
135 adult male size, MT increases 7.9 times. This is a far greater rate than the 2.6-2.9 times increase in slit
136 length to reach adult female size (1.4-1.9 fold increase to full adult male size) and HS10 width which
137 grows slightly more than slit length to 3.2 times the size of the first instar in adult females, and 1.8
138 times for adult males. In contrast, the cuticular pad at the distal end of the MT develops isometrically
139 with MT length ($\alpha = 0.99$, figure 5). Using MT length as a proxy, first instar spiders increase in size 12.7
140 times to reach the mean size of an adult female. The pad is pronounced in adults of both sexes, but in
141 the 5 early instars measured, it is small in size (Figure 3).

142

143 Discussion

144 The slit arrangement in the HS10 organ of *L. hesperus* resembles those in other web building species,
145 in particular *Zygiella x-notata* (Barth 2002). Similarly, the location of dendritic attachment to the outer
146 membrane, visible externally as the coupling cylinder (Figure 4), does not significantly deviate from
147 the basic pattern found in other species previously investigated (Barth 2002). It is possible that the
148 number of slits present in the HS10 organ of *L. hesperus* reflects the number of moults that the animal
149 has undergone. Kaston (1970) observed female *L. hesperus* moulting 9 times, occasionally up to 10,
150 while males can mature twice as fast moulting up to 7 times. We observed the addition of a single slit
151 per moult up to the 5th instar. If this trend continues to the final moult, then these females would have
152 undergone a maximum of 10 moults (23 slits, with first instars having the basic 13 slits), and males 7
153 (20 slits), which is in fact what we observed. Ecological conditions can influence the rate of
154 development in spiders, and the number of instars before the final moult. If it is the case that the
155 number of slits in the HS10 lyriform organ corresponds to the number of moults, as seems likely from
156 our results, it may be possible to use this as a tool in further studies where adults are available and
157 the number of moults are of interest in developmental studies.

158 The aspect ratio of the slits within a lyriform organ determine its sensitivity to vibrational stimuli, with
159 smaller slits requiring higher loads to generate enough deformation for a nervous response (Hössl et
160 al. 2006; Hössl et al. 2007; Hössl et al. 2009). Allometry is defined as differential growth rates of body
161 parts (Huxley et al. 1941). With the importance of morphology on the function of the lyriform organ
162 it is perhaps unsurprising that HS10 develops hypoallometrically (slower rate than body size)
163 compared with body size. However, it is somewhat surprising that the cuticular pad, an important
164 filter physically protecting the lyriform organ from high load stimuli, in contrast to HS10, grows
165 isometrically (same rate as body size). If the need to filter stimuli remains consistent from juvenile to
166 adult then it is curious that the pad should not scale hypoallometrically, like the HS10 organ, to
167 minimise changes in its filter properties.

168 The metatarsal pad distal of the HS10 lyriform organ in *L. hesperus* is small relative to that found in
169 the wandering spider *Cupiennius salei*, the subject of previous studies on this structure (McConney et
170 al. 2007; Young et al. 2014; Erko et al. 2015). In all but adult *L. hesperus* there is a very small area of
171 cuticle distal to HS10. In male and females this becomes a larger structure that resembles the
172 'appendix' area found in *Cupiennius* (Erko et al. 2015). In *Cupiennius* the metatarsal pad acts as a high-
173 pass filter, removing biologically irrelevant stimuli below 40Hz (McConney et al. 2007), its material
174 properties being complex and heterogeneous to perform this task (Young et al. 2014; Erko et al. 2015).
175 Unlike the distal portion of the pad which is soft and compliant, the 'appendix' adjacent to slit 1 of
176 HS10 is hard and sclerotized (Erko et al. 2015). Without analysis of the material properties of the pad

177 in adults, or indeed juvenile *L. hesperus*, it is not possible to infer whether their pad performs the same
178 function as in *Cupiennius* or whether it has a different structure specific to the web substrate that
179 transmits vibrational signals in *L. hesperus*. The angle of the joint on which the lyriform organ is located
180 also affects responses to vibrational stimuli (Finck 1981). The T of *Cupiennius* has a wider range of
181 angular motion before cuticular structures of the MT and T come into contact than that of the web-
182 dwelling species, *Nephila clavipes* (Barth 2002; Schaber et al. 2012). *L. hesperus* and *Cupiennius* inhabit
183 different substrate types, and show considerable differences in the angle of MT-T joint superficially
184 appearing similar to those observed in *Nephila*, which could reflect differences in pad structure and
185 function.

186 The lack of any significant pad in juvenile spiders may imply that they do not require much signal
187 filtering in order to respond to relevant stimuli, but there are also other explanations that are not
188 mutually exclusive. As well as acting as a filter it is thought that the pad serves to protect the HS10
189 organ from high amplitude, potentially damaging stimuli (Erko et al. 2015). This function is perhaps
190 more relevant to adults than juveniles, as in juveniles a damaged structure will be replaced with a fully
191 functioning new structure at the next moult. Cumulative damage to HS10 should therefore be more
192 of a problem in adults and could be an explanation for the greater change in morphology over
193 development of the pad relative to the lyriform organ itself.

194 Small spiders need to be able to detect prey and potential predators as much as far larger adult
195 spiders, and although behaviour and the overall composition of prey and predators may differ
196 somewhat between small spiders and large adults, there is overlap and detection of similar stimuli will
197 be required to survive (see Uma and Weiss 2012). However, very little is known about the ecology of
198 juvenile *L. hesperus* or indeed juvenile spiders in general. Further work should include
199 electrophysiological measurements of spiderling lyriform organs in response to vibrational stimuli in
200 order to determine whether the sensitivity of the organ changes throughout the development of the
201 animal. This would also give insight into the function of the cuticular pad through comparison of
202 responses between juveniles and adults whose pad sizes differ far more than the size of the lyriform
203 organ.

204

205 Compliance with ethical standards

206 *Conflict of interest*

207 The authors declare that they have no conflict of interest.

208 *Human and animal rights*

209 All applicable international, national, and/or institutional guidelines for the care and use of animals
210 were followed.

211 *Informed consent*

212 Informed consent was obtained from all individual participants included in the study.

213

214 References

- 215 Barth F, Pickelmann P (1975) Lyriform slit sense organs. *J Comp Physiol* 103:39–54. doi:
216 10.1007/BF01380043
- 217 Barth FG (2004) Spider mechanoreceptors. *Curr Opin Neurobiol* 14:415–22. doi:
218 10.1016/j.conb.2004.07.005
- 219 Barth FG (2012) Spider strain detection. In: *Frontiers in Sensing*. Springer-Verlag, Wien, pp 251–273
- 220 Barth FG (1971) Der sensorische Apparat der Spaltsinnesorgane (*Cupiennius salei* Keys., Araneae).
221 *Zeitschrift für Zellforsch* 112:212–246.
- 222 Barth FG (2002) *A Spider's World Senses and Behavior*. Springer, Berlin, Heidelberg
- 223 Barth FG, Ficker E, Federle H-U (1984) Model studies on the mechanical significance of compound
224 spider slit sensilla. *Zoomorphology* 104:204–215.
- 225 Barth FG, Geethabali (1982) Spider vibration receptors: threshold curves of individual slits in the
226 metatarsal lyriform organ. *J Comp Physiol A* 148:175–185. doi: 10.1007/BF00619124
- 227 Barth FG, Libera W (1970) Ein Atlas der Spahsinnesorgane von *Cupiennius salei* Keys. Chelicerata
228 (Araneae). *Zeitschrift für Morphol der Tiere* 68:343–369.
- 229 Barth FG, Stagl J (1976) The slit sense organs of arachnids. *Zoomorphologie* 86:1–23. doi:
230 10.1007/BF01006710
- 231 Elias DO (2003) Seismic signals in a courting male jumping spider (Araneae: Salticidae). *J Exp Biol*
232 206:4029–4039. doi: 10.1242/jeb.00634
- 233 Elias DO, Hebets EA, Hoy RR, Mason AC (2005) Seismic signals are crucial for male mating success in
234 a visual specialist jumping spider (Araneae: Salticidae). *Anim Behav* 69:931–938. doi:
235 10.1016/j.anbehav.2004.06.024
- 236 Erko M, Younes-Metzler O, Rack A, et al (2015) Micro- and nano-structural details of a spider's filter
237 for substrate vibrations: relevance for low-frequency signal transmission. *J R Soc Interface*
238 12:20141111–20141111. doi: 10.1098/rsif.2014.1111
- 239 Finck A (1981) The lyriform organ of the orb-weaving spider *Araneous sericatus*: vibration sensitivity
240 is altered by bending the leg. *J Acoust Soc Am* 70:70231–70233.
- 241 Hössl B, Böhm HJ, Rammerstorfer FG, et al (2006) Studying the deformation of arachnid slit sensilla
242 by a fracture mechanical approach. *J Biomech* 39:1761–8. doi: 10.1016/j.jbiomech.2005.05.031
- 243 Hössl B, Böhm HJ, Rammerstorfer FG, Barth FG (2007) Finite element modeling of arachnid slit
244 sensilla-I. The mechanical significance of different slit arrays. *J Comp Physiol A* 193:445–59. doi:
245 10.1007/s00359-006-0201-y
- 246 Hössl B, Böhm HJ, Schaber CF, et al (2009) Finite element modeling of arachnid slit sensilla: II. Actual
247 lyriform organs and the face deformations of the individual slits. *J Comp Physiol A* 195:881–94.
248 doi: 10.1007/s00359-009-0467-y
- 249 Huxley JS, Needham J, Lerner IM (1941) Terminology of relative growth-rates. *Nature* 148:225–225.
250 doi: 10.1038/148225a0
- 251 Kaston BJ (1970) *Comparative biology of American black widow spiders*. San Diego Soc Nat Hist

- 252 16:33–82.
- 253 Kasumovic MM, Andrade MCB (2004) Discrimination of airborne pheromones by mate-searching
254 male western black widow spiders (*Latrodectus hesperus*): species-and population-specific
255 responses. *Can J Zool* 82:1027–1034. doi: 10.1139/z04-081
- 256 Klärner D, Barth FG (1982) Vibratory signals and prey capture in orb-weaving spiders. *J Comp Physiol*
257 A 148:445–455.
- 258 Landolfi MA, Barth FG (1996) Vibrations in the orb web of the spider *Nephila clavipes*: cues for
259 discrimination and orientation. *J Comp Physiol A*. doi: 10.1007/BF00192316
- 260 Lohrey AK, Clark DL, Gordon SD, Uetz GW (2009) Antipredator responses of wolf spiders (Araneae:
261 Lycosidae) to sensory cues representing an avian predator. *Anim Behav* 77:813–821. doi:
262 10.1016/j.anbehav.2008.12.025
- 263 Maklakov A a., Bilde T, Lubin Y (2003) Vibratory courtship in a web-building spider: signalling quality
264 or stimulating the female? *Anim Behav* 66:623–630. doi: 10.1006/anbe.2003.2245
- 265 Masters WM, Markl H (1981) Signal transmission in spider orb webs. *Science* (80-) 213:363–365.
- 266 McConney ME, Schaber CF, Julian MD, et al (2007) Viscoelastic nanoscale properties of cuticle
267 contribute to the high-pass properties of spider vibration receptor (*Cupiennius salei* Keys). *J R*
268 *Soc Interface* 4:1135–43. doi: 10.1098/rsif.2007.1000
- 269 Molina J, Schaber CF, Barth FG (2009) In search of differences between the two types of sensory cells
270 innervating spider slit sensilla (*Cupiennius salei* Keys.). *J Comp Physiol A* 195:1031–41. doi:
271 10.1007/s00359-009-0477-9
- 272 Pringle JWS (1955) The function of the lyriform organs of arachnids. *J Exp Biol* 32:270–278.
- 273 Ross K, Smith R (1979) Aspects of the courtship behavior of the black widow spider, *Latrodectus*
274 *hesperus* (Araneae: Theridiidae), with evidence for the existence of a contact sex pheromone. *J*
275 *Arachnol* 7:69–77.
- 276 Schaber CF, Gorb SN, Barth FG (2012) Force transformation in spider strain sensors: white light
277 interferometry. *J R Soc Interface* 9:1254–64. doi: 10.1098/rsif.2011.0565
- 278 Schüch W, Barth FG (1985) Temporal patterns in the vibratory courtship signals of the wandering
279 spider *Cupiennius salei* Keys. *Behav Ecol Sociobiol* 16:263–271. doi: 10.1007/BF00310990
- 280 Uetz GW, Boyle J, Hieber CS, Wilcox RS (2002) Antipredator benefits of group living in colonial web-
281 building spiders: the “early warning” effect. *Anim Behav* 63:445–452. doi:
282 doi:10.1006/anbe.2001.1918
- 283 Uma DB, Weiss MR (2012) Flee or fight: ontogenetic changes in the behavior of cobweb spiders in
284 encounters with spider-hunting wasps. *Environ Entomol* 41:1474–80. doi: 10.1603/EN12126
- 285 Vibert S, Scott C, Gries G (2014) A meal or a male: the “whispers” of black widow males do not
286 trigger a predatory response in females. *Front Zool* 11:4. doi: 10.1186/1742-9994-11-4
- 287 Walcott C (1969) A spider’s vibration receptor: its anatomy and physiology. *Integr Comp Biol* 9:133–
288 144. doi: 10.1093/icb/9.1.133
- 289 Young SL, Chyasnavichyus M, Erko M, et al (2014) A spider’s biological vibration filter:
290 Micromechanical characteristics of a biomaterial surface. *Acta Biomater* 10:4832–4842. doi:

291 10.1016/j.actbio.2014.07.023

292

293

294 **Figure Legends**

295 **Fig. 1** A) Adult female *L. hesperus* on her web. B) Metatarsal lyriform organ (HS10) measured is located
296 on the dorsal side of the metatarsus at the metatarsus - tarsus joint (arrows). C) First pair of legs of a
297 second instar *L. hesperus*. Arrows indicate metatarsus length. MT = metatarsus, T = tarsus

298

299 **Fig. 2** SEM images of the black widow (*L. hesperus*) HS10 lyriform organ. A) first instar; B) second
300 instar; C) third instar; D) fourth instar; E) fifth instar; F) adult male; G) adult female; H) slit length for
301 slit 2 (grey) and 11 (white) in adult male and females. N= 7.

302

303 **Fig. 3** External morphology of the lyriform organ across all instars illustrated at the same scale. Adult
304 female slits are numbered

305

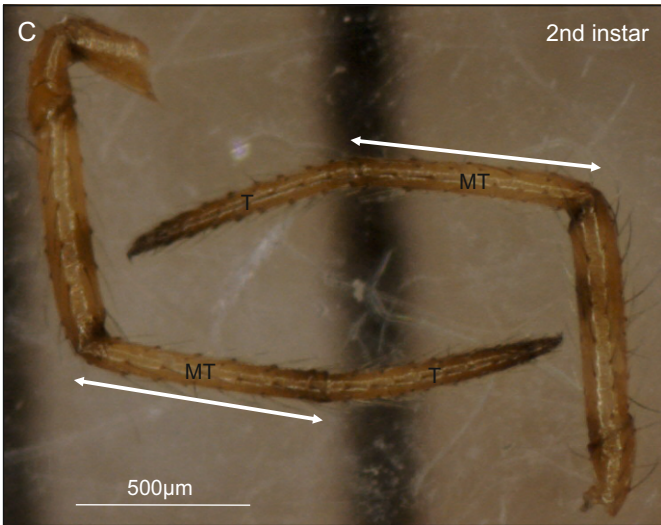
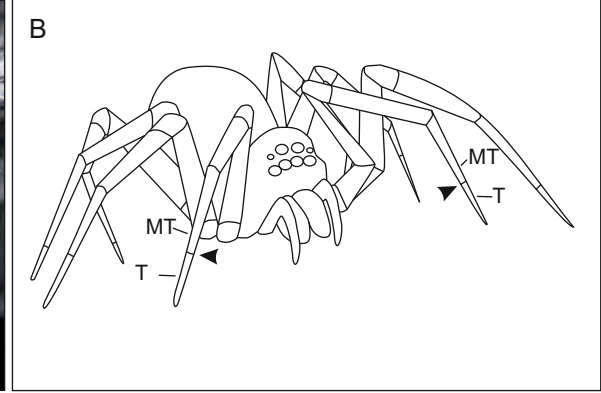
306 **Fig. 4** Position of coupling cylinder in HS10. A, B, C and D are first to fourth instar, respectively. E) Adult
307 male F) Adult female. No specimens had all coupling cylinders visible simultaneously, especially in
308 younger instars. Arrows indicate position of coupling cylinder. P indicates position of pad

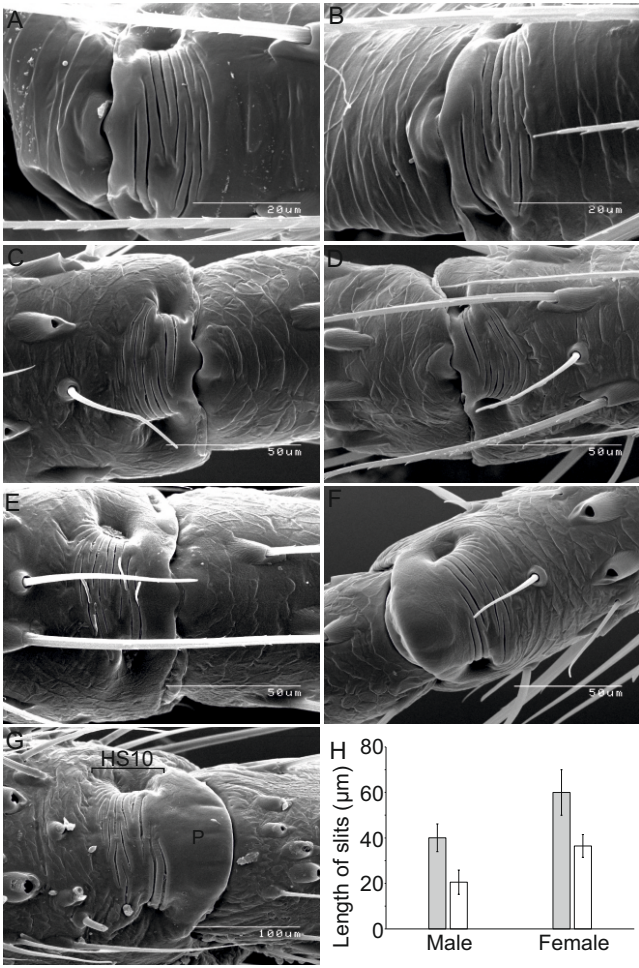
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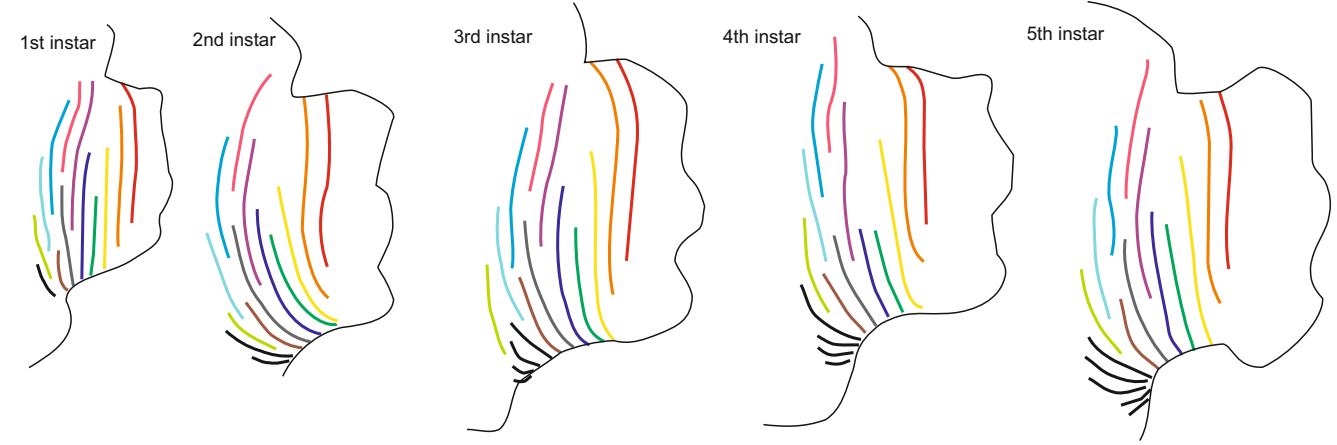
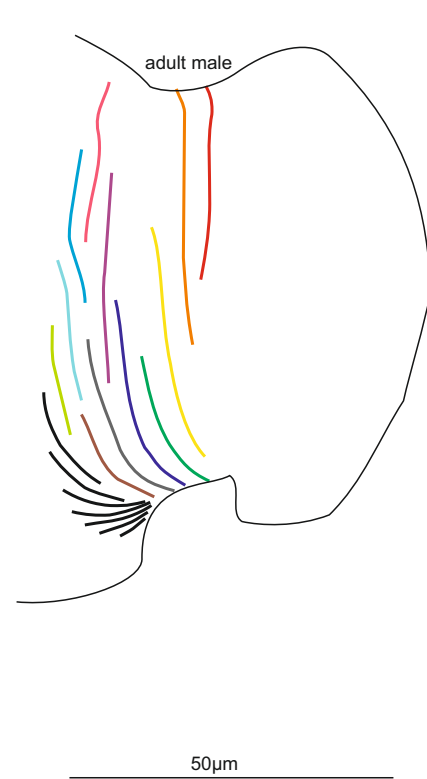
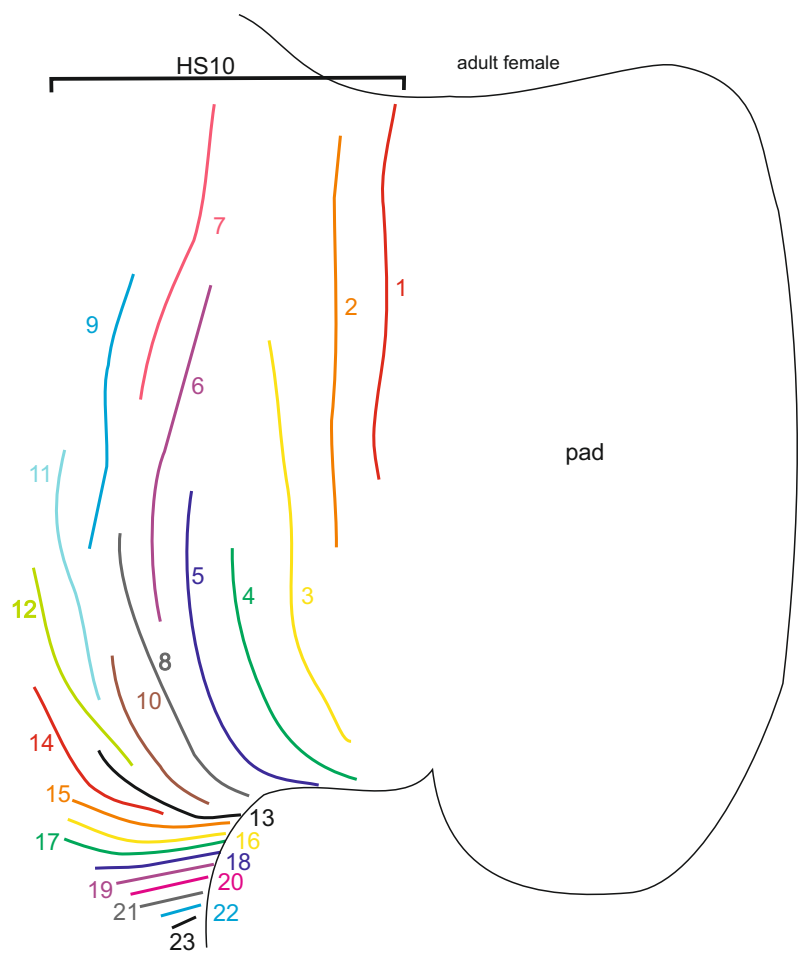
310 **Fig. 5** A) Log MT length against log HS10 width (black) and pad width (hollow). Straight line fits to the
311 data show isometric growth of the pad with MT (dashed) and hypoallometric growth of the HS10
312 (black). B) Log MT length against log slit length and pad width (hollow circles). Straight line fits to the
313 data show isometric growth of the pad with MT (dashed) and hypoallometric growth of slit 1 (grey
314 squares), slit 2 (white squares) and slit 9 (black squares)

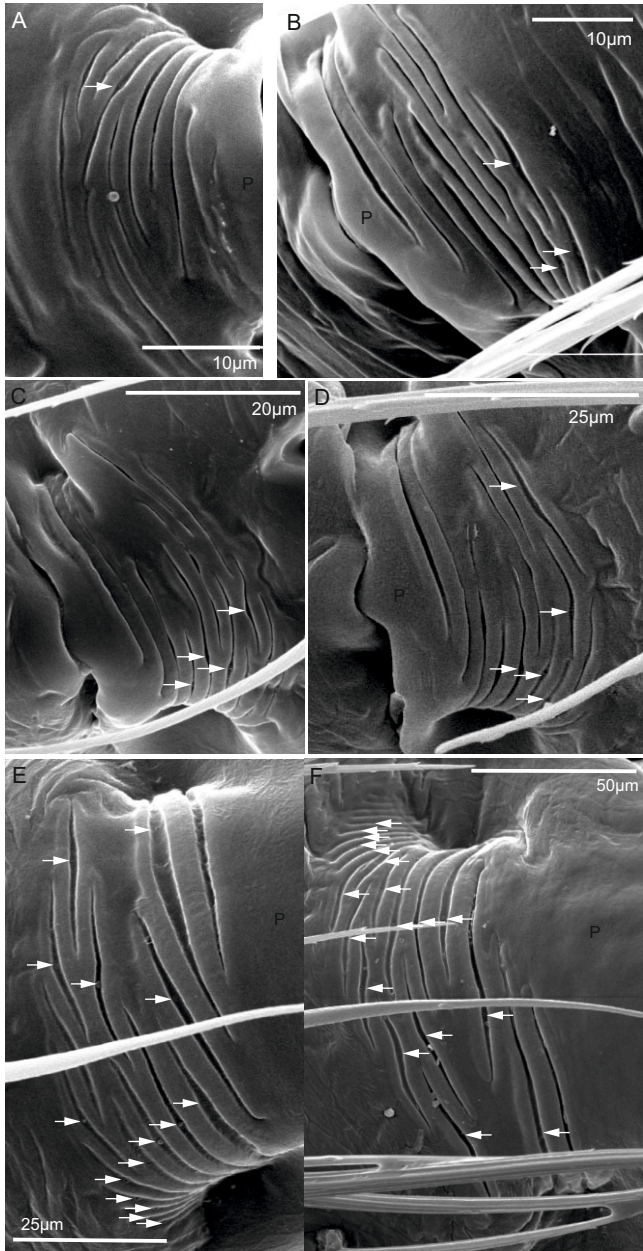
315

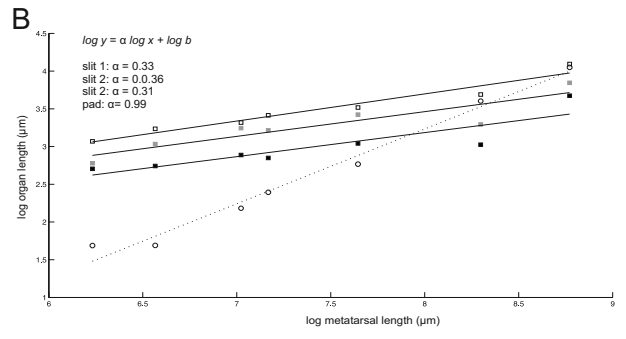
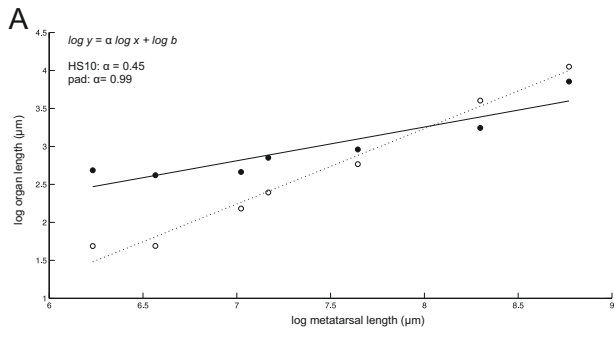
316 **Table 1** Measurements from the MT and HS10 across all instars and both adult sexes measured. Width
317 of pad was measured between its distal margin and slit 1. Width of HS10 is measured between slit 1
318 and 12. MT length is measured along its dorsal edge.











Instar	MT length (mm)	Pad width (μm)	HS10 width (μm)	Max. slits seen
1st	0.51 \pm 0.02 (n=10)	5.4 \pm 0.25 (n=6)	14.7 \pm 1.56 (n=6)	13 (n=6)
2nd	0.71 \pm 0.05 (n=10)	5.4 \pm 2.12 (n=9)	13.7 \pm 2.12(n=9)	14 (n=10)
3rd	1.12 \pm 0.03 (n=10)	8.9 \pm 1.25 (n=7)	18.1 \pm 1.39 (n=7)	15 (n=7)
4th	1.30 \pm 0.17 (n=4)	11.0 \pm 2.80 (n=4)	17.3 \pm 2.15 (n=3)	16 (n=3)
5th	2.09 \pm 0.08 (n=2)	15.9 (n=1)	22.9 (n=1)	16 (n=1)
Adult (m)	4.02 \pm 0.08 (n=9)	37.2 \pm 5.64 (n=9)	26.19 \pm 3.30 (n=9)	20 (n=9)
Adult (f)	6.44 \pm 0.32 (n=10)	57.4 \pm 11.16 (n=10)	47.17 \pm 6.63 (n=10)	23 (n=9)