1 # Chemical communication and ecology in Eupnoi harvestmen

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6 ## Abstract

7 Communication via chemical signals and cues is a widespread modality in animals. Producing,

8 transmitting, receiving and processing chemical compounds impose important challenges. Nonetheless,

9 certain arthropods rely almost exclusively on this channel for intra- and inter-specific communication.

10 Through a preliminary literature review, I summarize here the morphological, behavioral and evolutionary

11 implications of chemical communication in harvestmen (the arachnid order Opiliones), with particular

12 emphasis in one group: the Eupnoi or "Daddy Long-legs". This group has a unique secretory gland that

13 opens in an ozopore in their dorsum. While relying mostly on short-range olfaction and contact

14 chemoreception using different setae, some harvestmen are known to use chemicals in defense, alarm,

15 spatial marking, recruitment, or reproduction. I then propose future research direction on the

16 mechanisms of production and the evolutionary history of these traits. Specific questions can include (1)

17 are chemical signals used as alarm pheromones in Eupnoi aggregations? (2) Do harvestmen rely on

18 chemicals to mark their traditional roosting sites? If so, what are the chemicals involved and how do

19 those differ from the ones used as alarm signals? Also, (3) what are the specific functions of the chemical

20 communication during in female-male communication and multi-specific roosting aggregations? And (4)

21 to what extent the use of chemical defenses imposes a trade-off with other defense mechanisms such as

22 'playing dead' or voluntarily releasing legs. Overall, these harvestmen provide a unique opportunity to

23 test comprehensive and interdisciplinary hypotheses to understand the evolution of chemical

24 communication, as well as the importance of chemical ecology on species diversification in arthropods.

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26 **Key Words**: Arachnida, Daddy Long-legs, defenses, Opiliones, perception.

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I. Chemical communication in terrestrial environments

50   Communicating via chemical channels is challenging. However, this modality is thought to

51 be the most ancestral form of transmitting signals and cues (Bradbury & Vehrencamp 2011).

52 Molecules have a long path in these channels; organisms need to produce them, either

53 metabolically or aided by other organisms, or sequester them from the environment. After

54 synthesis, chemical compounds have to be stored, secreted, sent, and received (Agosta 1992).

55 Transmission can occur using current flows of air or water, by simple diffusion, or by deposition

56 on the substrate. Finally, received chemicals are to elicit neural responses and cognitive process

57 in the receiver.

58   Animals in terrestrial environments use either the airflow intended for olfactory

59   reception, and diffusion or direct deposition for contact reception to transmit their pheromones

60   (those chemicals intended for conspecifics) and allelochemicals (interspecific communication).

61   Substrate-born signals (or cues, depending on the context) have environmental constraints,

62   including the lack of temporal patterns and modulation (when compared with auditory and

63   visual channels), as well as the necessity for the receiver to physically contact the pheromone

64   source. Such pheromones are physiologically costly, and important trade-offs between their

65   composition, shape, molecular weight, and other chemical properties modulate how the

66   compounds persist, are transmitted and received in different topographic, climatic and

67   stratigraphic environments (Bradbury & Vehrencamp 2011). Lastly, the use of localized and

68   sessile signals is risky, because the signaler can be followed by an eavesdropping predator.

69   II. Chemical communication in arthropods

70   Chemical communication in invertebrates is associated with social interaction, including

71   reproduction. For instance, ants have numerous and highly context-specific glands and

72   pheromones (Hölldobler & Wilson 1990). Pseudo-social or no-social invertebrates have fewer

73   glands, which are used mostly in species recognition, and reproductive tasks. In arachnids,

74   chemical communication plays an important role in intra- and inter-specific interaction. In

75   spiders, this topics has been recently reviewed (Uhl & Elias 2011, Fischer 2019). Hence, this

76   review aims to focus on a particular group within the arachnid order of Opiliones, the Eupnoi.

77   Nonetheless, I will extract available information on all Opiliones to summarize our current

78   understanding of this topic.

79   In general, spider use chemicals to locate and evaluate potential mates, avoid predators

80   and attract prey by mimicking prey such as ants (revision in Uhl & Elias 2011). During male-

81   female interaction, both can recognize age and geographic precedence, suggesting the

82   specificity of the chemical signals. Surprisingly, the mechanisms and structures used to produce

83   pheromones are mostly unknown in spiders (Uhl & Elias 2011). Glands and spigots associated

84   with silk production, and the silk itself, are thought to convey information. Research has focused

85   on the sensory biology in spiders, especially in the physiological and neuro-ethological aspects of

86   receiving and processing mechanical stimuli involved in foraging (Barth 2002). As for reception,

87   tip pore sensilla in distal regions of legs and pedipalps are thought to perceive stimuli.

88   Certain clades of spiders are specialized in social life and rely on chemical communication

89   to distinguish between prey and conspecific, and even differentiate the latter in familiar and

90   unfamiliar individuals, which mediates agonistic behavior within the colony (Trabalon 2013).

91   However, solitary spider species also use chemical channels to mediate aggressive behavior. For

92   instance, theraphosids change the cuticular component ratios throughout maturation, which

93   increases their level of aggression (revision in Trabalon 2013).

94   Other arachnid orders have similar chemical communication mechanisms. Although they

95   do not produce silk, harvestmen, scorpions, pseudoscorpions and other arachnids possess

96   morphological and physiological adaptations that assist in producing, emitting and receiving

97   chemical signals.

III. Harvestmen systematics and biology

99   With over 6500 currently described species, the order Opiliones have four suborders

100   (from the basal to the more derived clade, and in increasing order of number of species):

101   Cyphoptalmi, Dypsnoi, Eupnoi and Laniatores (Giribet & Kury 2007, Giribet & Sharma 2015). The

102   presence of secretory gland and their respective lateral opening, the **ozopore**, are

103   synapomorphies of harvestmen. With more than two-thirds of those species, and notorious

104   reproductive and ecological processes, Laniatores have been the most studied group.

105   Interestingly, Eupnoi harvestmen are a widely distributed group with distinctive life history traits,

106   such as grouping behavior and autotomy, the voluntary release of legs (Machado & Macías-

107   Ordoñez 2007, Escalante et al. 2013, Domínguez et al. 2016).

108   Harvestmen are considered to be well defended despite lacking venom or silk.

109   Morphological, behavioral and chemical channels allow them anti-predator defense on different

110   contexts (day/night, active/roosting, solitary/aggregated) (Machado & Macías-Ordoñez 2007).

111   Traditionally, hierarchical "lines of defense" are thought to mediate their defense depending if

112   the interaction with predator escalates. Their defenses include aposematic coloration and

113   grouping, the hard spines and exoesqueleton, giving a pinch with the coxae and phemora or

114 chelicerae, performing thanatosis, stridulations within cheliceral segments, and lastly the

115 secretions of chemicals irritants (Gnaspini & Cavalheiro 1998, González et al. 2004, Pomini et al.

116 2010). Additionally, a trade-off between those strategies could allow species to rely more on

117 certain means of defense (Machado & Pomini 2008).

118   Chemical signaling is perhaps the best channel for communication for some harvestmen

119 (Machado & Macías-Ordoñez 2007). This because most harvestmen are nocturnal, have long

120 slender antenniform legs, and lack associated structures to perform visual or acoustic

121 communication. Also, since grouping behavior occur in certain clades, harvestmen can rely on a

122 combination of chemical cues from feces, pheromones, substrate impregnated with conspecifics'

123 odor, and cuticular compounds, as reported for other arthropods (cockroaches and spiders)

124 (Machado & Macías-Ordoñez 2007). Although these stimuli may act as chemical cues in

125 aggregations, their roles in mutual attraction remain to be experimentally tested (see Harvey et

126 al. 2017), as well as if scent-gland secretions functions as aggregation pheromones.

IV. Production of chemical signals in harvestmen

128   Here I will focus on Eupnoi harvestmen, the long-legged harvestmen, commonly known

129 as "Daddy Long-legs", to outline future research on chemical communication and ecology in this

130 group. As most research dealing with these topics has been done on another suborder (the

131 Laniatores), I also aimed to highlight the many unexplored avenues of research and unknown

132 topics in Eupnoi.

133   Pheromones and allelochemicals are produced by harvestmen in their unique secretory,

134 repugnatory or defensive glands. Glands are connected to the hemolymph, in which chemicals

135 travel, as well as the sequestrated compounds from food sources and symbiotic organisms.

136 Wouters et al. (2013) found that either microorganisms or enzymes are involved in the

137 production of pyranyl kenotes in one gonyleptid (Laniatores). This suggest that harvestmen

138 contain biosynthetic routes of secondary metabolites production. Additionally, harvestmen have

139 special cuticular cells, the oenocytes in the epidermis, which may be related to carbohydrate

140 production for use in the epicuticle and as pheromones (Shultz & Pinto-da-Roca 2007). The

141 chemical composition of the cuticle bears individual information. The production of compounds

142   deposited in the cuticle has proven to be sex-, age-, and nutrition dependent in harvestmen

143   (revision in Trabalon 2013); although no gland has been identified as the source yet.

144   Producing chemical defenses is physiologically costly. In *Acutiosoma longipes* Nazareth &

145   Machado (2015) found that females undergo crucial reproductive and defensive trade-offs when

146   producing the secretions. Additionally, producing secretions is condition-dependent, as poorly

147   fed individuals produced secretions with lower mass and concentration of benzoquinones

148   (Nazareth et al. 2016).

149   The secretory glands are sacs derive from the ectoderm, and have three layers (revision

150   in Gnaspini & Hara 2007). Although the gland lack accessory muscles, Clawson (1988) suggested

151   that secretion expulsion is facilitated by hydrostatic pressure from muscles compressing dorsal

152   and ventral exoskeleton. Secretion seems to occur by exocytosis from secretory granules, and

153   transport of fluids through glandular cells from hemocoel to the canaliculi (Clawson 1988). The

154   openings of each secretory gland, the **ozopore**, is located on the lateral portion of the prosoma,

155   either in the carapace or in the supracoxal pleural region (Shultz & Pinto-da-Rocha 2007). The

156   ozopores in Eupnoi are located between the coxa I and II, in the dorsal side.

157   Harvestmen release pheromones or allelochemicals directly through the ozopore. Most

158   of the chemicals released are intended for contact reception, although short-range olfaction is

159   also used (Willemart & Chelini 2007, Gainett et al. 2017). Harvestmen use many behaviors to

160   release chemicals, aided by morphological traits such as accessory appendages (Gnaspini &

161   Cavalheiro 1998, Schaider et al. 2011). For example, several ridges and aphophyses in the latero-

162   ventral side assist and regulate the process of releasing fluids (Gnaspini & Hara 2007).

163   V. Chemicals compounds

164   Pheromones are organic compounds with basic carbon skeleton. Some contain functional

165   groups with oxygen atoms, including alcohols, aldehydes or ketones (revision in Gnaspini & Hara

166   2007). Other pheromones are acidic or esters with two oxygen atoms (Bradbury & Vehrencamp

167   2011). Chemical properties of each compound determine their function and limitations. For

168   instance, airborne molecules evaporate easily (volatile). Also, airborne molecules are typically

169   small and light (maximum molecular weight: 300 dalton, and 20 carbon atoms) (Bradbury &

170   Vehrencamp 2011). Additionally, airborne pheromones are hydrophobic and lipophilic. Hence,

171   substrate-transmitted molecules have the opposite properties. Research technics to study

172   chemicals involved in harvestmen are noted in Box 1.

173   Heavy and complex non-volatile molecules can be highly specific. For example, closely

174   related species differ only in one functional group in the main component in sexual pheromones

175   or the ratios of components. If those blends contain the same compounds, accessory

176   components are important (Bradbury and Vehrencamp 2011). The size and polarity of the

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BOX 1. How to study chemical ecology and communication in harvestmen?

The first way to study chemical communication in harvestmen is by isolating and identifying the chemicals involved. There are three basic ways to do this (Raspotnig et al. 2010, 2015):

(1)   Directly dabbing secretions on small pieces of filter paper immediately after the emission from the ozopores after gentle squeezes to the harvestmen. Then, filter papers are extracted in hexane for 30 min.
(2)   Whole body extraction of individuals in 150 µl of hexane or methylene chloride for 30 min.
(3)   Extraction of excised single glands in methylene chloride for 30 h.

Several classic and modern research technics in chemistry are now available to study the chemical compositions of animal secretions, and to be able to infer their functions. As for harvestmen, extensive research has enabled several technics, even in species that release small volumes of chemicals, such as Eupnoi. Certain technics have proven successful in giving profiles and ratios of the components present in the chemical blends extracted from secretions, including:

-   Gas chromatography – mass spectrometry (GC-MS) (Raspotnig et al. 2015)
-   proton nuclear magnetic resonance spectroscopy (H NMR)
-   NMR (Rocha et al. 2011)
-   high resolution mass spectroscopy (HRMS) (Machado et al. 2005, Wouters et al. 2013)

Additionally, combining these with scanning electron microscopy (SEM) and magnetic resonance imaging (MRI) provide morphological confirmation of the associated structures, and their potential functions. See Fischer (2019) for detailed descriptions of available methods to study chemical compounds in spiders.

Finally, extensive field observations and behavioral ecology approaches using choice tests in experimental arenas and bioassays to look for the chemicals that elicit observed behaviors will complement the research on chemical communication in harvestmen.

177   molecules affect their persistence time in the environment. When vapor pressure decreases with

178   increasing size or polarity, the evaporation rate declines, and consequently, the signal can be

179   emitted for a longer time. Also, larger molecules diffuse slower, allowing the signal to stays for

180   longer (Trabalon 2013).

181   Although variable among clades, the secretions of harvestmen are usually a mixture of

182   compounds (see Table 10.1 in Gnaspini & Hara 2007). Raspotnig (2012) comprehensively

183   reviewed the diversity of scent gland-derived chemical components in harvestmen. The author

184   found a great variation on the number of components present in secretions between clades.

185   Furthermore, Raspotnig (2017) provided support for a hypothesis about the evolutionary

186   trajectory of chemical compound groups in harvestmen. For instance, naphthoquinones are

187   thought to be scent gland compounds of common ancestry, which have been lost in certain

188   groups. On the other hand, benzoquinones have appeared independently at least twice in

189   harvestmen. Ultimately, reconstructing the evolutionary trajectory of the chemical compounds

190   used secretions in harvestmen ('phylogenetic chemosystematic', Schaider *et al.* 2018) provides a

191   unique opportunity for very interesting interdisciplinary research.

192   Secretions in Cyphophthalmi harvestmen include naphthoquinones and methyl ketones,

193   in Laniatores alkylated benzoquinones, phenolics, vinyl ketones, and small tobacco alkaloids

194   (Raspotnig et al. 2015). Additionally, other compounds known contain alkaline benzoquinones,

195   quinones, phenols, naphthoquinone, anthraquinones, heptenones, hexenones, pentanones,

196   vynil ketones, phenylethylamine and bornyl esters (Ekpa et al. 1985, costa et al. 1993, Hara et al.

197   2005, Raspotnig 2012, Raspotnig et al. 2010, 2014a,b, 2015, Rocha et al. 2011, Wouters et al.

198   2013). Alkaloids, nicotinoid alkaloid anabaseine and nitrogen-containing components were found

199   in basal groups of cladonychiid harvestmen (Laniatores), and thought to be the ancestral

200   allomones in Laniatores. Then, those compounds were evolutionary "replaced" and reduced by a

201   phenol- and benzoquinone-rich chemistry in derived clades (Raspotnig et al. 2011).

202   Notorious exceptions to the patterns listed above exist. For instance, volatile secretions

203   composed mostly of octanes in a naphthoquinone matrix in some Dyspnoi species (Raspotnig et

204   al. 2014). Along with Dyspnoi, the Eupnoi harvestmen scent gland have been poorly investigated,

205   compared with Laniatores (Raspotnig et al. 2014). Sclerosomatidae Eupnoi species secrete

206   mostly short-chain acyclic ketones and alcohols. Leiobuninae species have highly volatile, short

207   acyclic ethyl ketone-rich secretions. Phalangiidae produce bezoquinones, caprylic acid, and

208   naphthoquinones, considered to be rare as natural products (revision in Hara et al 2005,

209   Gnaspini & Hara 2007, Raspotnig et al 2010, 2015). For example, the phalangiid *Rialena*

210   *triangularis* have benzoquinone-based chemical defenses, along with naphthoquinone and

211   caprylic (=octanoic) acid (Raspotnig et al. 2015).

VI. Transmission of chemical signals in harvestmen

213   Chemical communication is challenging for animals in terrestrial environments since the

214   medium (either air or solid substrate) impose stronger barriers for long distance perception than

215   water. Additionally, the prevalence time is variable between microclimates. For instance, alarm

216   or aggregation signals transmit between 1-10 cm in small colonial insects (Bradbury &

217   Vehrencamp 2011). Environmental factors affect the chemical transmission, for instance (1)

218   ambient wind and currents, (2) temperature, which increases the pressure of volatiles and higher

219   temperatures cause faster spread and sooner fade (3) humidity and rain, (4) vegetation

220   structure, since complex tridimensional environment, and (5) topography and surface structure.

221   The transmission of substrate-born semiochemicals varies with the morphological

222   arrangement of an animal's secretory structures, its behaviors, and function. Harvestmen have

223   two main strategies of transmitting defensive compounds: (1) create a chemical shield around

224   the body, and (2) direct the liquid toward the offending agent, i.e. 'jet emission' (Segovia et al.

225   2015). These strategies are variable among harvestmen (Gnaspini & Hara 2007). Eupnoi

226   harvestmen have shown to emit a secretion globule that then evaporates, as well as the

227   emission of a fine spray that moistens its dorsum, displacement of liquid through ventral

228   integumentary grooves, and delivering the secretion by retracting a leg toward the harvestmen's

229   body (revision in Gnaspini & Hara 2007). Additionally, inter-sexual variation in jet emission was

230   recently reported for *Phareicranaus calcariferus* (Moore et al. 2018), specifically in the

231   propensity to jet, and the composition (enteric v. creamy secretions).

232   Harvestmen also use colorless enteric fluids secreted through the mouth opening to

233 assist the transmission of chemical signals (Acosta et al. 1993). These fluids are often mixed with

234 the white-milky, yellowish secretions from the secretory gland, and displaced towards the end of

235 the body by lateral channels or sallow slits (Clawson 1988, Segalerba & Toscano-Gadea 2016).

236 This process dilutes the secretion and increases the evaporation surface. Mixing the compounds

237 is useful because (1) it dilutes the costly (for production and storage) compounds produced in

238 the glands, and (2) some compounds, like benzoquinones, are unstable in water (Gnaspini &

239 Hara 2007). During agonistic interactions, Eupnoi harvestmen can use the coxal aphophyses

240 direct the secretions towards the ventral side of the body (Shultz & Pinto-da-Rocha 2007).

241   VII. Chemical reception in harvestmen

242   Receiving chemical stimuli is complex. First, adequate sensory structures are required.

243 Then animals then have to be able to discriminate between innumerable chemical compounds in

244 the environment and couple the relevant ones that will elicit appropriate responses. Finally,

245 associated neuron and neural pathways for those relevant stimuli should quickly transmit precise

246 information to central nervous systems.

247   Chemical channels used by arthropods, however, seldom transmit reliable information

248 about the source or location of the emitting source, especially because chemical compounds do

249 not follow any intensity pattern or gradients. Therefore, relying on olfactory reception (smell) is

250 not as distributed as contact reception (taste) in arthropods. The olfaction in Lepidopterans and

251 hymenopterans is assisted by an extensive development of antennae or other hair-like

252 projections throughout their body that provide a maximized exposure volume for sensilla and

253 other structures and cells to airborne chemicals. Certain receptors in forelegs and near the

254 mouth assist in foraging, whereas other receptors (such as bristles with pores) in legs and dorsal

255 and ventral areas serve for communication.

256   The sensory capacities among harvestmen are varied, and each group seems to rely

257 differently on certain modalities. Some gonyleptids (Laniatores) used olfaction when food was

258 placed in their container (Santos & Gnaspini 2002, Willemart 2002). While hunting, live prey is

259 searched using direct physical contact with mechanoreceptors. Willemart and Chelini (2007)

260 experimentally found on a gonyleptid that both close-range olfaction and contact

261 chemoreception are important while foraging. Depending on the intensity of the stimuli, these

262 harvestmen used leg II to smell and leg I to taste. As for olfaction on Eupnoi, Chelini et al. (2009)

263 found that the response of *Eumesosoma roeweri* to predatory cues (spider scent) was not

264 different than to conspecific or control treatments. This suggests that chemical reception (at

265 least long-range olfaction) is not extensively developed in Eupnoi (but see Gainett et al. 2017).

266 However, in long-legged Eupnoi distant mechanoreception might also be important. Detection of

267 vibration through the substrate was also experimentally demonstrated in phalangiid Eupnoi

268 harvestmen (Immel 1955).

269 VI. a. Sensory structures

270   Harvestmen have a unique, elaborated, and diverse array of hair-like structures and slits

271 for chemosensory reception, mostly located in the distal end of legs, pedipalps, and chelicerae

272 (Shultz & Pinto-da-Rocha 2007). Willemart et al. (2009) provide a very extensive and detailed

273 revision and analysis of those structures, and the demonstrated and assumed sensory capacities.

274 The authors conclude that harvestmen seem to be strongly dependent on contact

275 chemoreception for reproductive interactions, hunting, and predator avoidance. However, close-

276 range olfaction assists certain groups (especially Laniatores) while scavenging or foraging for

277 food with a strong odor (Willemart et al. 2009). Additionally, Gainett *et al.* (2017) recently

278 proposed the importance of several pored-sensilla on olfaction reception for this group of

279 arachnids.

280   While harvestmen pedipalps act primary as tactile organs, harvestmen have sensory

281 structures in their legs (especially the forelegs, I and II), prosoma and opisthosoma, in both

282 ventral and dorsal surfaces (Shultz & Pinto-da-Rocha 2007). The cuticular surface have setae and

283 seta-covered aphophyses associated with prey capture. Harvestmen setae are diverse in form,

284 shape and sensitivity, but in general are hair-like projections of the cuticle with basal articulation

285 (Willemart et al 2009). In Eupnoi setae are thought to be the most important and widespread

286 sensory structure (Box 2).

BOX 2. Main sensory structures of Eupnoi harvestmen

- Slit (*lyrifissures*) sensilla: measure cuticular strain, self-generated muscular or haemolymphal movement (propioreceptors) or from external sources (exteroception). Located mostly in the basal segments of all appendages, and near the articulations. Not arranged in true lyriform organs, but occur either in solitary or groups.

- Campaniform sensilla: circular or oval modifications of slit sensilla. Located in all legs, with concentration near the plane of autotomy. Leg II in *Phalangium* had similar numbers than other legs.

- Sensilla chaetica: located in the whole body. Contain a socket membrane, and this setae are probably gustatory contact mechanoreceptor because they have an internal lumen associated with a pore tip.

- Sensilla basiconica: located on the dorsal side of the metatarsus of all legs, and also in the dorsal side of the pedipalps of *Phalangium opilio*. A short shaft inserts in a large sockets. Its function is unknown.

- Spicer's tarsal organ: A longitudinal series of microdenticles on the ventral surface of the pedipalps. Their pores lack innervation, and this organ is thought as an olfactory receptor. However, without further examination, this structure can also be a glandular opening.

- Other structures in harvestmen include scattered macrosetae or spines, clumps of microsetae at the tip of pedipalps and legs, microsetae with final projections in pedipalps (plumose or pinnate setae) present exclusively in Eupnoi, and setae associated with gland in Dyspnoi that assist the secretion of adhesive fluids to capture prey (Shultz & Pinto-da-Rocha 2007).

See Gainett et al. (2017) for more description of sensilla associated with olfaction.

287

VII. b. Neural reception

289   Once the chemical compound has been coupled from the medium to the sensors, binding

290   proteins attach it to the sensory cell's membrane (for example, the G protein coupled receptor

291   GPCR; similar to rhodopsin in photoreceptor cells). This coupling then depolarizes the membrane

292   of those neurons and fire action potentials (Barth 2002, Shultz & Pinto-da-Rocha 2007).

293   Reception proteins are sensitive to specific functional groups, molecule sizes, or shapes.

294   Therefore, the receptor is more intricate than a simple lock-key system. Additionally, the

295   receptor responds to a variety of ligands, and the match with the cell's requirements affect the

296 excitatory output (amplitude, rate, and duration of neural spike trains) (Bradbury & Vehrencamp
297 2011).

298   The electric impulse then heads to a glomerulus (which centralizes the impulses coming
299 from many receptor cells). The glomeruli save information of where the receptor was, although
300 no evidence has been found about a chemotopic ordering in the CNS, as for the case of tactile or
301 visual receptors (Bradbury & Vehrencamp 2011). The way how arthropods can discern between
302 chemical compounds is currently not resolved. However, the interaction between many receptor
303 cells and glomeruli, and the action of inhibitory interneurons suggest that multi-source pathways
304 allow for the specificity of the responses to different compound blends. Harvestmen sensillae
305 can have one or more basal neurons (Shultz & Pinto-da-Rocha 2007). However, in spiders, tarsal
306 chemoreceptor sensory hairs are usually innervated by up to 21 sensory cells (Trabalon 2013).
307 This may activate the olfactory bulb with only a specific signature pattern of glomeruli activity,
308 analogous to a tri-dimensional bar code (Bradbury & Vehrencamp 2011).

309 VIII. Phylogenetic implications and approaches
310   Chemical compounds and the ratios of each in a mix, known as the "pheromone blend"
311 or "odor signature" (Bradbury & Vehrencamp 2011), can be species-specific, especially in those
312 clades with complex molecules. Therefore, secretions can be informative traits to elucidate
313 phylogenetic relationships, as well as the evolutionary trajectory of production mechanisms and
314 functions. Different states of the trait can inform of speciation, radiation, and even convergence
315 in pheromone composition. Additionally, like many other phenotypic traits, chemical compounds
316 are expected to differ greatly between species if it's driven by sexual selection than if they are
317 involved in alarm signaling. But how to make species-specific chemical compounds? Animals can
318 (1) modify the molecule properties. Large molecules with the unique structure or chemical
319 composition, for example, adding amino acids; and (2) change the blend of compounds. Infinite
320 unique mixtures can ten arise when the main and minor components are present in different
321 ratios.

322   Traits related to the chemical communication and ecology of harvestmen have been used
323 in systematic studies. For example, the chemical compounds, the morphology of the gland

324   opening in the tegument, the behaviors employed during predation attempts to evaporate or

325   jettison the secretions and their combinations apparently have phylogenetic signals (Acosta et al.

326   1993, Hara & Gnaspini 2003; Gnaspini & Hara 2007, Pomini et al. 2010). For example, the

327   chemical composition of the secretion has been used, along with morphometrical, genetic,

328   ecological and/or behavioral data to elucidate the phylogenetic patterns in the families

329   Gonyleptidae (Hara et al. 2005, Caetano & Machado 2013) and Phalangiidae (Wachter et al.

330   2015). However, chemical compounds also bring intriguing results, mostly because of the lack of

331   comprehensive phylogenetic and pheromone identity information. In 22 species of Gonyleptidae

332   37 compounds were found, but only half of them were identified (Hara et al. 2005). Some of

333   them revealed symplesiomorphies, while others autopomorphies. Interestingly, one acyclic

334   compound that was also found in a sclerosomatid Eupnoi (Hara et al. 2005). In Dyspnoi

335   harvestmen Shear et al. (2014) found different chemicals in species of Travunioidea, suggesting

336   phylogenetic signal of chemical compounds, and separation of these during clade diversification.

337   In another clade of eastern US species the major component was anabaseine, an alkaloid related

338   to nicotine. In the western species, the major component was a phenylethylamine. In Eupnoi, no

339   phylogenetic effort including chemical data has been made, although systematics of

340   Sclerosomatidae has been recently studied (see Hedin et al. 2012b).

341   IX. Functions of chemical communication in harvestmen

342   Harvestmen engage in both intra- and inter-specific chemical communication using

343   volatile and non-volatile pheromones. For several life history activities, the chemical channel is

344   their only mean of transmitting signals. Fischer (2019) extensively reviewed the importance and

345   functions of chemical communication for spiders. In Opiliones, predator and parasite deterrence

346   and alarm signaling are the most frequently studied functions. Secretions can have more than

347   one function. For instance, in Eupnoi the secretions are thought to serve to mark trails as a mean

348   of intraspecific communication with the clear fluid, and as defense with the milky one, as

349   suggested for *Leiobunum aldrichi* (Bishop 1950).

350   For harvestmen intra-specific communication occurs during the scent-marking of

351   grouping sites, prey attraction, and various reproductive tasks (marking of oviposition sites,

352   parental care, and male-female courtship interactions) (Juberthie et al. 1991, Gnaspini & Hara

353   2007). However, the functions of those chemicals vary among suborders. For instance, whereas

354   all harvestmen use chemicals as defense, only Laniatores are known so far to use them

355   extensively in alarm (Machado et al. 2005, Da Silva Fernandes & Willemart 2014), and only

356   Eupnoi species used them to mark roosting sites (Donaldson & Grether 2007). Below I will

357   expand on most of those functions.

358   IX. a. Defense

359   The chemical secretions can be useful to deter certain types of potential predators

360   (revision in Gnaspini & Hara 2007). For example, benzoquinones of the harvestmen *Goniosoma*

361   *longipes* (Gonyleptidae) may serve as a chemical shield. When tested in lab trials, such shield

362   deterred ants, wandering spiders and frogs but not opossums (Machado et al. 2005). Secretions

363   have also experimentally demonstrated to be successful to deter flatworm attacks in *Myschonyx*

364   *cuspidatus* (Gonyleptidae) (Silva et al. 2018). Besides the chemical shielding and jettison, some

365   behaviors may enhance the secretion as defense. The "bobbing" behavior of intense and

366   frequent vibrations (Gnaspini & Hara 2007) may have two mechanisms of anti-predator defense:

367   (1) confuse predators, and (2) release and diffused unnoticed secretions (as suggested by Chelini

368   et al. 2009). However, chemical secretions did not increase survival in *M. cuspidatus* harvestmen

369   exposed to wandering spiders that had their gland opening clogged (Dias & Willemart 2013).

370   IX. b. Alarm signaling

371   The function of quinones as alarm pheromones was recorded in the gonyleptid

372   *Goniosoma proximum*. By exposing aggregations with the exudate 73.3% of the aggregations

373   responded (Machado et al. 2002). Also, the time of reaction was inverse to group size (Machado

374   et al. 2002). Gregariousness is prerequisite for the evolution of alarm signals, as noted in many

375   other arthropods. Since the alarmed harvestmen bump into each other while fleeing, the alarm

376   reaction is also mechanically spread, resulting in a general erratic scattering of the group, as

377   occur in fish and insects (revision in Machado & Macías-Ordoñez 2007). Alarm pheromone can

378   simultaneously act as a defense mechanism, and in fact could be a by-product of the defensive

379   function. Evolutionary, once grouped, harvestmen might have taken advantage of the chemical

380   channel and transformed the defensive response and use it as intra-specific communication.

381 However, Segovia et al. (2015) found that the chemical secretions of *D. pectinifemur* do not

382 function as an alarm signal.

IX. c. Site marking and recruitment

384 Harvestmen secretions may function as site marking to attract conspecifics. For instance,

385 Schaider & Raspotnig (2009) found the presence of an atrium outside the ozopore and the

386 possibility of solid secretions of chemicals in the Dypsnoi *Trogulus tricarinatus* (Trogulidae),

387 suggesting site marking in this soil-dwelling species. Species of *Leiobunum* have been seen

388 rubbing the defense tubercle over the area where lay eggs, potentially to mark sites and repel

389 others (Gnaspini & Hara 2007). Also, Donaldson & Grether (2007) suggested that the Eupnoi

390 *Prionostemma* can mark the roosting site continuously. However, the site marking does not

391 necessarily have to involve communication.

IX. d. Reproductive tasks

393 The occurrence of sexually dimorphic glands was reported in Cosmetidae and

394 Gonyleptidae (Da Silva Fernandes & Willemart 2014, Dias & Willemart 2016). Males rub parts of

395 the legs that matched with the gland openings, which was interpreted as the release of a

396 pheromone into the substrate, which may amplify the signal towards females. Additionally, the

397 authors found the males were waving the leg, which may be important for short-range

398 communication. Also, males of *Iporangaia pustulosa* can control the release of secretions of the

399 metatarsal gland IV (Murayama & Willemart 2015). However, the phenotypic traits or individual

400 identification of those chemical cues remain to be studied. Additionally, Dias & Willemart (2016)

401 did not find evidence of contact chemical release during mating in two species of Opiliones,

402 despite having sexually dimorphic glands.

403 Ultimately, chemical signals can simultaneously serve for different purposes. For

404 instance, *Formica* ants spray formic acid used in defense and recruiting colony members to the

405 source of danger (Wilson 1971). However, these differences could be achieved with small

406 modifications in the chemical compounds, their blends or ratios. Therefore, behavioral essays

407 and proper identification of the chemicals used in different biologically relevant context are

408 necessary to test the multifunctionality of chemical communication in harvestmen.

409   Marking the substrate with individual information have been found for reproductive tasks

410   in Eupnoi harvestmen. Willemart & Hebets (2012) found that the Eupnoi *Leiobunum vittatum*

411   rubs their body in the substrate. The authors found sex-specific responses to conspecific cues,

412   suggesting the possibility of sexual communication mediated by chemical cues. Males exposed to

413   conspecific cues performed two behaviors never recorded in females. Males spent more time,

414   engage in more scrapping with leg I, and did more pedipalps tapping when faced with

415   conspecific vs control cues. Females spent more time tapping their palps in the presence of male

416   cues rather than female cues, suggesting their ability to distinguish them. But there was no

417   difference between winning or losing males. Certain Eupnoi (*Leiobunum vittatum* and

418   *Phalangium opilio*) apparently can differentiate males and females after contact (Macías-

419   Ordoñez 1997; Willemart et al. 2006).

420   Expanding of the importance of intraspecific communication, Fowler-Finn et al. (2014)

421   found a behavioral interplay with repeated leg touching and grabbing between male and female

422   *Leiobunum vittatum*. Also, the production and offer of a nuptial gift –probably derived from

423   enteric fluids secreted by the mouth– by the male was key in continuing the mating. This nuptial

424   gift can be high in enzyme content (Kahn et al. 2018). Additionally, Kahn et al. (2018) found that

425   aminoacid content of gifts is variable among taxa and better predicted by reproductive

426   morphology and behavior, instead of the phylogeny. Overall, this recent information also

427   suggests the importance of chemical communication for reproductive purposes.

428   Additionally, pheromones of *Prionostemma* harvestmen seem to be sex-specific and

429   females appear to be attracted to female scent, while males are attracted equally to male and

430   female scent (G. Grether, *pers. comm*.). The sex ratio in aggregations of *Prionostema* is

431   commonly 1:1, although some groups are either male or female skewed (Grether et al. 2014b).

432   Harvey et al. (2017) experimentally demonstrated that this skew is caused by sex-specific

433   differential recruitment through chemical signals. Together, these data suggest that

434   sclerosomatids can form aggregations for several reasons, and females can return to the more

435   aggregations more frequently, can be more sensitive to disturbance, or have different

436   microhabitat preferences than males (as suggested by Grether et al. 2014b).

X. Chemical communication in aggregating Eupnoi

Since many species of harvestmen form aggregations, the use of chemical as communication seems to follow an evolutionary pathway favoring initial steps to social life. Also, harvestmen often form multispecific aggregations (Grether et al. 2014b, Harvey et al. 2017, Domínguez et al. 2016, I. Escalante *pers. obs.*). Some species apparently do not release pheromones in the groups, while other do. The differential release of alarm pheromones may explain multispecific aggregations; species that do not release pheromones can take advantage of the ones that do, and their presence favors other species by diluting the effect of risk in the aggregations (Machado & Macías-Ordoñez 2007). However, this idea remain untested.

In aggregations, the use of pheromones may be key in three contexts: (1) communication during recruitment, (2) defense and (3) alarm signals. However, evidence on the multifunctional of pheromones in this context is still preliminary. The attraction of conspecific to daytime roosting sites in spiny palms in an undescribed species of the Neotropical *Prionostemma* (Sclerosomatidae) genus, which inhabits the rainforest of Central America, have been investigated. These sclerosomatids leave the groups at dusk and forage on the ground (Wade et al. 2011), and at day re-group, although not necessarily in the same site (Donaldson & Grether 2007). Harvestmen apparently mark the substrate (palm fronds) with pheromones (Donaldson & Grether 2007, Grether and Donaldson 2007). Such sites persist as a grouping location for even several years (Teng et al. 2012). *Prionostemma* harvestmen have also high site tenancy to particular "traditional" sites, even though sever predations and detrimental changes in preferred ecological factors were simulated and experimentally tested (Grether et al. 2014a); suggesting that roosting site can become "ecological traps". Additionally, new unmarked individuals frequently join the aggregation (Grether et al. 2014a). Nonetheless, Grether et al. (2014b) found a decrease in recruitment over time after experimentally removing individuals. They suggest that marking pheromones can also include alarm informational. Nonetheless, what is noticeable in these findings is the function of pheromones in communication. It is worth nothing that the untested proposed mechanism involved contact chemoreception of potentially heavy and non-volatile pheromones. Donaldson & Grether (2007) report frequent observations of *Prionostemma* harvestmen rubbing their sides and posterior end of the carapace against palm

466   fronds, which could be interpreted as the scent-marking behavior. However, other species have

467   been found to not rely on conspecific markings or plant volatile compounds to select roosting

468   sites (i.e. the Eupnoi *Jussara*, Pagoti et al. 2017). Regardless of this, in arthropods, constant

469   marking of a site creates a long-term and spatial overlap of single puffs that allows conspecific to

470   find and follow the intended sites or trials (reviewed for ants and copepods in Bradbury &

471   Vehrencamp 2011).

472   ## XI. Conclusions and future directions

473   Besides anecdotal observations of reduced recruitment (Grether et al. 2014b), and

474   research on distant Laniatores harvestmen (Machado et al. 2002), the alarm function of

475   pheromones in Eupnoi harvestmen remains untested. The presence of alarm pheromones will be

476   beneficial for group living and will provide information on the evolutionary maintenance of this

477   trait. By exposing groups to concentrated pheromones, the alarm function of their secretions

478   could be correlated with (1) their fleeing behavior, (2) site tenancy, measured as the return

479   throughout the days, (3) the

480   transmission of the alarm signal by either olfactory or contact receptions, (4) the potential

481   importance of bobbing behavior (Gnaspini & Hara 2007, Escalante et al. 2019) as a way to

482   disseminate the alarm pheromone (an untested strategy so far), and finally (5) the importance of

483   the position in the group. Therefore, future research will provide important insights into the

484   interaction between behavior, chemical communication, morphology (in the form of the

485   extremely elongated legs in Eupnoi) in harvestmen. Consequently, this research will benefit the

486   knowledge of the factors mediating the grouping behavior as a widespread trait in animals.

487   As for the defense function of pheromones in harvestmen, both the translucent and the

488   milky secretion have been seen in the Neotropical *Prionostemma* and the North American

489   *Nelima paessleri* (Escalante, *pers. obs.*). Both systems provide the opportunity to test the

490   importance of chemical communication during mating, as well as during recruitment and

491   defense. These Eupnoi harvestmen frequently autotomy their legs (Domínguez et al. 2016,

492   Escalante et al. *In prep.*), which brings questions regarding the potential trade-offs of defensive

493   strategies in this group. Finally, these clades are also known to form multispecies aggregations,

494   which will allow testing for the importance of chemical ecology and chemo-systematics on

495   species diversification and the overall evolutionary trajectory of these arachnids.

496   Proper extraction and identifications of the compounds are necessary in order to

497   understand the nature of these chemical signals. The context-dependent differences in the

498   major and minor chemical compounds (and/or their proportions in the blends) in the secretions

499   will inform the chemical variation of these signals. Specifically, to identify the chemicals released

500   as alarm pheromones during a disturbance to the groups (perhaps by emulating a predatory

501   attempt), and the one deposited in the roosting sites at day, that can serve as intra-specific

502   communication to mark the sites. This will test for the multifunctionality of secretions as well as

503   the diversity of compound used by *Prionostemma* and other Eupnoi harvestmen. Ultimately, this

504   will suggest interesting coupling mechanisms in harvestmen, as well as their ability to fine-scale

505   discrimination using the elaborate, diverse, and chemically different secretions.

506   The use of intersexual chemical communication has been suggested in harvestmen in the

507   form of pheromones and nuptial gifts. Although Eupnoi species have shown to discriminate

508   between male and female chemical signals, the properties of their signals, their use and

509   importance have not been addressed. These topics can be studied by conducting reproductive

510   trails (as in Fowler-Finn et al. 2014) and extract the secretions and nuptial gifts as those are

511   presented during the interactions. Then, chemical analyses will provide the identity of the

512   compounds in the secretion. Finally, to test the function of the secretions in communication can

513   be investigated with ablation experiments and removal of nuptial gifts. Consequently,

514   information of this kind will inform of the importance of courtship and fitness of the chemical

515   traits on Eupnoi harvestmen as well as in invertebrates that use chemical channels for

516   reproduction.

517   ## XII. Acknowledgements

XIII. References

Acosta, L. E., Poretti, T. I., & Mascarelli, P. E. (1993). The defensive secretions of *Pachyloidellus goliath* (Opiliones, Laniatores, Gonyleptidae). *Bonn. Zool. Beitr*, *44,* 19-31.

Agosta W.C. (1992). Chemical Communication: The Language of Pheromones. Rockefeller University Press, New York, pp. 13–32.

Barth, F. G. (2002). *A spider's world: senses and behavior*. Springer Science & Business Media.

Bishop, S. C. (1950). The life of a harvestman. *Nature Magazine 276*, 264–267.

Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication*. Sinauer.

Caetano, D. S., & Machado, G. (2013). The ecological tale of Gonyleptidae (Arachnida, Opiliones) evolution: phylogeny of a Neotropical lineage of armoured harvestmen using ecological, behavioural and chemical characters. *Cladistics*, *29*(6), 589-609.

Chelini, M. C., Willemart, R. H., & Hebets, E. A. (2009). Costs and benefits of freezing behaviour in the harvestman *Eumesosoma roeweri* (Arachnida, Opiliones). *Behavioural Processes*, *82*(2), 153–159.

Clawson, R. (1988). Morphology of defense glands of the Opilionids (Daddy Longlegs) *Leiobunum vittatum* and *Leiobunum flavum* (Arachnida, Opiliones, Palpatores, Phalangiidae). *Journal of Morphology*, *196*, 363–381.

Dias, B. C., & Willemart, R. H. (2013). The effectiveness of post-contact defenses in a prey with no pre-contact detection. *Zoology*, *116*, 168–174.

Dias JM & Willemart RH. (2016). Do sexually dimorphic glands in the harvestman Gryne perlata (Arachnida: Opiliones) release contact pheromones during mating? European Journal of Entomology 113: 184–191.

Domínguez M, Escalante I, Carrasco-Rueda F, et al. (2016). Losing legs and walking hard: Effects of autotomy and different substrates in the locomotion of harvestmen in the genus Prionostemma. Journal of Arachnology 44: 76-82.

Donaldson, Z. R., & Grether, G. F. (2007). Tradition without social learning: scent-mark-based communal roost formation in a Neotropical harvestman (*Prionostemma* sp.). *Behavioral Ecology and Sociobiology*, *61*, 801-809.

Ekpa, O., Wheeler, J. W., Cokendolpher, J. C., & Duffield, R. M. (1985). Ketones and alcohols in the defensive secretion of *Leiobunum townsendi* Weed and a review of the known exocrine secretions of Palpatores (Arachnida: Opiliones). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *81*, 555-557.

Escalante, Ignacio. Albín, Andrea. Aisenberg A. (2013). Lacking sensory (rather than locomotive) legs affects locomotion but not food detection in the harvestman Holmbergiana weyenberghi. Canadian Journal of Zoology 91: 731.

Escalante I, Badger MA & Elias DO. (2019). Variation in movement: multiple locomotor gaits in Neotropical harvestmen. Biological Journal of the Linnean Society: 127:943-507.

Fernandes, N. D. S., & Willemart, R. H. (2014). Neotropical harvestmen (Arachnida, Opiliones) use sexually dimorphic glands to spread chemicals in the environment. *Comptes Rendus - Biologies*, *337*, 269–275.

Fischer A. (2019). Chemical communication in spiders – a methodological review. The Journal of Arachnology 47: 1.

Fowler-Finn, K. D., Triana, E., & Miller, O. G. (2014). Mating in the harvestman *Leiobunum vittatum* (Arachnida: Opiliones): from premating struggles to solicitous tactile engagement. *Behaviour*, *151*, 1663-1686.

Gainett G, Michalik P, Müller CHG, et al. (2017). Ultrastructure of chemoreceptive tarsal sensilla in an armored harvestman and evidence of olfaction across Laniatores (Arachnida, Opiliones). Arthropod Structure & Development.

Giribet G & Sharma PP. (2015). Evolutionary Biology of Harvestmen (Arachnida, Opiliones). Annual Review of Entomology 60: 157–175.

Giribet, G. & A. Kury. (2007). Phylogeny and Biogeography. *In*: Pinto-da-Rocha, R., Machado, G., & Giribet, G. (Eds.). *Harvestmen: the Biology of Opiliones*. Harvard University Press.

565 Gnaspini, P. & A.J. Cavalheiro. (1998). Chemical and behavioral defenses of a Neotropical cavernicolous harvestman:
566   *Goniosoma spelaeum* (Opiliones, Laniatores, Gonyleptidae). Journal of Arachnology *26*, 81–90.

567 Gnaspini, P. & M. R. Hara (2007). Defense mechanisms. *In*: Pinto-da-Rocha, R., Machado, G., & Giribet, G. (Eds.).
568   *Harvestmen: the Biology of Opiliones*. Harvard University Press.

569 González, A., Rossini, C., & Eisner, T. (2004). Mimicry: Imitative depiction of discharged defensive secretion on
570   carapace of an opilionid. *Chemoecology*, *14*, 5–7.

571 Grether, G. F., & Donaldson, Z. R. (2007). Communal roost site selection in a Neotropical harvestman: habitat
572   limitation vs tradition. Ethology *113*, 290–300.

573 Grether, G. F., Levi, A., Antaky, C., & Shier, D. M. (2014a). Communal roosting sites are potential ecological traps:
574   experimental evidence in a Neotropical harvestman. *Behavioral Ecology and Sociobiology*, *68*, 1629–1638.

575 Grether, G. F., Aller, T. L., Grucky, N. K., Levi, A., Antaky, C. C., & Townsend, V. R. (2014b). Species differences and
576   geographic variation in the communal roosting behavior of *Prionostemma* harvestmen in Central American
577   rainforests. Journal of Arachnology *42*, 257–267.

578 Hara, M. R., Cavalheiro, A. J., Gnaspini, P., & Santos, D. Y. a C. (2005). A comparative analysis of the chemical nature
579   of defensive secretions of Gonyleptidae (Arachnida: Opiliones: Laniatores). *Biochemical Systematics and Ecology*,
580   *33*, 1210–1225.

581 Hara, M. R., & Gnaspini, P. (2003). Comparative study of the defensive behavior and morphology of the gland
582   opening area among harvestmen (Arachnida, Opiliones, Gonyleptidae) under a phylogenetic perspective.
583   *Arthropod Structure and Development*, *32*, 257–275.

584 Harvey BD, Vanni KN, Shier DM, et al. (2017). Experimental test of the mechanism underlying sexual segregation at
585   communal roosts of harvestmen (Prionostemma spp.). Ethology 123:516-525.

586 Hedin, M., Starrett, J., Akhter, S., Schönhofer, A. L., & Shultz, J. W. (2012a). Phylogenomic resolution of paleozoic
587   divergences in harvestmen (Arachnida, Opiliones) via analysis of next-generation transcriptome data. *PloS One*,
588   *7*, e42888.

589 Hedin, M., Tsurusaki, N., Macías-ordóñez, R., & Shultz, J. W. (2012b). Molecular phylogenetics and evolution
590   molecular systematics of sclerosomatid harvestmen (Opiliones, Phalangioidea, Sclerosomatidae): Geography is
591   better than taxonomy in predicting phylogeny. *Molecular Phylogenetics and Evolution*, *62*, 224–236.

592 Hölldobler, B. & E. O. Wilson (1990). *The ants*. Harvard University Press.

593 Immel, V. 1955. Einige bemerkungen zur biologie von *Platybunus bucephalus* (Opiliones, Eupnoi). – Zoologisches
594   Jahrbuch, Abteilung Fuer Systematik 83: 475–484.

595 Juberthie C., Lopez A., Juberthie-Jupeau, L. (1991) Les glandes odorantes des Ischyropsalididae souterrains
596   (Opilions): ultrastructure et role. Mem Biospeol 18:39–46.

597 Kahn PC, Cao DD, Burns M, et al. (2018). Nuptial gift chemistry reveals convergent evolution correlated with
598   antagonism in mating systems of harvestmen (Arachnida , Opiliones). Ecology and Evolution 2018: 1–8

599 Machado, G., Bonato, V., & Oliveira, P. S. (2002). Alarm communication: A new function for the scent-gland
600   secretion in harvestmen (Arachnida: Opiliones). *Naturwissenschaften*, *89*, 357–360.

601 Machado, G., Carrera, P. C., Pomini, A. M., & Marsaioli, A. J. (2005). Chemical defense in harvestmen (Arachnida,
602   Opiliones): Do benzoquinone secretions deter invertebrate and vertebrate predators? *Journal of Chemical
603   Ecology*, *31*, 2519–2539.

604 Machado, G. & R. Macías-Ordoñez. (2007). Social behavior. *In*: Pinto-da-Rocha, R., Machado, G., & Giribet, G. (Eds.).
605   *Harvestmen: the Biology of Opiliones*. Harvard University Press.

606 Machado, G., & Pomini, A. M. (2008). Chemical and behavioral defenses of the Neotropical harvestman *Camarana
607   flavipalpi* (Arachnida: Opiliones). *Biochemical Systematics and Ecology*, *36*, 369–376.

608 Macías-Ordóñez, R. (1997). *The mating system of Leiobunum vittatum Say 1821 (Arachnida: Opiliones: Palpatores):*
609 *resource defense polygyny in the striped harvestman* (Doctoral dissertation, Lehigh University).

610 Moore AMK, Townsend VR, Moore MK, et al. (2018). Sexual Variation in Jet Emissions from Scent Glands in a
611 Neotropical Harvestmen (Opiliones : Cranaidae) from Trinidad , W . I . 18: 14–18.

612 Murayama, G. P., & Willemart, R. H. (2015). Mode of use of sexually dimorphic glands in a Neotropical harvestman
613 (Arachnida: Opiliones) with paternal care. *Journal of Natural History*, (Feb 2015), 1–11.

614 Nazareth TM & Machado G. (2015). Egg production constrains chemical defenses in a neotropical arachnid. PLoS
615 ONE 10: 1–14.

616 Nazareth TM, Sudatti DB & Machado G. (2016). Chemical Defense as a Condition-Dependent Trait in Harvestmen.
617 Journal of Chemical Ecology 42: 1047–1051.

618 Pagoti GF, Peñaflor MFGV, Marabesi MA, et al. (2017). Proximate factors and potential benefits influencing selection
619 of Psychotria suterella for shelter by the harvestman Jussara spec. Entomologia Experimentalis et Applicata: 1–
620 10.

621 Pomini, A. M., Machado, G., Pinto-da-Rocha, R., Macías-Ordóñez, R., & Marsaioli, A. J. (2010). Lines of defense in the
622 harvestman *Hoplobunus mexicanus* (Arachnida: Opiliones): Aposematism, stridulation, thanatosis, and irritant
623 chemicals. *Biochemical Systematics and Ecology*, *38*, 300–308.

624 Raspotnig G. (2012). Scent gland chemistry and chemosystematics in harvestmen. Biologia Serbica 34: 5–18.

625 Raspotnig, G., Leutgeb, V., Schaider, M., & Komposch, C. (2010). Naphthoquinones and Anthraquinones from Scent
626 Glands of a Dyspnoid Harvestman, *Paranemastoma quadripunctatum*. *Journal of Chemical Ecology*, *36*, 158–162.

627 Raspotnig G, Bodner M, Schäffer S, et al. (2015). Chemosystematics in the Opiliones (Arachnida): A comment on the
628 evolutionary history of alkylphenols and benzoquinones in the scent gland secretions of Laniatores. Cladistics 31.

629 Raspotnig G, Schaider M, Föttinger P, et al. (2017). A Model for Phylogenetic Chemosystematics: Evolutionary
630 History of Quinones in the Scent Gland Secretions of Harvestmen. Frontiers in Ecology and Evolution 5

631 Raspotnig, G., Schaider, M., Föttinger, P., Komposch, C., & Karaman, I. (2011). Nitrogen-containing compounds in
632 the scent gland secretions of European Cladonychiid harvestmen (Opiliones, Laniatores, Travunioidea). *Journal of*
633 *Chemical Ecology*, *37*, 912–921.

634 Raspotnig, G., Schaider, M., Stabentheiner, E., Leis, H. J., & Karaman, I. (2014a). On the enigmatic scent glands of
635 dyspnoan harvestmen (Arachnida, Opiliones): First evidence for the production of volatile secretions.
636 *Chemoecology*, *24*, 43–55.

637 Raspotnig, G., Schaider, M., Föttinger, P., Leutgeb, V., & Komposch, C. (2014b). Benzoquinones from scent glands of
638 phalangiid harvestmen (Arachnida, Opiliones, Eupnoi): a lesson from *Rilaena triangularis*. *Chemoecology*, *25*, 63–
639 72.

640 Rocha, D. F. O., Hamilton, K., Gonçalves, C. C. S., MacHado, G., & Marsaioli, A. J. (2011). 6-alkyl-3,4-dihydro-2H -
641 pyrans: Chemical secretion compounds in Neotropical harvestmen. *Journal of Natural Products*, *74*, 658–663.

642 Santos, F. H., & Gnaspini, P. (2002). Notes on the Foraging Behavior of the Brazilian Cave Harvestman Goniosoma
643 Spelaeum (Opiliones, Gonyleptidae). *Journal of Arachnology*, *30*, 177-180.

644 Schaider, M., Komposch, C., Stabentheiner, E., & Raspotnig, G. (2011). Functional anatomy of scent glands in
645 *Paranemastoma quadripunctatum* (Opiliones, Dyspnoi, Nemastomatidae). *Journal of Morphology*, *272*, 1182–
646 1191.

647 Schaider M, Novak T, Komposch C, et al. (2018). Methyl-ketones in the scent glands of Opiliones: a chemical trait of
648 cyphophthalmi retrieved in the dyspnoan Nemastoma triste. Chemoecology 28: 61–67.

649 Schaider, M., & Raspotnig, G. (2009). Unusual organization of scent glands in *Trogulus tricarinatus* (Opiliones,
650 Trogulidae): evidence for a non-defensive role. *Journal of Arachnology*, *37*, 78–83.

651   Segalerba A & Toscano-Gadea CA. (2016). Description of the defensive behaviour of four neotropical harvestmen
652   (Laniatores: Gonyleptidae) against a synchronic and sympatric wolf spider (Araneae: Lycosidae). Arachnology 17:
653   52–57.

654   Segovia JMG, Hara MR, Pagoti GF, et al. (2015). The Scent Glands of the Neotropical Harvestman Discocyrtus
655   pectnifemur: Morphology, Behavior and Chemistry. Journal of Chemical Ecology 41: 716–723.

656   Silva MS, Willemart RH & Carbayo F. (2018). Sticky flatworms (Platyhelminthes) kill armored harvestmen (
657   Arachnida , Opiliones) but are not immune to the prey' s weapons. Journal of Zoology 306: 88-94.

658   Shultz, J.W & R. Pinto-da-Rocha. (2007). Morphology and Functional Anatomy. *In*: Pinto-da-Rocha, R., Machado, G.,
659   & Giribet, G. (Eds.). *Harvestmen: the Biology of Opiliones*. Harvard University Press.

660   Teng, B., Dao, S., Donaldson, Z. R., & Grether, G. F. (2012). New communal roosting tradition established through
661   experimental translocation in a Neotropical harvestman. *Animal Behaviour*, *84*, 1183-1190.

662   Trabalon, M. 2013. Chemical Communication and Contact Cuticular Compounds in Spiders. *In* Nentwig, W.
663   (ed). *Spider Ecophysiology*. Berlin/Heidelberg: Springer.

664   Uhl, G. & D. O. Elias 2011. Communication. *In*: Herberstein, M. E. (Ed.). *Spider Behaviour: Flexibility and Versatility*.
665   Cambridge University Press.

666   Wade, R. R., Loaiza-Phillips, E. M., Townsend, V. R., & Proud, D. N. (2011). Activity patterns of two species of
667   Neotropical harvestmen (Arachnida: Opiliones) from Costa Rica. *Annals of the Entomological Society of America*,
668   *104*, 1360-1366.

669   Wachter GA, Muster C, Arthofer W, et al. (2015). Taking the discovery approach in integrative taxonomy: Decrypting
670   a complex of narrow-endemic Alpine harvestmen (Opiliones: Phalangiidae: Megabunus). Molecular Ecology 24:
671   863–889.

672   Wilson, E. O., & Regnier Jr, F. E. (1971). The evolution of the alarm-defense system in the formicine ants. *American
673   Naturalist 1971*, 279-289.

674   Willemart, R. H. (2002). Cases of intra-and inter-specific food competition among Brazilian harvestmen, in captivity
675   (Opiliones, Laniatores, Gonyleptidae). *Revue arachnologique*, *14*, 49-58

676   Willemart, R. H., & Chelini, M. (2007). Experimental demonstration of close-range olfaction and contact
677   chemoreception in the Brazilian harvestman, *Iporangaia pustulosa*. *Entomologia experimentalis et applicate*
678   123:1–7.

679   Willemart, R. H., Farine, J.-P., & Gnaspini, P. (2009). Sensory biology of Phalangida harvestmen (Arachnida,
680   Opiliones): a review, with new morphological data on 18 species. *Acta Zoologica*, *90*, 209–227.

681   Willemart, R. H., Farine, J. P., Peretti, A. V., & Gnaspini, P. (2006). Behavioral roles of the sexually dimorphic
682   structures in the male harvestman, *Phalangium opilio* (Opiliones, Phalangiidae). *Canadian journal of zoology*, *84*,
683   1763-1774.

684   Willemart, R. H., & Hebets, E. A. (2012). Sexual differences in the behavior of the harvestman *Leiobunum vittatum*
685   (Opiliones, Sclerosomatidae) towards conspecific cues. *Journal of Insect Behavior*, *25*, 12–23.

686   Wouters, F. C., Rocha, D. F., Gonçalves, C. C., Machado, G., & Marsaioli, A. J. (2013). Additional vinyl ketones and
687   their pyranyl ketones in gonyleptid Harvestmen (Arachnida: Opiliones) suggest these metabolites are widespread
688   in this Family. *Journal of natural products*, *76*, 1559-1564.