

1 **Differential investment in visual and olfactory brain regions is linked to the sensory**
2 **needs of a wasp social parasite and its host**

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4 **Running title:** Brain investment in a social parasite and host

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6 Allison N. Rozanski¹, Alessandro Cini^{2,3}, Taylor E. Lopreto¹, Kristine M. Gandia¹, Mark E.
7 Hauber⁴, Rita Cervo² & Floria M. K. Uy^{1,5*}

8
9 ¹ Department of Biology, University of Miami, Coral Gables, FL 33146, USA

10 ² Department of Biology, University of Florence, Sesto Fiorentino (FI), 50019, Italy

11 ³ Centre for Biodiversity and Environment Research, University College London, Gower
12 Street, London, WC1E 6BT, UK

13 ⁴ Department of Evolution, Ecology and Behavior, School of Integrative Biology,
14 University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

15 ⁵ Department of Biology, University of Rochester, Rochester, NY 14627, USA

16
17 *Author for correspondence: floria.uy@rochester.edu Tel: +1-786-301-3975

18 Department of Biology, University of Rochester, Rochester, NY 14627, USA

19 ORCID: 0000-0002-2918-0418

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34

35

36 **ABSTRACT**

37 Obligate insect social parasites evolve traits to effectively locate and then exploit their hosts,
38 whereas hosts have complex social behavioral repertoires, which include sensory recognition to
39 reject potential conspecific intruders and heterospecific parasites. While social parasite and host
40 behaviors have been studied extensively, less is known about how their sensory systems function
41 to meet their specific selective pressures. Here, we compare investment in visual and olfactory
42 brain regions in the paper wasp *Polistes dominula*, and its obligate social parasite *P. sulcifer*, to
43 explore the link among sensory systems and brain, and behavior. Our results show relative
44 significant volumetric differences between these two closely-related species, consistent with
45 their very different life-histories. Social parasites show proportionally larger optic lobes and
46 central complex to likely navigate long-distance migrations and unfamiliar landscapes to locate

47 the specific species of hosts they usurp. Contrastingly, hosts have larger antennal lobes and
48 calyces of the mushroom bodies compared with social parasites, as predicted by their sensory
49 means to maintain social cohesion via olfactory signals, allocate colony tasks, forage, and
50 recognize con- and heterospecific intruders. Our present work suggests how this tradeoff
51 between visual and olfactory brain regions may facilitate different sensory adaptations needed to
52 perform social and foraging tasks by the host, including recognition of parasites, or to fly long
53 distances and successful host localizing by the social parasite.

54

55 **Keywords:** sensory brain regions, coevolution, host exploitation, *Polistes dominula*, *Polistes*
56 *sulcifer*, sensory systems, social parasite

57

58

59 **INTRODUCTION**

60 Social or brood parasitism, in which a parasitic individual takes advantage of the parental care
61 of a host, is a reproductive strategy that has evolved independently in diverse lineages, and is
62 well-represented in birds and social insects (Antonson, Rubenstein, Hauber, & Botero, 2020;
63 Buschinger, 2009; Cini, Sumner, & Cervo, 2019; Kilner & Langmore, 2011). The significant
64 costs of social parasitism to host species result in a coevolutionary arms race, where the
65 parasite must locate and successfully exploit parental care by the host, while the host, in turn,
66 must recognize and reject potential parasites (Feeney, Welbergen, & Langmore, 2012; Hauber,
67 Moskát, & Bán, 2006; Lenoir, d'Ettorre, Errard, & Hefetz, 2001). In particular, deceiving hosts
68 is critical to obligate social parasites, a type of insect brood parasite that has lost the worker
69 caste and solely depends on exploiting their social host for brood care (Rabeling, 2020).

70 Although the adaptive behaviors of host and obligate social parasites have been studied
71 extensively (Cervo, 2006; Lhomme & Hines, 2019; Loope, Lopez-Osorio, & Dvořák, 2017;
72 Nehring, Dani, Turillazzi, Boomsma, & d'Ettorre, 2015), the evolution and function of sensory
73 systems to facilitate their behavioral interactions and arms-race remain poorly understood
74 (Aidala et al., 2012; Stevens, 2013). Hosts have large sensory repertoires that facilitate general
75 foraging decisions, social interactions, task allocation, and the recognition of nestmates as well
76 as intruders. In contrast, the main selective pressure on obligate social parasites is to find and
77 then deceive their hosts: therefore, fine-tuned sensory systems are critical to mediate both the
78 enemy-recognition by the host, and the successful deception and exploitation by the social
79 parasite (Stoddard & Hauber, 2017).

80 However, developing brain tissue needed to process multiple and complex sensory
81 stimuli can be energetically expensive (Kotrschal et al., 2013; Niven & Laughlin, 2008;
82 O'Donnell, Clifford, & Molina, 2011). In addition, variation in the demands of specific
83 sensory stimuli may drive differential investment in specific sensory brain regions (Arganda,
84 Hoadley, Razdan, Muratore, & Traniello, 2020; Barton, Purvis, & Harvey, 1995; Keesey,
85 Grabe, Knaden, & Hansson, 2020). For example, nocturnal lineages of mammals, birds, and
86 insects showed larger olfactory over visual brain structures compared with diurnal lineages
87 (Barton et al., 1995; Corfield, Wild, Hauber, Parsons, & Kubke, 2008; O'Donnell et al., 2015;
88 Sheehan, Kamhi, Seid, & Narendra, 2019; Stöckl et al., 2016). In insects, the size of sensory
89 brain regions is typically positively related to their relative importance and specific needs of
90 each species (El Jundi, Huetteroth, Kurylas, & Schachtner, 2009; Gronenberg & Hölldobler,
91 1999). Specifically in social Hymenoptera, volumetric differences in brain regions are
92 associated with age, colony size, foraging, caste, social interactions, and the need for

93 distinguishing colony members from intruders (Arganda et al., 2020; Ehmer, Reeve, & Hoy,
94 2001; O'Donnell, Donlan, & Jones, 2007; Rehan, Bulova, & O'Donnell, 2015; Seid, Goode,
95 Li, & Traniello, 2008; Seid & Junge, 2016). Therefore, in systems where brood parasites
96 attack social insects, hosts are predicted to use their specialized sensory systems to recognize
97 and reject intruders (Cervo, Bertocci, & Turillazzi, 1996; Cini, Bruschini, Signorotti, et al.,
98 2011; Lenoir et al., 2001). In turn, parasites would use their own sensory systems to first
99 navigate to locate potential hosts (Cervo et al., 1996; Cervo & Turillazzi, 1996), and then
100 identify the correct host species, replace the host queen, and exploit the host workers for the
101 foreign brood care (Cervo, 2006; Nehring et al., 2015; Pollock, Hoover, Uy, & Hauber, 2021)
102 (Cini, Bruschini, Poggi, & Cervo, 2011; Nehring et al., 2015; Ortolani, Zechini, Turillazzi, &
103 Cervo, 2010).

104 To explore adaptive investment in specific brain regions by hosts and obligate brood
105 parasites, we here take advantage of a unique system composed of two closely-related paper
106 wasp species in Southern Europe (Choudhary, Strassmann, Queller, Turillazzi, & Cervo,
107 1994). *Polistes sulcifer* is the obligate social parasite of *Polistes dominula* (Cervo, 2006;
108 Cervo & Turillazzi, 1996; Ortolani et al., 2010). Paper wasps use cuticular hydrocarbons
109 (CHCs) as odor signals that indicate fertility and dominance (Dapporto, Dani, & Turillazzi,
110 2007), and to distinguish nestmates from conspecific and heterospecific intruders (Bruschini et
111 al., 2011; Dani, 2006; Dani, Jones, Destri, Spencer, & Turillazzi, 2001; Lorenzi, Bagnères,
112 Clément, & Turillazzi, 1997; Mora-Kepfer, 2014). Therefore, the use of CHCs recognition and
113 visual inspection of facial markings may also be used to detect potential parasites by the host
114 *P. dominula* (Cervo, Cini, & Turillazzi, 2015; Cini, Ortolani, Zechini, & Cervo, 2015;
115 Ortolani et al., 2010; Sledge, Dani, Cervo, Dapporto, & Turillazzi, 2001; Turillazzi et al.,

116 2000). Paper wasp hosts also have sensory needs according to division of labor. Subordinate
117 workers forage to collect nest material and prey, while foundresses remain on the nest and
118 interact with these incoming foragers(Queller et al., 2000).

119 In contrast, the social parasite *P. sulcifer* must overcome different and complex
120 sensory challenges. A *P. sulcifer* female emerges from its overwintering site at the top of high
121 mountains and migrates long distances to lower elevations across unfamiliar terrain to locate
122 the nests of *P. dominula* (Cervo, 2006), requiring navigational abilities. After finding a host
123 colony, the parasite usurps and functionally replaces the host foundress to take reproductive
124 control (Cervo, 2006), and acquires the CHCs of the colony (Bagnères & Lorenzi, 2010;
125 Dapporto, Cervo, Sledge, & Turillazzi, 2004; Sledge et al., 2001; Turillazzi et al., 2000).
126 However, host workers may eventually detect the parasite usurper , perhaps due to an
127 imperfect chemical and/or behavioral integration into the host colony (Cini, Branconi,
128 Patalano, Cervo, & Sumner, 2020; Cini, Bruschini, Poggi, et al., 2011; Sledge et al., 2001).
129 Nonetheless, when successful, the parasite usurper becomes the sole egg layer (Cervo, 2006;
130 Turillazzi et al., 2000). After the adult female and male parasite brood emerge from the host
131 nest, they migrate to the top of mountains to mate, and fertilized females overwinter to start
132 the search and usurpation cycle the following spring (Cervo, 2006). Therefore, *P. sulcifer*'s
133 sensory needs may reflect a strong need in vision needed to migrate long distances to an
134 unfamiliar mountain to mate and overwinter, to then, in the following spring, subsequently
135 migrate again and locate host nesting sites and a host colony.

136 We hypothesize that relative proportions of select brain regions reflect the differential
137 investment by hosts and parasites to meet their specific sensory needs. In particular, here we
138 focus on insect brain regions with known functions. First, the antennal lobes (AL) receive

139 olfactory stimuli from the antennae (Anton & Homberg, 1999). Second, the optic lobes (OL) are
140 known to process visual input directly from the eyes and are associated to visual ecology needs.
141 The OL are divided into the lamina (LA), medulla (ME) and lobula (LO), which provide contrast
142 enhancement, color vision and motion detection, and shape discrimination, respectively
143 (Arganda et al., 2020; Gronenberg & Liebig, 1999; Strausfeld, 1989; Yang, Lin, & Hung, 2004;
144 Yilmaz, Grübel, Spaethe, & Rössler, 2019). Third, the calyces (CAL) of mushroom bodies (MB)
145 act as learning centers and integrate olfactory information in the Lip (LI) and visual information
146 in the collar (CO). (Ehmer & Hoy, 2000; Strausfeld, Hansen, Li, Gomez, & Ito, 1998). Finally,
147 the Central complex (CX), is implicated in migration and spatial navigation (Heinze & Homberg,
148 2007; Honkanen, Adden, da Silva Freitas, & Heinze, 2019).

149 Given the known functions of these specific brain regions and the broad knowledge
150 about the natural history of this social parasite-host system, we first predicted that *P. sulcifer*
151 would show greater relative volumes in the brain regions necessary for navigation compared
152 with its host. For example, studies in migratory desert locusts and monarch butterflies showed
153 a proportional increase in the OL and CX compared with their non-migratory conspecifics
154 (Heinze, Florman, Asokaraj, El Jundi, & Reppert, 2013; Ott & Rogers, 2010). Second, we
155 expected that hosts would have larger AL compared with its parasite, due to the different and
156 complex olfactory stimuli they encounter and must assess (Dani, 2006). Finally, because
157 social interactions increase the CAL volume in *Polistes* wasps (Ehmer et al., 2001; Molina &
158 O'Donnell, 2007), hosts would show an increase in volume of the LI and CO, compared with
159 the social parasite.

160

161 **MATERIALS AND METHODS**

162 **Field collection and usurpation experiments**

163 In Spring 2016 and 2017, we collected host colonies from unparasitized populations in the
164 surroundings of Florence (Tuscany, Italy). Although most populations of *P. dominula*
165 experience low to null pressure from *P. sulcifer* parasites, previous experimental work in this
166 population found that hosts do respond to simulated attacks by these social parasites (Cini,
167 Nieri, Dapporto, Monnin, & Cervo, 2014). Colonies had 2-4 foundresses and brood, but no
168 adult workers. We fixed each nest to the ceiling of a glass cage (15 cm x 15 cm x 15 cm) and
169 maintained it under controlled laboratory conditions with ad libitum sugar, water, fly maggots
170 as larvae food, and paper for nest building. We individually marked colony members with
171 enamel paint dots (Testor ©) on the thorax and wings. We also collected *P. sulcifer* females
172 emerging from their overwintering sites at 2050 m altitude on Monti Sibillini (Umbria-Marche,
173 Italy). We kept them in the same type of glass cages, inside a fridge at 4°C with ad libitum
174 water and sugar, until it was time to end diapause. We simulated spring conditions by exposing
175 the parasites to direct natural and artificial sunlight, and low-elevation natural temperature
176 according to our established protocol (Cini et al., 2020; Cini, Bruschini, Poggi, et al., 2011;
177 Ortolani, Turillazzi, & Cervo, 2008).

178 We ran experimental trials during the last week of May, when usurpation usually occurs
179 in the field (Cervo et al., 1996; Ortolani et al., 2008; Turillazzi, Cervo, & Cavallari, 1990). For
180 usurpation trials, we randomly chose host colonies and introduced a single social parasite
181 female inside the glass cage of a putative host nest (Cini, Bruschini, Poggi, et al., 2011). We
182 only chose parasites that showed usurpation behavior, confirmed by the clear attempts to land
183 on the colony and the attacks toward the host foundresses. Our final sample size consisted of
184 usurping parasites that were collected as they attacked the host nest (N =4) and post-usurpation

185 parasites collected one week after successful integration (N =6). In unparasitized colonies, we
186 collected host queens (N =7) and workers confirmed to have the typical active worker behavior
187 one week after their emergence (N =4). Specimens were treated with an aldehyde-based
188 fixative (Prefer, Anatech Ltd, Battle Creek, USA) and kept refrigerated at 4°C until the
189 embedding of the wasp head capsules, which were used to quantify whole brain (WB) and
190 distinct brain regions. Glyoxal is the active ingredient in this fixative, and provides the
191 morphology equivalent to formaldehyde fixation.

192

193 **Histology and quantification of brain structures**

194 We employed an established histological protocol for *Polistes* brains to test for relative
195 volumetric differences in brain structures that receive and process sensory information (Ehmer
196 & Hoy, 2000; Molina & O'Donnell, 2008; O'Donnell et al., 2011; O'Donnell et al., 2007;
197 O'Donnell, Bulova, DeLeon, Barrett, & Fiocca, 2019). We dehydrated each head capsule
198 through a series of ascending ethanol concentrations and acetone, and implemented Embed 812
199 resin kit concentrations (Electron Microscopy Sciences, Hatfield, USA). The samples were
200 placed in an open-air rocking shaker (Thermo Fisher Scientific, Waltham, USA) and alternated
201 with placement under vacuum to improve infiltration. We placed the embedded head capsules in
202 plastic molds filled with resin in a 60°C oven to polymerize for 72 hours. This reliable resin
203 medium facilitates accurate measurements of brain regions by avoiding distortion of the tissue
204 during the dehydration and embedding steps (Ocampo, Sánchez, & Barrantes, 2020).

205 To facilitate subsequent quantification of each brain region, heads were sectioned into 17
206 µm-thick coronal axis sections and stained with the NISSL stain toluidine blue. We then
207 photographed each consecutive brain section per specimen by using a Canon camera (EOS 5D

208 Mark III) mounted on a Leica DM IL LED microscope at 4x magnification and a 1000-micron
209 scale. We used the image AxioVision Software (version 4.8; Zeiss) to quantify all well-defined
210 brain regions. We focused on the AL, and the three substructures of the OL that receive visual
211 information (LA, ME and LO). We also traced two CAL substructures: LI and CO. Finally, we
212 followed the methods by Sheehan et al. 2019, which quantified the CX, the subesophageal zone
213 (SEZ), and the remaining tissues classified as central brain (CB) (as depicted in Fig. 3a). We
214 traced every other section in each specimen, as established by the reported accuracy of less than
215 3.5% error for 34 micro meter thick sections (Ehmer et al., 2001). Furthermore, we traced one
216 hemisphere of the brain for each specimen, quantified area and calculated volume of each brain
217 region by using the clearly defined boundaries for each brain region used in previous *Polistes*
218 neuroanatomy studies (Molina & O'Donnell, 2008; Molina & O'Donnell, 2007; O'Donnell et al.,
219 2011). Tracing and quantification of brain structures were performed blindly to the 2 species and
220 4 categories. Each image was standardized using a 100 μm scale, and head width was measured
221 as a proxy for body size. Finally, we used the software RECONSTRUCT to generate the 3D
222 brain reconstructions for these two species (Fiala, 2005).

223

224 **Statistical analyses**

225 Observed differences in proportional allocation in different brain regions can arise from
226 changes in allometric scaling through grade shifts (e.g, change in elevation) and/or changes in
227 the slope of the covariance between brain regions (Eberhard & Wcislo, 2011; O'Donnell et al.,
228 2013; Ott & Rogers, 2010; Seid, Castillo, & Wcislo, 2011; Sheehan et al., 2019; Stöckl et al.,
229 2016). Thus, we here explored whether 1) hosts and parasites differed in the relative size of
230 specific brain regions, and 2) changes in grade shift and/or slope explained the investment in

231 specific sensory regions compared with non-sensory regions. To test for volumetric differences
232 in visual and olfactory regions between host and social parasites, we first quantified absolute
233 volume for each brain region and for the whole brain (WB). For the specimens used in this
234 study, the OL contribute on average to 42% of the total brain in *P. dominula* and 46% in *P.*
235 *sulcifer*, which can influence the scaling of relative brain regions. Therefore, we normalized
236 individual brain regions by CB volume to control for the effect of the OL in relative neuropil
237 scaling, and avoid distortions of brain volume and size per species (Ott & Rogers, 2010;
238 Sheehan et al., 2019; Stöckl et al., 2016).

239 To determine the relationship in the investment between sensory brain regions and central
240 brain, we implemented Standardized Major (SMA) regression analyses, using the SMATR v.3
241 package for R (Warton, Duursma, Falster, & Taskinen, 2012; Warton, Wright, Falster, &
242 Westoby, 2006). We utilized the scaling relationship between brain regions x and y , using the
243 allometric equation $y = a \cdot x^\beta$ (Dubois, 1897; Huxley & Teissier, 1936). We then used the linear
244 equation $\log(y) = \beta \log(x) + \log(a)$, where $\log(a) = \alpha$, as this logarithmic transformation estimates
245 β from the slope and α from intercept of a regression (Huxley & Teissier, 1936), used in previous
246 studies that calculated proportional volumetric differences in brain regions (Ott & Rogers, 2010;
247 Sheehan et al., 2019; Stöckl et al., 2016). Given our sample size and because neuropils did not
248 differ significantly between host queens and host workers or between usurping and post-
249 usurpation parasites, we pooled all within-species samples for the interspecies comparison.

250 First, we tested for a Common Slope between host and social parasite ($H^0 = \beta_{host} =$
251 $\beta_{parasite}$) by using a log-likelihood test. Specifically, we ran the following comparisons in neural
252 tissue volume: 1) WB and body size, 2) CB and WB, 3) pooled sensory regions and CB, and 4)
253 each sensory region normalized by the CB (Table 1). We defined pooled sensory regions as the

254 combination of antennal lobes, optic lobes, lip and collar. Second, if the host and parasite shared
255 a Common Slope, we then tested for a Slope Index (SI), a Common Shift and a Grade Shift
256 Index (GSI) for the four comparisons described above. The Slope Index ($SI = \beta_{\text{host\&par}}$) tested if
257 volume in a brain region was isometric, calculated by a log-likelihood test. Therefore, if $\beta \neq 1$,
258 the proportion of sensory brain region (brain region y) and central brain (x) is allometric,
259 meaning that x/y would change with size. The Common Shift ($H^0 = \text{equal axis between host and}$
260 parasite) indicated any shift along the major axis, calculated by a Wald Test. The Grade Shift
261 Index (GSI) quantified how much larger a sensory brain region (region y) is for a given size of
262 central brain (region x) for hosts compared with parasites ($H^0 = \alpha_{\text{host}} = \alpha_{\text{parasite}}$). GSI represents
263 changes in elevation (intercept α) with no changes in the slope (β), reflecting volumetric
264 differences between the two species as $e^{\alpha_{\text{host}} - \alpha_{\text{parasite}}}$, calculated by a Wald test. If the GSI > 1,
265 hosts had a larger brain region than parasites, and if GSI < 1, parasites had a larger brain region
266 than hosts. Log-likelihood tests and Wald tests were implemented in the SMATR package.
267 Finally, we compared investment between the host and social parasite in specific sensory regions
268 by normalizing each brain region by the CB, and running Mann-Whitney U tests.

269

270 **RESULTS**

271 **Contribution of allometry to host and social parasite differences**

272 Hosts and parasites did not share a common allometric slope when we compared differences
273 between absolute whole brain and body size ($P = 0.026$, Figure 1a, Table 2). Hosts showed an
274 isometric pattern, maintaining a consistent volume in specific brain regions. In contrast, the
275 social parasites showed a hyperallometric relationship, with more variation in WB volume while
276 showing similar head width. Both the relationship between WB and CB (Figure 1b, and between

277 CB and pooled sensory brain regions (Figure 1c) (See Table 2) shared a common slope,
278 respectively. Hosts showed a grade shift, with increased CB volume compared with social
279 parasites, as indicated by significant differences in elevation ($GSI > 1$, $P = 0.014$). Parasites also
280 showed higher volume of pooled sensory brain regions compared with hosts, with the OL
281 representing $> 65\%$ of these structures ($GSI < 1$, $P = 0.016$). We found no effect of body size on
282 brain region volume (SI) in the CB or pooled sensory regions ($P = 0.25$ and $P = 0.9$, respectively).

283 Next, hosts and social parasites shared a common slope in the relationship between each
284 sensory brain region and CB, but had unique differences in the GSI and SI depending on the
285 specific region (Table 2, Figure 2, Figure 3f). The LA was significantly larger in parasites
286 compared with hosts ($GSI < 1$, $P < 0.001$) and allometric, as indicated by significant differences in
287 the SI ($P < 0.001$) (Table 1, Figure 2). The ME and LO also had increased volume in parasites
288 compared with hosts ($GSI < 1$, $P < 0.001$ and $P = 0.01$, respectively), but were isometric (Figure
289 2, Table 2). However, the AL showed an allometric increase in the hosts compared with the
290 parasites (Figure 2). Both the CO and LI, that process visual and olfactory stimuli respectively,
291 also showed this allometric pattern. The LI also had a significant grade shift ($GSI > 1$, $P < 0.001$)
292 in the hosts compared with parasites (Figure 2, Table 2). Finally, the CX had a similar pattern of
293 allometry to the optic lobes, with larger volume by parasites compared with hosts ($GSI < 1$,
294 $P = 0.045$).

295

296 **Differential relative investment and tradeoffs between sensory brain regions**

297 When comparing the relative volume in sensory regions normalized by the CB, we found
298 significant differences between hosts and social parasites (Figure 3a). Hosts had increased AL
299 volume ($U = 19.00$, $df = 1$, $P = 0.011$, Figure 3b,) and a larger CAL than social parasites ($U = 17.00$,

300 df =1, P=0.007) Both CAL substructures, LI and CO, were significantly larger in the host than
301 in the parasite (U=8.00, df =1, P=0.001 and U=9.76, df=1, P=0.03 respectively, Figure 3c). In
302 contrast, OL volume was larger in social parasites compared with hosts (U=103.00, df =1,
303 P=0.001)). Specifically, social parasites showed larger LA (U=105.00, df =1, P<0.001, Fig. 2D),
304 ME (U=95.00, df =1, P=0.005, Figure. 3d) and LO (U=91.00, df =1, P=0.011, Figure 3d). Social
305 parasites also showed higher volume in the CX, compared with the hosts (U=79.00, df =1,
306 P=0.025, Figure 3e).

307

308

309 **DISCUSSION**

310 Social parasites have evolved sensory and behavioral strategies to effectively locate host nests
311 then exploit the worker force, while hosts have evolved complex behavioral repertoires needed to
312 recognize nestmates versus conspecific competitors and heterospecific intruders (Bagnères &
313 Lorenzi, 2010; Brandt, Foitzik, Fischer-Blass, & Heinze, 2005; Cervo, 2006; Cini, Patalano, et
314 al., 2015; Cini, Sumner, et al., 2019). In this study, hosts and parasites showed significant
315 differences in the investment towards visual and olfactory brain regions, which supports the
316 hypothesis that expensive brain tissue is allocated according to specific sensory needs (Keeseey et
317 al., 2020). For instance, the most important selective pressure in the social parasite is to navigate
318 and usurp a host nest (Cini, Patalano, et al., 2015; Cini, Sumner, et al., 2019; Grüter, Jongepier,
319 & Foitzik, 2018). Notably, *P. sulcifer* shows a large volume of the optic lobes, which coincides
320 with the selective pressures for migration and host detection. In *Polistes*, the optic lobe and eye
321 morphology are positively correlated due to the retinotopic organization of this optic neuropil:
322 the more ommatidia that form the eye, the larger the optic lobe (Ehmer et al., 2001). Large

323 volume of the lamina, medulla and lobula may reflect *P. sulcifer*'s use of visual cues to localize
324 host nests (Cervo, 2006), as optic lobe allocation consistently reflects the visual ecology of
325 different Hymenopteran species (Arganda et al., 2020; Gronenberg, Ash, & Tibbetts, 2008;
326 Gronenberg & Liebig, 1999; Yang et al., 2004; Yilmaz et al., 2019). Once in close proximity,
327 the parasite uses olfactory cues to distinguish *P. dominula* from sympatric *P. gallicus* or *P.*
328 *nimpha*, as the parasite's large size can hinder effective usurpation in the latter two species
329 (Cervo, 2006; Cervo & Turillazzi, 1996; Cini, Bruschini, Signorotti, et al., 2011).

330 Similarly, the central complex is significantly larger in the social parasite compared with
331 hosts that search for prey and nest building materials with a familiar home radius of few hundred
332 meters around their nest (Prezoto, Maciel, Detoni, Mayorquin, & Barbosa, 2019; Ugolini &
333 Cannicci, 1996) and have an average dispersal rate of less than a kilometer from their natal nest
334 (Bluher, Miller, & Sheehan, 2020). Our result shows similar findings to within-species
335 comparisons in monarch butterflies and desert locusts, in which migratory individuals had larger
336 central complex volume, when compared with non-migratory conspecifics (Heinze et al., 2013;
337 Ott & Rogers, 2010). In these insects, the central complex has been suggested to act as an
338 internal compass that codes for spatial directions (Heinze & Homberg, 2007; Heinze & Reppert,
339 2011, 2012; Homberg, Heinze, Pfeiffer, Kinoshita, & El Jundi, 2011) and towards path
340 integration in *Cataglyphis* ants who have outstanding navigation skills (Habenstein, Amini,
341 Grübel, El Jundi, & Rössler, 2020). These studies suggest the potential role of this brain region
342 in long-term migration and navigation of unfamiliar landscapes in the social parasites. In our
343 dataset, social parasites also show smaller volume in the antennal lobes and in the calyx,
344 compared with their hosts. The low investment in sensory processing is remarkably similar to
345 neural tissue allocation in the obligate parasitic ant *Polyergus mexicanus* compared with its host

346 (Sulger, McAloon, Bulova, Sapp, & O'Donnell, 2014).

347 In contrast, parasite attacks do not represent the strongest selective pressure in the
348 sensory system of hosts, as incidence of obligate parasitism is almost null in most host
349 populations (RC & AC, pers. comm.). Our results of high volume in olfaction and sensory
350 processing regions are consistent with other studies that found preferential brain investment in
351 brain regions associated needed for effective communication, maintenance of division of labor,
352 learning and memory (Farris, Robinson, & Fahrbach, 2001; Gronenberg, Heeren, & Hölldobler,
353 1996; Jaumann, Seid, Simons, & Smith, 2019; O'Donnell et al., 2015; Rehan et al., 2015; Seid,
354 Harris, & Traniello, 2005; Smith, Seid, Jiménez, & Wcislo, 2010). As predicted, hosts had larger
355 antennal lobes than their social parasites. Colony members consistently communicate as they
356 rear the brood, forage for prey and materials to build their nest among colony members and
357 foraging. Host queens also use olfaction to assess fertility and policing attempts by subordinate
358 workers, and to maintain their dominance in the nest hierarchy (Dapporto et al., 2010). The
359 antennal lobes in *P. dominula* may facilitate identifying non-nestmate conspecifics or social
360 parasites, as recent work in paper wasps showed both an expansion and rapid evolution in the 9-
361 exon odorant receptors, responsible for detecting CHCs (Legan, Jernigan, Miller, Fuchs, &
362 Sheehan, 2021). If successful during usurpation, *P. sulcifer* will adopt the odor of the host colony
363 and become chemically integrated, therefore, it is critical for the host to detect any intruders
364 approaching the nest, including non-nestmate conspecific and social parasites (Cervo, 2006; Cini
365 et al., 2020; Turillazzi et al., 2000).

366 Hosts have large mushroom body calyces, which function as centers for learning and
367 sensory integration (Heisenberg, Heusipp, & Wanke, 1995) (Farris et al., 2001; Gronenberg et
368 al., 1996; Jaumann et al., 2019; O'Donnell et al., 2015; Rehan et al., 2015; Seid et al., 2005;

369 Smith et al., 2010). In *Polistes*, social experience influences the relative volume of sensory brain
370 regions (Jernigan, Zaba, & Sheehan, 2021). Specifically, *P. dominula* single foundresses wasps
371 had smaller antennal lobes and mushroom bodies than multiple foundresses (Ehmer et al., 2001).
372 These findings suggest that increased lip and collar volume may be a response to the important
373 role of integrating olfactory and visual information, respectively, to discriminate nest intruders,
374 which was found in experimental behavior trials in this host-parasite system (Cini et al., 2015).
375 In addition, an increase in the collar volume may not be influenced by visual acuity, but instead
376 towards a computational demand for visual signals among colony members in our host species
377 (Ehmer et al., 2001). For example, both vision and olfaction are used in nestmate recognition by
378 *P. dominula* (Cini et al., 2019). Another possibility is that larger volume in the calyx by hosts is
379 due to the visual and olfactory demands of foraging (Ehmer et al., 2001) and catching prey. This
380 pattern is consistent with the observed larger calyx in the host ant *Formica fusta* compared with
381 workers of its obligate social parasite *Polyergus mexicanus*, which rarely forage (Sulger et al.,
382 2014). These two hypotheses may not be mutually exclusive, and further experiments that
383 include molecular, cellular and circuitry approaches (Godfrey & Gronenberg, 2019; Godfrey,
384 Swartzlander, & Gronenberg, 2021) would elucidate the effect of these two selective pressures
385 towards sensory investment.

386 In summary, we found significant differences, in the volume of visual and olfactory
387 brain regions between these two closely-related species. These results raise intriguing questions
388 about the sensory needs driving differential allocation of brain tissue between and within
389 species in this host and social parasite system. A recent study found reversible brain plasticity in
390 brain volume in gamergates that transition back to a subordinate role in the socially flexible ant,
391 *Harpergnathos saltator* (Penick et al., 2021). These changes in brain size were proposed as an

392 energy-saving mechanism in ants (Gronenberg et al., 1996; Gronenberg & Liebig, 1999).
393 Therefore, the switch from a searching solitary parasite to invading and exploiting a host
394 colony, may influence changes in the brain. One possibility is that a social parasite could
395 increase whole brain volume and/or specific brain regions to integrate as a parasite queen in a
396 social host nest. Alternatively, social parasites with larger brains may have higher success in
397 usurping a host colony, compared with those with smaller brains. Finally, future work
398 comparing host populations highly attacked or not attacked by *P. sulcifer* will effectively test
399 the role of these social parasites as a selective pressure on the differential allocation in specific
400 brain sensory regions in their hosts.

401

402 **AUTHORS CONTRIBUTIONS**

403 FMKU, MEH, RC and AC contributed to study concept and design. AC and RC collected hosts
404 and social parasites in the wild, and ran usurpation trials in laboratory conditions. ANR, TEL and
405 KMG collected volumetric data. ARN and FMKU analyzed the data and wrote the first draft of
406 the manuscript. All authors contributed to data interpretation, drafting the final version of the
407 manuscript and approved its submission.

408

409 **CONFLICT OF INTEREST**

410 Authors declare no conflict of interests.

411

412 **LITERATURE CITED**

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703

704 **TABLE 1** Data used in allometric analyses for the social host *Polistes dominula* (N=11) and the obligate social parasite *P. sulcifer*
705 (N=10). Brain regions included are the lobula (LO), medulla (ME), lamina (LA), optic lobes (OL), antennal lobes (AL), collar (CO),
706 lip (LI), calyx (CAL), central brain (CB), central complex (CX), whole brain (WB), combined sensory regions (SENS). Head width
707 (HW) was used as a proxy for body size.

Species	LO (μm^3)	ME (μm^3)	LA (μm^3)	OL (μm^3)	AL (μm^3)	CO (μm^3)	LI (μm^3)	CAL (μm^3)	CB (μm^3)	CX (μm^3)	WB (μm^3)	SENS (μm^3)	HW (μm)
<i>P. dominula</i>	25866642.4	58358246	25087719.4	109312607.8	14417853	21202423.12	15632664.5	36835087.62	87691130.6	1526094.76	253528012.5	16583688.19	1666.96
<i>P. dominula</i>	22436137.6	63635046	24612736	110683919.6	14496491.6	20797245.8	14422079.54	35219325.34	93036814.6	1410327.82	258724139.3	16568732.47	1667.43
<i>P. dominula</i>	17041568.4	49983553	20558236	87583357.4	11744137.2	15590598.27	11069522.8	26660121.07	87511779.58	1104368.28	218087268.3	13057548.87	1384.88
<i>P. dominula</i>	17366295.6	50314050	18453605.4	86133951	12117413	17097003.02	12961068.26	30058071.28	82944422.9	1396554.76	218472988.8	13552856.59	1362.67
<i>P. dominula</i>	21885089.4	61345486	25090932.4	108321507.8	14643660.6	19781725.64	14849153.88	34630879.52	100225447.9	1547332.52	263877974.9	16365252.71	1572.12
<i>P. dominula</i>	17321993.6	50609884	22784168.4	90716046	11801763.8	16341027.64	11962388.64	28303416.28	77198019.66	1521790.7	212864583.7	13566656.4	1479.84
<i>P. dominula</i>	19702037.8	58033716	22804367.8	100540121.6	12726961.6	16533561.82	13285643.48	29819205.3	89611154.46	1472132.68	237606501.3	14799534.69	1467.15
<i>P. dominula</i>	21440702.6	62001856	25513807.4	108956366	15113988.72	21239782.32	15453528.02	36693310.34	91975249.6	1429054.68	259422019.9	16744677.03	1672.68
<i>P. dominula</i>	25482190.8	70084744	28243075.8	123810010.6	15351646	22876870.08	15332209.73	38209079.81	98153097.54	2098659.52	281670350	18351725.25	1648.84
<i>P. dominula</i>	21720672.2	57230398	24591843	103542913.2	16773169	19834063.2	15079834.7	34913897.9	95249127.96	1679176.36	256096370.5	16084724.25	1677.28
<i>P. dominula</i>	22031330.2	61654036	25401491.8	109086858	16329390.8	23188088.74	15919180.12	39107268.86	98037783.14	1953048.74	268810701.1	17077291.79	1776.99
<i>P. sulcifer</i>	22776401.1	66321080	32924122.7	122021603.8	10125696.4	18159024.98	11047246	29206270.98	90515062.48	1280561.72	257076062.4	16656100	1717.89
<i>P. sulcifer</i>	23808277.64	66394111.66	31996786.64	122199175.9	11197386.6	16442369.4	11654225.22	28096594.62	88950805.78	2063096.54	256596150.7	16764534.5	1651.94
<i>P. sulcifer</i>	23908828.22	68555608.28	27862839.08	120327275.6	13303092.11	18420115.4	11842612.42	30262727.82	93633157.09	1552439.32	263900181.6	17026702.45	1697.79
<i>P. sulcifer</i>	25107500.6	69501304	33727418.6	128336223.2	15104112.4	20677341.74	12985663.86	33663005.6	99341971.12	1657040.32	282288120.2	18294614.91	1792.09
<i>P. sulcifer</i>	23526609.4	66653464	34043554	124223627.4	16915656.2	21437671.5	14291129.56	35728801.06	95720654.07	1801277.16	242990974.6	18355982.7	1826.56
<i>P. sulcifer</i>	20755840.6	60714846.35	24697171.6	106167858.6	11744280	14798489.8	11043301.66	25841791.46	80653032	1792861.82	229757508	14910447.6	1629.9
<i>P. sulcifer</i>	19284738.8	55043444.9	25691161.94	100019345.6	10516018.44	14213571.82	8473311.7	22686883.52	72783445.04	1366523.92	210008946.8	13722550.18	1641.09
<i>P. sulcifer</i>	19060842.34	48682422.3	23900647.82	91643912.46	9458719.42	16569625.28	10379754.6	26949379.88	81559487.98	1769551.42	214588745.5	13302925.75	1700.98
<i>P. sulcifer</i>	21909453.46	63399137.68	31217468.9	116526060	12132729.66	14566223.56	10312809.28	24879032.84	89215401.94	1879786.22	248516243.5	15930084.16	1672.51
<i>P. sulcifer</i>	19124996.6	51406504	20778046	91309546.6	11970709.8	13356670.5	10328152.46	23684822.96	76028675.17	1602885.12	208031260.8	13200258.57	1582.03

709 **TABLE 2** Standardized Major Axis regressions to test for allometry in *Polistes dominula* (host)
710 and *Polistes sulcifer* (social parasite). Volume of brain regions that receive and process sensory
711 stimuli was compared with central brain volume. Scaling of each brain region was calculated
712 with a Grade Shift Index ($GSI = e^{\alpha_{host} - \alpha_{par}}$) that compares differences in elevation between the
713 two species. If $GSI > 1$, a specific brain region is larger in the host. If $GSI < 1$, a specific brain
714 region is larger in the social parasite. The Slope Index (SI) calculates if the allometric scaling of
715 each brain region to the central brain deviates from $\beta = 1$. Each statistical test was implemented
716 as recommended by the SMATR 3 package in R.

Brain region	Common Slope		Isometry			Common Shift		Common Elevation		
	Log likelihood	P	Log likelihood	P	SI	Wald test	P	Wald test	P	GSI
Whole Brain	4.926	0.026								
Central Brain	0.131	0.717	0.261	0.25	0.9	0.545	0.461	5.974	0.014	1.02
Sensory regions	0.335	0.562	0.372	0.1	1.25	0.219	0.589	5.845	0.016	0.97
Optic Lobe	0.212	0.645	0.376	0.09	1.35	0.019	0.891	18.660	<0.001	0.93
Antennal Lobe	0.042	0.836	0.684	<0.001	1.84	3.098	0.078	1.388	0.238	1.03
Lamina	0.141	0.707	0.586	<0.001	1.82	0.551	0.458	28.780	<0.001	0.90
Medulla	0.123	0.726	0.327	0.15	1.28	0.015	0.903	11.220	<0.001	0.95
Lobula	3.890	0.050	0.394	0.08	1.37	0.019	0.891	6.625	0.01	0.96
Calyx	0.168	0.682	0.674	<0.001	1.66	3.851	0.042	5.891	0.012	1.04
Collar	0.177	0.674	0.722	<0.001	1.8	2.943	0.086	2.089	0.148	1.03
Lip	0.768	0.087	0.7174	<0.001	1.91	6.925	<0.001	16.27	<0.001	1.07
Central Complex	0.848	0.357	0.521	0.02	1.75	0.009	0.357	3.547	0.045	0.93
	$P \leq 0.01$	$P \leq 0.05$	$P > 0.05$							

717

718 **FIGURE LEGENDS**

719

720 **FIGURE 1** Role of allometry in volume of whole brain (WB), central brain (CB) and
721 combined sensory brain regions between host and social parasite. Relationship between (a)
722 head width (as a proxy for body size) and WB, (b) WB and CB and (c) CB and combined
723 sensory regions. Species are depicted as: host (grey circles) and parasites (black circles).
724 Standardized major axis fits are log transformed per species with the lines based on intercepts
725 and slopes (grey for hosts and black for social parasites). The dotted line represents the
726 common slope. For full statistical tests, see Table 2.

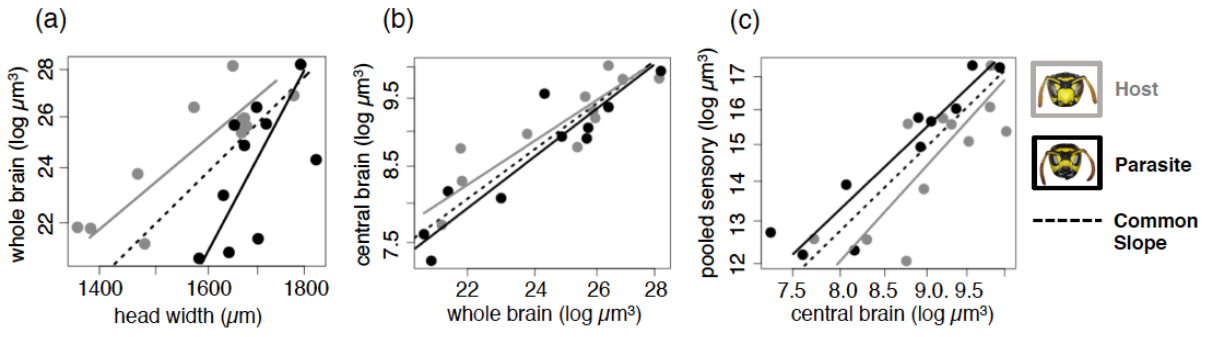
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728 **FIGURE 2** Scaling relationship between the volume of log transformed sensory brain regions
729 and the Central Brain. The dotted line indicates the common allometric slope for the host and
730 parasite species. Grey lines depict the allometric slope for the host and the black line for the
731 parasites. Each dot represents an individual. The dotted line depicts the common slope. See
732 Table 2 for full Standardized Major Axis Tests and inference for allometric lines.

733

734 **FIGURE 3** Organization of brain regions in *Polistes dominula* (host) and *P. sulcifer* (social
735 parasite). (a) Frontal view of 3D reconstructed brain regions for the host (grey and to the left)
736 and the parasite (black and to the right). Black scale bar = 1 mm. (b) Antennal lobes (AL). (c)
737 Substructures of the calyx (CAL): lip (LI) and collar (Co). (d) Substructures of the OL: lamina
738 (LA), medulla (ME) and lobula (LO) in sequence. (e) Central complex (CX). All brain regions
739 are normalized by the central brain shown in light grey. The subesophageal zone (ZEZ) is shown
740 in dark pink and the peduncle (PED) in light pink. Each dot represents an individual and is grey
741 for hosts (H) and black for parasites (P). Each box plot shows the median, 25th and 75th

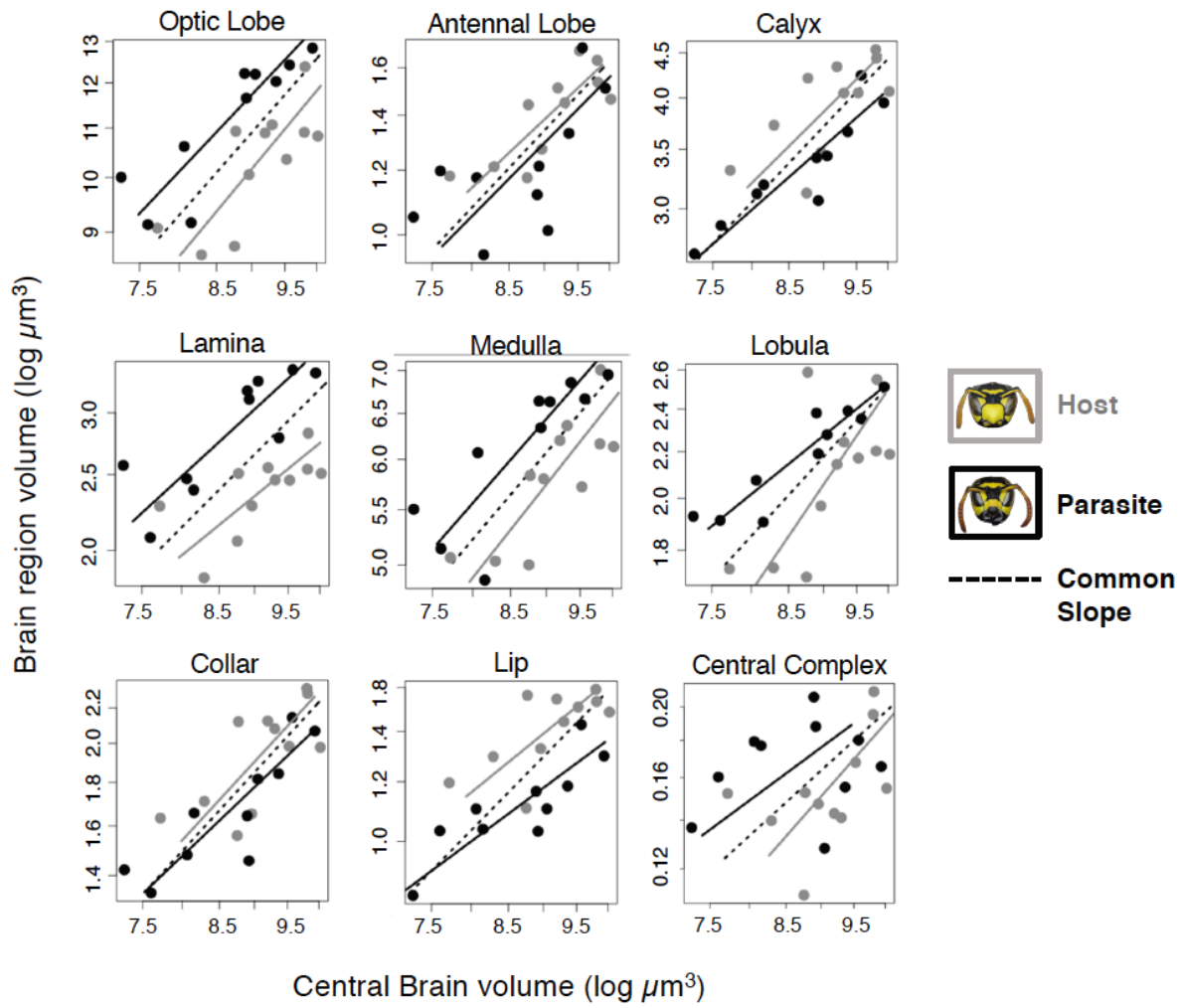
742 percentiles and the whiskers show the 5th and 95th percentiles. (f) The Grade Shift Index (GSI)
743 was calculated by scaling differences in normalized sensory brain regions between species. If
744 $GSI > 1$, a brain region is larger in hosts compared with parasites, and vice versa for $GSI < 1$.
745 The Slope Index (SI) is represented by the deviation of the estimated common allometric slope β
746 from 1. Statistical results based on Mann-Whitney U tests (* $P < 0.05$, ** $P < 0.01$). Full
747 statistical tests can be found in the Results section and Table 2.
748



749

750 Figure 1.

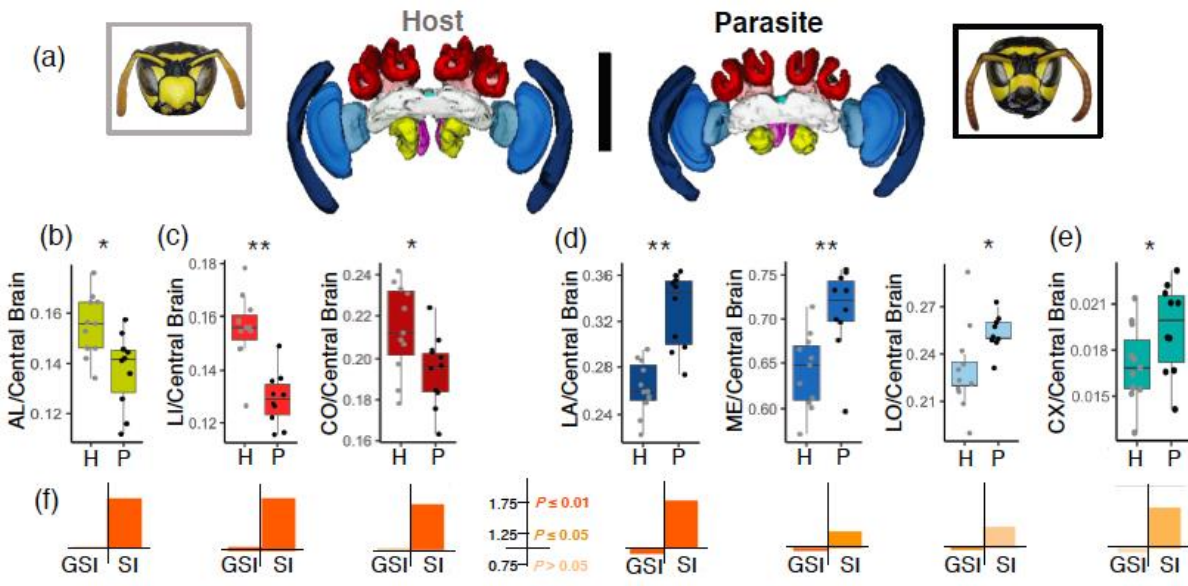
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752

753 Figure 2.

754



755

756 Figure 3.