Diversity of compounds in femoral secretions of Galápagos iguanas (genera: *Amblyrhynchus* and *Conolophus*), and their role in sexual communication in lek-mating marine iguanas (*Amblyrhynchus cristatus*) (#18500)

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Diversity of compounds in femoral secretions of Galápagos iguanas (genera: *Amblyrhynchus* and *Conolophus*), and their role in sexual communication in lek-mating marine iguanas (*Amblyrhynchus cristatus*)

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Background. Chemical signals are widely used in the animal kingdom, enabling communication in various social contexts, including mate selection and the establishment of dominance. Femoral glands, which produce and release waxy secretions into the environment, are organs of central importance in lizard chemical communication. Galápagos marine iguanas (*Amblyrhynchus cristatus*) is a squamate reptile with lek-mating system. Although the lekking behavior of marine iguanas has been well-studied, their potential for sexual communication via chemical cues has not yet been investigated. Here we describe the diversity of the lipophilic fraction of males' femoral gland secretions among 11 island populations of marine iguanas, and compare it with the composition of its sister species, the Galápagos land iguana (*Conolophus subcristatus*). We also conducted behavioural observations in marine iguana territorial males in order to explore the possible function of these substances in the context of male dominance in leks. **Methods.** Femoral secretions were analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS), and Gas chromatographwitha flame ionizationdetector (GC-FID) in order to characterize the lipophilic composition. To understand the potential role of femoral secretions in marine iguana intraspecific communication, territorial males were sampled for their femoral glands and monitored to record their head bob rate – a territorial display behaviour in males – as well as the number of females present in their leks. **Results.** We found that the gland secretions were composed of ten saturated and unsaturated carboxylic acids ranging in chain lengths between C_{16} and C_{24}, as well as three sterols. Cholesterol was the main compound found. Intriguingly, land iguanas have a higher diversity of lipophilic compounds, with structural group of lipids (i.e. aldehydes) entirely absent in marine

iguanas; overall the chemical signals of both species were strongly differentiated. Lipid profiles also differed among populations of marine iguanas from different islands, with some islands demonstrating a high diversity of lipophilic compounds (i.e. full spectra of compounds), and others lacking one or more compounds. Among the compounds most frequently found missing were 11- and 13- eicosenoic acids. Gland secretions of males with a better body condition and with a higher dominance status (i.e. those accompanied by females and with higher head bob display) were proportionately richer in C_{20}-unsaturated fatty acids (11-eicosenoid acid). **Discussion.** Land and marine iguanas strongly diverged in their chemical composition of the femoral glands likely due to ecological differences between both species. Despite that marine iguana populations varied in their femoral gland composition that was not related to their genetic structure. Our results indicated that 11-eicosenoic acid may play an important role in intraspecific chemical communication in marine iguanas.

1 **Diversity of compounds in femoral secretions of Galápagos iguanas (genera:**

2 **Amblyrhynchus and Conolophus), and their role in sexual communication in lek-mating**

3 **marine iguanas (Amblyrhynchus cristatus)**

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INTRODUCTION

Intraspecific chemical communication is a widespread phenomenon among vertebrates (Wyatt 2003). Although reptiles rely heavily on visual signals to communicate with conspecifics, they also use chemical cues in sexual interactions, as well as in a broader social context (Mason & Parker 2010). Squamate reptiles (lizards and snakes) hold the best promise for researchers aiming to study chemical communication. This is mainly due to highly-developed chemosensory capacity, which is used for intraspecific communication. For instance, substrate-deposited scents are used by snakes to locate partners and/or avoid rivals (LeMaster & Mason 2003; Shine et al. 2005). Lizards have the ability to produce scent marks that are recognized by conspecifics (Carazo et al. 2008; Khannoon et al. 2011; Martins et al. 2006). The popularity of lizards as models to study chemical communication has been increasing steadily. However, the functions of the specific compounds that make up chemical signals remain poorly known.

One of the most important organs involved in chemical communication are femoral glands, which are epidermal skin glands situated ventrally on the hind limbs of males (Mayerl et al. 2015). Femoral glands have been reported from Mantellid frogs and although the phylogenetic origin is different than in lizards they serve for similar functions of intraspecific communication (Poth et al. 2013; Poth et al. 2012). Reptiles release waxy secretions from their femoral glands, these are composed of a mixture of proteins and lipids (Weldon et al. 2008). For example, desert iguanas (*Dipsosaurus dorsalis*) have poorly volatile femoral secretions, composed of a 20% lipid fraction and 80% of protein material (Alberts 1990). Proteins absorb ultraviolet light that could be used by desert iguanas to locate pheromones of low volatility (Alberts 1989). Inter-species variability in proteins from femoral glands suggests that proteins could be used for species discrimination in sympatrically occurring lizard species (Alberts 1991). A recent study on Turks and Caicos iguanas (*Cyclura carinata*) showed that genetic differentiation among lineages from different islands is reflected by the pattern of proteins in femoral secretions (Welch et al. 2017). In addition, the lipophilic compounds in the femoral secretions play an important role in many types of sexually-mediated behaviour. For example, femoral glands in males of some lizard species emit odours that could communicate specific information about the traits of the signaller, and may therefore guide female mate choices (López & Martín 2005; Martín & López 2006; Martín & López 2011; Olsson et al. 2003). A link

55   between chemical cues and dominance has also been demonstrated. For instance, femoral gland

56   secretions of male *Lacerta monticola* convey information about the individual's dominance

57   status (Moreira et al. 2006). This situation is hardly surprising, given that the function and

58   activity of femoral glands are regulated by androgens (Fergusson et al. 1985). In green iguanas,

59   *Iguana iguana*, the level of testosterone is positively correlated with the amount of lipids in the

60   femoral secretions of dominant males (Alberts et al. 1992). In this framework, several studies

61   have described the lipid components of the femoral glands in various species of lizards (reviewed

62   in (Martín & López 2014; Weldon et al. 2008)). Although most research on the specific

63   chemicals that compose femoral secretions has focused on lacertids (Martín & López 2014), a

64   few older studies about iguanids are also available (Alberts 1990; Alberts et al. 1992; Weldon et

65   al. 1990). The femoral secretions of the green iguana *I. iguana* are known to contain a high

66   diversity of volatile lipids that appear to be received by chemoreception (Alberts 1993; Alberts et

67   al. 1992). ~~The lipophilic components found in green iguanas~~ include several saturated and

68   unsaturated fatty acids of different chain lengths (C_{14}-C_{26}), as well as sterols (Alberts et al. 1992;

69   Weldon et al. 1990).

70   Despite increasing interest in the chemical ecology of lizards, there are no such studies on

71   one of the best known reptile species in the world: the marine iguana (*Amblyrhynchus cristatus*).

72   This species is endemic to the Galápagos Islands - a world-renowned biodiversity hotspot

73   formed by 13 major islands (>10 km^2), 6 smaller ones and numerous islets in the eastern Pacific.

74   One of the most interesting aspects of the species' behavioural ecology is its lek-mating system.

75   A lek is defined as a mating system in which there is no parental care, males aggregate in

76   clusters, and females benefit from no resources other than the gametes available in the male

77   territories (Höglund & Alatalo 1995). ~~Although lek-mating behaviour has not been studied in~~

78   ~~detail for most of reptiles, territorial settling has been described in~~ *Anolis aeneus* ~~lizards and~~

79   ~~could also occur in other species of iguanids (see (Stamps 1988)). In contrast,~~ marine iguana

80   lekking has received considerable attention over the past decades, and the system is reasonably

81   well described. At the beginning of the mating season, male iguanas establish small clustered

82   territories along the rocky coastline. These territorial males display head bobbing behaviour in

83   order to attract females to their territories, presumably to mate and also to intimidate competing

84   males that are approaching the territory of the dominant male (Wikelski et al. 1996). However,

85   males that cannot establish territories (marginal males) have far less reproductive success than

86 territorial ones, and basically rely on forced copulations with females that remain outside of the
87 lek territories (Partecke et al. 2002; Wikelski et al. 1996). Apparently, females may benefit from
88 mating within specific males´ territories, as the territorial male protects them from harassment by
89 others (Trillmich & Trillmich 1984; Wikelski et al. 1996). The importance of chemical signals in
90 lekking species has been verified in insects. The mosquito *Aedes aegypti* uses an aggregation-
91 pheromone to mediate lek establishment (Cabrera & Jaffe 2007) and males of the sand fly
92 *Lutzomyia longipalpis* may use pheromones to attract females to their leks (Jones & Hamilton
93 1998). Therefore, the lek-mating system offers a valuable natural setup to investigate questions
94 regarding chemical signalling.

95   Several factors suggest that marine iguanas may rely heavily on pheromones for
96 intraspecific communication. Firstly, they possess sexually dimorphic femoral glands that are
97 more developed in males and are almost absent, and likely far less functional, in females.
98 Secondly, observations have revealed a "substrate-licking" behaviour in marine iguanas;
99 (Carpenter 1966) observed that male iguanas protrude their tongue onto the lava rocks as if
100 testing for scent marks. Further, female iguanas have been reported to lick the sand intensively
101 when exploring new areas to lay eggs (Rauch 1988). Tongue licking is a chemoreceptive
102 response to recognition of marked substrates in iguanids (De Fazio et al. 1977; Krekorian 1989)
103 and suggests that *A. cristatus* might use conspecific olfactory cues for intraspecific
104 communication.

105   Our study is the first to analyze the lipophilic fraction of femoral gland secretions in
106 Galápagos iguanas. By performing intensive sampling of all major island populations and
107 subspecies of marine iguanas (see (Miralles et al. 2017)), we seek to characterize the lipophilic
108 profile of femoral glands, as well as to explore the variation between populations originating
109 from distinct islands of the archipelago. In addition to sampling marine iguanas, we also
110 collected femoral secretions of land iguanas (*Conolophus subcristatus*). *Conolophus* is the sister
111 lineage of *Amblyrhynchus,* having diverged around 4.52 million years ago, most likely on the
112 Galápagos Archipelago (MacLeod et al. 2015). Therefore, we compare the chemical profile of
113 marine iguanas with their closest taxon, to investigate the level of divergence in chemical signals
114 between the two.

115   A further aim of this study was to understand the functions of femoral gland compounds
116 in marine iguanas. For this purpose, we collected behavioural observations in one population of

117 *A. cristatus*. Morphological and behavioural data of territorial males were collected during the

118 breeding season and analyzed together with data on gland chemistry. Body size, as well as the

119 body condition index, of male marine iguanas were estimated as determinant factors for female

120 mate choice and male dominance hierarchy (Wikelski et al. 1996). Territorial males were

121 regularly monitored to estimate their head-bobbing rate, and the presence of females in their

122 territories was recorded. Head-bobbing is a behaviour typically displayed by territorial males to

123 defend their territories from other males, as well as to attract females during the breeding season

124 (Wikelski et al. 1996). Dominant males, i.e. those with high display levels (i.e. frequent head

125 bobbing) and who are accompanied by females, should release certain chemicals in their

126 territories that inform conspecifics of their status.

127

128 ## METHODS AND MATERIALS

129

130 **Pheromone sampling**

131

132 During December and January 2014 - 2016 (a period coinciding with the mating season of

133 marine iguanas), an overall of 196 iguanas were captured in 11 islands of the Galápagos

134 archipelago in order to sample their femoral glands. Of those, a subsample of 134, representing

135 all major island populations and subspecies (see Table S1 and Fig. 1 for an overview), were used

136 to examine the variation of chemical profiles among populations. However, due to uneven

137 sampling effort, the number of obtained samples per population was unequal. Therefore a subset

138 of 107 samples, with even sample size for each population was randomly selected and used in

139 statistical analyses in order to avoid any effects of an unbalanced design. The remaining samples

140 (62) were taken from territorial males of a single population (i.e. *A. cristatus mertensi* from La

141 Lobería on San Cristóbal) and used to understand the function of chemical signals in

142 intraspecific communication. In this way, chemical composition of femoral secretions could be

143 analyzed in relation to individual morphology and behaviour (see below: **Morphological dataset**

144 and **Behavioral dataset**). The marine iguanas were captured using a pole with a lasso, and their

145 femoral secretions were obtained by gently squeezing the femoral glands. These were then

146 placed in a 2 ml glass vial with dichloromethane. All samples were stored cold (in portable

147   coolers at approximately 4 – 8 ºC) during the field work, and subsequently transported to
148   laboratories in Germany for chemical analysis.

149   In addition, during January 2016, we also sampled the femoral glands of three individuals
150   of land iguana *C. subcristatus*, which are housed in a spacious outdoor enclosure at the Charles
151   Darwin Research Station on Santa Cruz Island.

152   The Galápagos National Park authority granted the research permission for this study
153   (permit numbers: PC-22-14, PC-08-15 and PC-09-16).

154

155   **Chemical analysis and lipid characterization**

156

157   The chemical analyses were performed by Gas Chromatography coupled with Mass
158   Spectrometry (GC-MS), and Gas chromatograph with a flame ionization detector (GC-FID). GC-
159   MS analyses of natural compounds were performed on a GC 7890A/MSD 5975C from Agilent.
160   Mass spectrometry was performed in an electron ionization mode (EI) with 70 eV. Fused-silica
161   capillary columns and HP-5MS (Agilent Technologies, 30 m, 0.25 mm i.D. 0.25 μm film
162   thickness) were used, with helium as the carrier gas. GC-FID was performed using a HP-5
163   column (Agilent Technologies, 30 m, 0.25 mm i.D. 0.25 μm film thickness) using hydrogen (H_2)
164   as the carrier gas with a flame ionization detection (FID) system.

165   The temperature program for the GC-MS was: 50-5-5-320 (50 °C starting temp., hold
166   time 5 min then 5 °C/min increase up to 320°C). For GC-FID with autosampler the temperature
167   program 125-5-5-320 (125 °C starting temp., hold time 5 min, the 5 °C/min increase up to
168   320°C) was used.

169   The samples collected in December 2014 and January 2015 were preserved in 500 μL
170   dichloromethane. Aliquots of 20 μL from each sample were taken for derivatizations, and 0.8 μL
171   of internal standard (tridecyl acetate) was added. The samples collected in December 2015 and
172   January 2016 were stored in 200 μL dichloromethane. Aliquots of 5 μL were taken for
173   derivatizations and 0.4 μL of internal standard (tridecyl acetate) was added, adding extra 20 μL
174   dichloromethane.

175   Derivatizations of the samples were performed using diazomethane (CH_2N_2), synthesized
176   as an etheric solution from Diazald following Black's procedure for converting carboxylic acids
177   into their corresponding methyl ester (Black 1983). Elucidation of the structure was performed

178   by mass spectral comparison with data bases, and comparison with reference compounds
179   identified 13 major compounds. Fatty acids were identified as their corresponding methyl esters
180   (Table 1). In contrast to marine iguanas, land iguana samples also feature aldehydes in addition
181   to carboxylic acids (Table 1). Besides the additional aldehydes, hexadecyl hexadecanoate and di-
182   (9-octadecenoyl)-glycerol were also found (Table 1). The position of the double bonds was
183   confirmed by dimethyl disulfide (DMDS)/I_2-derivatization of methylated natural samples
184   according to published procedures (Bruns et al. 2013; Buser et al. 1983).

185

186   **Morphological dataset**

187

188   Morphological data ~~and femoral secretions~~ were collected in a group of territorial males to
189   explore whether the chemical compounds might inform of certain morphological traits relevant
190   for mate choice and/or dominance status. For this part, we focused on a single population (i.e. *A.*
191   *cristatus mertensi* from La Lobería, San Cristóbal Island).
192   During December 2014 and January 2015, a total of 53 male marine iguanas were
193   measured for their snout-ventral length (SVL) using a metric-tape ($\pm$ 1 mm), and for head width
194   (distance across the jaw at the widest point) using a calliper ($\pm$ 0.1 mm). Body mass was
195   recorded using a portable field scale ($\pm$ 10 g). In addition, femoral gland secretions were also
196   taken from all captured iguanas.
197   We estimated the relative head size as the residuals of a linear regression of log-
198   transformed head size versus SVL. The body condition was calculated as the residuals from a
199   linear regression between body mass and SVL (both log-transformed) (Krebs & Singleton 1993;
200   Wikelski & Romero 2003).

201

202   **Behavioural dataset**

203

204   In order to understand the role of chemical cues in marine iguana lek-systems, we performed an
205   observational study at the La Lobería colony in San Cristóbal during the period from 14/12/2015
206   until 06/01/2016.

207   The study consisted of two parts performed in parallel: 1) a count to estimate the rough

208   number of iguanas in the study area ~~was conducted~~; 2) the behaviour of territorial males in

209   selected leks was recorded and their femoral glands were sampled.

210

211   **Marine iguana count**

212

213   A daily count was performed to provide a rough overview of the number of marine iguanas in

214   our study site (in total 12 days). In each count, the observer walked along the coastline, counting

215   all iguanas visible within the range of a ~1.5 km transect. The marine iguanas were classified in

216   three categories on the basis of their external features: territorial males, female-sized individuals

217   and juveniles, following the classification characteristics of (MacLeod et al. 2016). Territorial

218   males have enlarged dorsal crests, large body size and conspicuous breeding coloration. Female-

219   sized individuals lack such features, with their mostly dark and uniform coloration that contrasts

220   with the colourful red and green patches of the males. However, female-mimicking males or

221   sneakers cannot be identified by external features (Wikelski et al. 1996) and therefore we were

222   not able to distinguish sneaker males from females and both were classified as "female-sized"

223   individuals. Juveniles can be easily distinguished by their relatively smaller size (approximately

224   less than 70 cm in total length). Average numbers and standard deviations (SD) are given for

225   each category in the results section. The marine iguana count performed in this study intended to

226   provide a general idea of the number of iguanas in our study area but population size for *A.*

227   *cristatus* in this island has been already estimated from a mark-recapture study (see (MacLeod et

228   al. 2016)).

229

230   **Monitoring of territorial males**

231

232   ~~In parallel to the census,~~ the behaviour of 9 territorial males was monitored in their lek-mating

233   areas. For this, we selected two different sites at the "La Lobería" breeding colony on San

234   Cristóbal (subspecies *A. c. mertensi*) and an additional site on the neighbouring island of Santa

235   Cruz (*A. c. hassi*). Territorial males from each site were photographed at the beginning to allow

236   identification from digital images, and marked with numbers on the flanks using non-permanent

237   white paint to allow individual recognition during the observations. Territorial males show

238   strong site-fidelity and occupy the same territorial areas during the entire reproductive season

239   (Wikelski et al. 1996). We made an overall of 55 observations on focal territorial males, each

240   lasting between 15 and 60 minutes, during which we noted the number of head bobs in each

241   observation period. Observations were made between 8 am and 6 pm local time. The head

242   bobbing rate was then calculated by dividing the total number of head bobs recorded by the total

243   time of the observation in minutes. We also monitored the presence or absence of females in the

244   male territory (females were considered as 'present' when one or more females were within

245   approximately two body lengths of the focal male).

246

247   **Statistical analysis**

248

249   The proportions of the compounds were re-standardized by calculating the relative contribution

250   of each compound (peak area of a focal compound) with respect to the total peak area for all

251   substances. Statistical analyses were performed with the interface Rstudio in R software version

252   3.3.2 (R Development Core Team 2013), as well as with Statistica v8.0 (Statsoft Inc., Tulsa, OK,

253   USA).

254   A permutational multivariate ANOVA (PERMANOVA) was used to test for significant

255   differences in chemical profiles among the different islands. Multiple pairwise comparisons were

256   performed by correcting for multiple testing on the basis of the Benjamini-Hochberg (B-H)

257   method, which controls for false-discovery rate (Benjamini & Hochberg 1995). We used

258   SIMPER (similarity percentage analysis) to assess which compounds contributed most to

259   differences among populations. This analysis allows identifying which compounds contribute

260   more to the observed pattern of similarity.

261   Furthermore, the relationship between morphological and behavioural variables, and the

262   amount of specific lipids was examined in marine iguanas. In order to explore the degree of

263   collinearity in chemical composition data, a Principal Component Analysis (PCA) was

264   performed on the 13 lipophilic compounds, including those from the individuals for which

265   morphological or behavioural data had been gathered. We used the PCA to reduce the

266   dimensionality of the data, and to detect co-linearity among the levels of the various compounds.

267   Therefore, to avoid including co-linearity among independent variables in subsequent model

268   development, only those compounds that correlated best with each PC factor (i.e. variables with

269 the highest loadings) were used, and the remaining compounds were discarded (following
270 (Dormann et al. 2013); see Table S2). Compounds that correlated strongly with each PC factor
271 were selected as independent variables for further modelling, these were: tetracosanoic acid,
272 hexadecanoic acid, 11-eicosenoic acid and 9-octadecenoic acid.

273   In order to test the relationship between morphology and proportion of the volatile
274 compounds in secretions, we built three linear models which considered body size (SVL), body
275 condition, and relative head size as dependent variables, with the proportion of selected
276 compounds as explanatory variables. The probability that the chemical profile of a given male
277 influenced whether or not he was accompanied by females was modelled using a mixed effects
278 logistic regression (binomial family, "glmer" function of package: "lme4"; (Bates et al. 2014)).
279 The identity of the male was considered as a random factor. The best model, including the best
280 subset of independent variables, was selected following the AIC corrected for small sample size
281 with the use of the "glmulti" package (Calcagno 2013). As some iguanas had no 11-eicosenoic
282 acid, we tested whether the presence of this chemical explained their head bobbing behaviour by
283 performing a non-parametric Mann-Whitney U-test.

284

285 **RESULTS**

286

287 **Diversity of lipids in femoral glands of Galápagos iguanas**

288

289 A total of 13 lipids from marine iguanas were identified and quantified (Table 1). We
290 characterized 10 carboxylic acids with chain lengths ranging between C_{16} and C_{24}, as well as 3
291 steroids. The most common compound in the femoral glands was cholesterol (30.73 %) followed
292 by hexadecanoic acid (23 %). C_{20}-unsaturated fatty acids, such as 11-eicosenoic acid (0.34 %)
293 and 13-eicosenoic acid (0.1 %), were the rarest compounds in marine iguana secretions.

294   A total of 20 volatile compounds were found in the femoral glands of land iguanas. Of
295 these compounds, 8 were found in *C. subcristatus* but not in *A. cristatus* (Table 1). The exclusive
296 compounds included saturated and unsaturated carboxylic acids, as well as aldehydes.
297 Interestingly, aldehydes were totally absent in marine iguana femoral glands, and thus were

298 specific to land iguanas. 13-Eicosenoic acid was absent in land iguanas, but present in some

299 populations of marine iguanas (Table 1, 2).

300 Lipophilic profiles varied greatly among island populations of marine iguanas (Table 2,

301 Fig. 2). All populations shared 8 compounds, these were: Hexadecanoic acid, 9-octadecenoic

302 acid, octadecanoic acid, 5, 8, 11, 14-eicosatetraenoic acid, eicosanoic acid, docosanoic acid,

303 tetracosanoic acid and cholesterol. Marine iguanas from some islands (e.g. Isabela, Santa Cruz,

304 San Cristóbal (La Lobería), Floreana and Santiago) had the full range of lipids, thus presenting a

305 high chemical diversity. However, other islands lacked one or more compounds (e.g. Española,

306 Marchena, Pinta, and Genovesa). This case was especially striking in Pinta and Genovesa, in

307 which the diversity of lipids was the lowest of the entire archipelago, with only 8 and 9 lipophilic

308 compounds, respectively. C_{20}-unsaturated fatty acids (i.e. 11- and 13- eicosenoic acids) were

309 most often absent in marine iguana populations (Table 2). Accordingly, the chemical

310 composition was dependent on population origin (PERMANOVA, Pseudo $F_{11,\ 95}$ = 7.78, P =

311 0.001, Fig 2). Pairwise comparisons revealed significant differences between lipids of some

312 populations (Table S3). Surprisingly, secretion chemistry differed between populations

313 inhabiting different islands, but belonging to the same genetic cluster (e.g. Fernandina-Isabela

314 and Española-Floreana, see Table S3). Conversely, some populations belonging to different

315 genetic clusters had similar chemical profiles (e.g. Pinta-Santa Fe and San Cristóbal-Fernandina).

316 In the case of the iguanas from Punta Pitt and La Lobería, which inhabit the same island (San

317 Cristóbal) but are genetically distinct, pairwise comparisons showed that their chemical

318 secretions are rather similar (corrected P = 0.28).

319

Morphological and behavioural data

321

322 The body size (SVL) of focal marine iguanas ranged from 35 cm to 54 cm, and body mass

323 ranged from 3 kg to 7.75 kg. The body condition index ranged from -0.40 (worst condition) to

324 0.26 (best condition). Male iguanas had a minimum head width of 3.6 cm and a maximum of 7.8

325 cm.

326 The average number of marine iguanas observed in the study area per study day was 135.

327 Female-sized were the most abundant group (average $\pm$ SD= 78 $\pm$ 8), followed by territorial

328 males (57 $\pm$ 8) and juveniles (10 $\pm$ 4). The head bobbing rate of the focal males ranged from 0.73

329   to 2.40 head bobs per minute. Some males were never accompanied by females, while others had

330   females in their territories during all the observations (raw behavioural data is shown in Table

331   S4).

332

333   Functional correlates of femoral secretions with behaviour

334

335   The PCA summarizes the variation of the 13 chemical compounds in four PCs, accounting for a

336   total of 75 % of the variation. The first PC (eigenvalue = 3.96, variance = 30.4 %) correlated best

337   with tetracosanoic acid (coefficient loading = 0.89). The second PC (2.39, 17.23 %) correlated

338   most with hexadecanoic acid (0.89). The third PC (1.98, 15.21 %) showed the highest correlation

339   with 11-eicosenoic acid (0.89). Finally, the fourth PC (1.6, 12.3 %) correlated with 9-

340   octadecenoic acid (0.78).

341   The best explanatory model for male body condition included two variables: 11-

342   eicosenoic acid and tetracosanoic acid (adjusted $R^2 = 0.24$, $F_{2,\,46} = 8.41$, $P < 0.001$; 11-eicosenoic

343   acid: estimate = 16.18, t = 3.64, $P < 0.001$; tetracosanoic acid: estimate = 5.31, t = 2.32, $P = 0.02$;

344   Fig. 3). Body size (SVL) and relative head size were both independent of the proportions of the

345   chemicals found in the femoral glands (all $P > 0.10$).

346   The best model (i.e. lowest AICc, see Table 3) that predicted the probability of having

347   females in a male's territory included only one of the chemical compounds, i.e. 11-eicosenoic

348   acid. Males with higher proportions of 11-ecoisenoic acid were more likely to be accompanied

349   by females (estimate $\pm$ SE = 496.6 $\pm$ 166.5, Z = 2.98, $P = 0.003$; Fig. 4).

350   Furthermore, presence of 11-eicosenoic acid in femoral glands also correlated with an

351   increased head bobbing rate. Territorial males with 11-eicosenoic acid showed more head bobs

352   per minute (median = 1.84) than those lacking that compound (median = 1) (See Table S4).

353   However, this difference was only marginally significant (Mann Whitney U test: Z = -1.94, N =

354   7, $P = 0.05$, Fig. 5).

355

356   DISCUSSION

357

358   Although many researchers have focused their attention on the ecology, behaviour and
359   ~~micro~~evolution of the Galápagos iguanas, studies on the chemical communication in these iconic
360   reptiles have so far been neglected. Our study is the first to survey the lipophilic compounds of
361   Galápagos iguanas, and to explore their possible function for intraspecific communication in *A.*
362   *cristatus*. Unlike many studies focusing on the chemistry of femoral gland secretions (reviewed
363   in (Weldon et al. 2008)), we surveyed not one but all major island populations of marine iguanas,
364   providing a complete overview of the intraspecific variation of lipophilic substances in this
365   species. In the following section, we discuss the general patterns of lipophilic diversity between
366   land and marine iguanas, as well across marine iguana populations. Furthermore, we describe the
367   composition of specific lipid compounds and their possible functional correlates for chemical
368   communication in the context of lek-mating.

369

370   **Diversity of femoral lipids in Galápagos iguanas**

371

372   The lipid profiles of marine and land iguanas were significantly different. A total of 13 and 20
373   different lipids were found in marine iguanas and land iguanas, respectively (Table 1). In detail,
374   eight compounds (fatty acids and aldehydes) were found in land iguanas but not in marine
375   iguanas. Most surprisingly, many of these compounds are aldehydes, a group of lipids that is
376   completely absent in marine iguanas, making these substances a characteristic difference
377   between land and marine iguanas. It is likely that land iguanas might have one or more enzymes
378   that catalyze the production of aldehydes. Given that land and marine iguanas diverged around
379   4.5 million years ago on the Galápagos archipelago (MacLeod et al. 2015), enzymes catalyzing
380   aldehyde synthesis might have evolved independently in the land iguana lineage. Alternatively,
381   genes for these enzymes might have been lost in the marine iguana lineage, or are now down-
382   regulated. A transcriptome/genome analysis of both land and marine iguanas, as well as of
383   closely related out-group taxa, such as members of the genus *Ctenosaura* from Central America,
384   could shed light on these alternative explanations. Interestingly, aldehydes are present in the
385   lizard *Psammodromus algirus* (Martín & López 2006), but not in a phylogenetically close
386   species, *P. hispanicus* (López & Martín 2009). The presence of aldehydes in *P. algirus* could
387   have a functional environmental constraint, as this species inhabits grassy areas where substrate
388   scent marking is difficult, and therefore the presence of highly volatile substances like aldehydes

389 would be more suitable for short-range communication (Martín & López 2014). In the case of

390 Galápagos iguanas, the ecological differences between marine and land iguanas are ~~striking~~.

391 Marine iguanas live in humid coastal areas, and some adults dive to feed on algae (Trillmich &

392 Trillmich 1986). Conversely, land iguanas, are strictly terrestrial and are associated with a dry

393 and hot environment for most of the year, feeding mainly on *Opuntia* cactus and other vegetation

394 that provides a water source in the arid lowlands where they live (Traveset et al. 2016).

395 Ecological factors might also be possible drivers for the observed divergence in chemical signals

396 between both species. For example, in marine iguanas, marking rocky substrates could be

397 essential for establishing territories by dominant males. For this purpose, aldehydes may be

398 poorly suited since they volatilize too rapidly in the high humidity conditions, while carboxylic

399 acids and steroids would persist longer. A recent study on chemical signal functionality of

400 terrestrial vertebrates (amniotes) found that aldehydes are emitted from the sender's body rather

401 than from scent marks, likely due to their susceptibility to oxidation and degradation that would

402 limit their persistence in scent marks (Apps et al. 2015). Interestingly, aldehydes occurring in

403 body odours are much shorter than those found in land iguanas, confirming that aldehydes are

404 part of femoral gland secretions. Further research should clarify their potential role in

405 intraspecific communication.

406   In general terms, the femoral lipids present in *A. cristatus* are similar to those found in

407 other iguanids (Alberts et al. 1992), and are composed of ten carboxylic acids and three sterols.

408 The carboxylic acid chain lengths ranged between C_{16} and C_{24}. The most abundant compound

409 was cholesterol. This sterol is also one of the most common compounds found in the femoral

410 glands of many other lizard species (Martín & Lopez 2014). Cholesterol is thought to play an

411 important role in stabilizing other molecules, such as fatty acids found in gland secretions

412 (Escobar et al. 2003; Weldon et al. 2008). This would be especially important in hot habitats,

413 such as those occupied by the marine iguanas, where high temperatures may contribute to the

414 rapid degradation of chemical signals; therefore cholesterol could act as ~~a pheromone~~ protector.

415 However, the chemical composition of the femoral glands varies greatly among populations of

416 marine iguanas across the archipelago. For example, cholesterol accounted for almost 40 % of

417 the gland secretions in marine iguanas from Isabela and Santa Cruz, but was less than 20 % of

418 secretions from Pinta and Española iguanas. Other compounds, such as 11- and 13-eicosenoic

419 acids, were absent in some of the northern islands (Marchena, Pinta, Genovesa), as well as in

420 Santa Fe and northern San Cristóbal, but were present in low proportions in the rest of the
421 islands. Similarly, other studies on Mediterranean lizards showed high variation among island
422 populations within the same species. For instance, populations of the Balearic lizard *Podarcis*
423 *lilfordi* from different islets showed strong differences in the lipid composition of their femoral
424 secretions (Martín et al. 2013). Further, a study comparing gland chemistry of different
425 populations of the Skyros wall lizard (*Podarcis gaigeae*), found that islet populations diverged
426 considerably more than mainland populations (Runemark et al. 2011). However, contrary to the
427 latter study, which suggested that chemical profile variation reflects genetic differentiation
428 between populations, the observed variation in marine iguana lipid profiles does not correlate
429 with the underlying genetic population structure and ongoing differentiation/speciation
430 processes. Based on analysis of microsatellite loci, most islands of the archipelago harbour a
431 distinct genetic cluster of marine iguanas, with some exceptions where islands share a cluster
432 (MacLeod et al. 2015). This is the case for iguanas from Española and Floreana, as well as for
433 the populations from Isabela and Fernandina. In our study, the chemical signatures of the iguanas
434 from these islands differed statistically. Moreover, a remarkable peculiarity of the genetic
435 structure of marine iguanas is found in the island of San Cristóbal, where two genetically distinct
436 subspecies are in an ongoing speciation process within the same island (MacLeod et al. 2015;
437 Miralles et al. 2017). One of the most important mechanisms driving speciation though chemical
438 signals is a mutation in a receptor that switches the specificity for a given pheromone compound
439 (Leary et al. 2012). This process promotes the development of divergent ~~pheromones~~, thereby
440 establishing pre-mating barriers and precluding gene flow between populations. However, we
441 found no evidence that the ongoing speciation process in the marine iguanas of San Cristóbal is
442 reflected in the variation of lipophilic compounds. The femoral secretions of the two main
443 colonies of the distinct genetic clusters, i.e. La Lobería and Punta Pitt, have similar chemical
444 profiles. We therefore conclude that the profiles of lipophilic substances do not correlate with the
445 genetic diversity of populations and therefore, lipophilic compounds might play only a minor
446 role in prezygotic isolation between marine iguana populations.
447
448 **Behavioural and functional correlates of lipophilic ~~substances~~**
449

450   We found that marine iguanas with better body condition had higher amounts of 11-eicosenoic
451   acid and tetracosanoic acid (see Fig. 3). Saturated and unsaturated fatty acids, such as 11-
452   eicosenoic acid and tetracosanoic acid, are part of the energetic dietary lipids and fat stores of
453   lizards (Geiser & Learmonth 1994; Simandle et al. 2001). Therefore, femoral secretions rich in
454   11-eicosenoic acid and tetracosanoic acid might reflect male fat reserves, suggesting that only
455   marine iguanas in an optimal body condition can afford to allocate these lipids to their femoral
456   glands. Further support for this hypothesis comes from the fact that the presence of females in
457   leks correlates with levels of 11-eicosenoic acid in the territorial male's secretions. Male iguanas
458   that had 11-eicosenoic acid in their secretions had a higher head bobbing rate when compared to
459   those that lacked this compound, though the effect was only marginal. Female marine iguanas
460   choose territorial males for mating on the basis of their head bobbing behaviour - the greater the
461   investment in the display, the higher the reproductive success of the males (Vitousek et al. 2008).
462   Thus, dominant males may also enhance their mating probability by releasing high amounts of
463   11-eicosenoic acid within their territories. Evidence to support this idea is provided by other
464   reptile studies which show that unsaturated fatty acids may function as sexual attractors. For
465   instance, male rock lizards *Iberolacerta cyreni* produce scent marks with more oleic acid to
466   attract females and thereby increase their mating opportunities (Martín & Lopez 2010). A similar
467   mechanism is likely to occur in the lek-mating system of marine iguanas, where scent marking
468   would be essential in order to establish male territories (Partecke et al. 2002; Wikelski et al.
469   1996). Therefore, the results of this study suggest the presence of an underlying mechanism,
470   whereby scent marks are produced by males to increase their mating opportunities and therefore
471   their reproductive success. In order to attract females to their own territories, males would
472   provide reliable information on their condition by the allocation of more 11-eicosenoic acid in
473   their femoral gland secretions. Further experimental research is needed to understand how
474   female marine iguanas use the information contained in male gland secretions. An experiment
475   testing female response to different concentrations of 11-eicosenoic acids could elucidate
476   whether such compound is a chemosignal involved in mate choice.

477

478   **CONCLUSIONS**

479

480   Our study is the first comprehensive analysis of lipophilic compounds of femoral glands in male
481   Galapagos iguanas. Evolutionary divergence between land and marine iguanas is reflected by a
482   remarkable divergence of lipophilic substances that might have altered in response to
483   environmental constraints and the different life histories of both species. Differences in
484   composition of lipophilic substances seem not to be of central importance for the maintenance of
485   genetic population structure, or for the ongoing differentiation and speciation processes in
486   marine iguanas. However, males might use certain compounds, such as fatty acids like 11-
487   eicosenoic and tetracosanoic acids to signal a good body condition and to successfully attract
488   females for lek-mating. The composition and type of lipophilic substances in the femoral
489   secretions of marine iguanas seem to be more influenced by habitat conditions than by genetic
490   differentiation.

491

492   **ACKNOWLEDGEMENTS**

498

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636 **Table 1**

637 Relative amounts (Mean $\pm$ SD) of major compounds present in marine and land iguana

638 secretions, with the retention indices (RI) of methyl esters of the fatty acids and cholesterol

639 derivatives. Retention indices have been calculated using a method developed by Van den Dool

640 and Kratz (1963). Compounds specific for land iguanas have been given in bold (aldehydes are

641 marked with an*).

642

643

Lipids (Mean $\pm$ SD)			
Marine iguana	Land iguana	Compound name	RI
-	2.59 $\pm$ 1.65	**Hexadecanal***	**1813**
-	0.55 $\pm$ 0.74	**7-Hexadecenoic acid**	**1904**
-	1.48 $\pm$ 1.08	**9-Hexadecenoic acid**	**1908**
23.02 $\pm$ 12.47	19.99 $\pm$ 12.19	Hexadecanoic acid	1928
-	0.48 $\pm$ 0.83	**9-Octadecenal***	1993
-	0.27 $\pm$ 0.47	**11-Octadecenal***	1997
-	0.59 $\pm$ 0.67	**Octadecanal***	2016
-	1.55 $\pm$ 0.37	**Di-(9-Octadecenoyl)-glycerol**	2473
13.59 $\pm$ 4.96	20.40 $\pm$ 12.34	9-Octadecenoic acid	2099
1.4 $\pm$ 1.25	4.72 $\pm$ 2.80	11-Octadecenoic acid	2104
12.22 $\pm$ 7.35	8.37 $\pm$ 4.21	Octadecanoic acid	2125
4.75 $\pm$ 2.86	1.92 $\pm$ 1.06	5,8,11,14-Eicosatetraenoic acid	2258
0.34 $\pm$ 0.99	0.26 $\pm$ 0.15	11-Eicosenoic acid	2299
0.1 $\pm$ 0.22	-	13-Eicosenoic acid	2306
3.54 $\pm$ 2.83	0.55 $\pm$ 0.31	Eicosanoic acid	2326
2.39 $\pm$ 1.78	0.47 $\pm$ 0.16	Docosanoic acid	2501
2.11 $\pm$ 1.68	0.55 $\pm$ 0.37	Tetracosanoic acid	2536
30.73 $\pm$ 13.39	29.67 $\pm$ 31.81	Cholesterol	3133
4.64 $\pm$ 4.46	0.11 $\pm$ 0.19	Cholestanol	3143
1.11 $\pm$ 1.39	1.32 $\pm$ 0.75	Cholestanone	3187
-	4.11 $\pm$ 5.3	**Hexadecyl hexadecanoate***	**3361**

644 **Table 2**

645 Percentage (Mean ± SD) of the lipophilic compounds indentified and quantified in the distinct

646 populations of marine iguanas (*A. cristatus*). Most influential compounds contributing to

647 dissimilarity among populations are marked in bold. *Abbreviations for the populations:*

648 *IS=Isabela, MAR=Marchena, PIN=Pinta, GEN=Genovesa, SAN=Santiago, ESP=Española,*

649 *FL=Floreana, SFE=Santa Fe, FDA=Fernandina, CRUZ=Santa Cruz, SRL= San Cristóbal (La*

650 *Lobería), SRPC= San Cristóbal (Punta Pitt).*

651

	IS	MAR	PIN	GEN	SAN	ESP	FL	SFE	FDA	CRUZ	SRL	SRPC
Hexadecanoic acid	13.17 ± 2.81	29.17 ± 15.15	39.04 ± 11.77	32.75 ± 16.65	29.68 ± 7.82	38.77 ± 9.79	11.51 ± 2.41	26.05 ± 9.93	18.91 ± 3.15	14.92 ± 3.06	15.84 ± 3.15	21.46 ± 12.49
9-Octadecenoic acid	14.61 ± 2.53	12.48 ± 6.61	9.89 ± 7.97	11.25 ± 7.05	13.08 ± 4.54	17.53 ± 4.50	12.07 ± 1.75	15.83 ± 2.97	13.68 ± 2.27	15.16 ± 2.22	11.05 ± 6.42	15.16 ± 5.90
11-Octadecenoic acid	**2.60 ± 0.65**	**0.61 ± 0.95**	**0.00 ± 0**	**0.00 ± 0**	**1.98 ± 1.89**	**0.32 ± 0.90**	**1.94 ± 0.68**	**0.64 ± 1.03**	**2.03 ± 0.79**	**2.05 ± 0.38**	**2.59 + 0.96**	**0.87 ± 0.94**
Octadecanoic acid	7.98 ± 1.42	17.42 ± 13.11	23.02 ± 12.47	13.86 ± 9.2	14.28 ± 4.04	19.80 ± 5.85	6.77 ± 1.08	11.24 ± 3.37	10.78 ± 1.88	8.32 ± 1.48	8.90 + 1.06	12.75 ± 8.91
5,8,11,14-Eicosatetraenoic acid	5.76 ± 1.01	4.49 ± 3.73	2.75 ± 4.29	2.22 ± 3.5	3.53 ± 2.52	4.47 ± 4.92	4.94 ± 0.81	2.95 ± 3.15	6.28 ± 1.02	6.04 ± 0.88	6.26 + 1.21	5.23 ± 2.95
11-Eicosenoic acid	**1.00 ± 0.24**	**0.00 ± 0**	**0.00 ± 0**	**0.00 ± 0**	**0.12 ± 0.31**	**1.20 ± 3.39**	**0.39 ± 0.35**	**0.00 ± 0**	**0.08 ± 0.28**	**0.62 ± 0.47**	**0.51 + 0.38**	**0.00 ± 0**
13-Eicosenoic acid	**0.59 ± 0.24**	**0.00 ± 0**	**0.00 ± 0**	**0.00 ± 0**	**0.08 ± 0.22**	**0.00 ± 0**	**0.19 ± 0.27**	**0.00 ± 0**	**0.00 ± 0**	**0.19 ± 0.24**	**0.04 + 0.13**	**0.00 ± 0**
Eicosanoic acid	2.75 ± 0.57	3.68 ± 3.09	4.50 ± 6.01	1.94 ± 3.19	6.56 ± 2.09	6.23 ± 5.06	2.80 ± 1.52	1.22 ± 1.97	4.12 ± 1.05	3.11 ± 0.5	3.57 + 0.82	3.30 ± 2.11
Docosanoic acid	1.94 ± 0.34	2.39 ± 2.75	1.79 ± 2.79	0.87 ± 1.84	3.91 ± 2.12	1.94 ± 2.90	3.12 ± 0.58	1.66 ± 2.16	2.82 ± 0.80	2.29 ± 0.33	2.71 + 0.68	2.87 ± 1.87
Tetracosanoic acid	**1.81 ± .28**	**1.98 ± 2.27**	**0.73 ± 1.79**	**0.86 ± 1.79**	**4.02 ± 2.01**	**1.49 ± 2.80**	**2.76 ± 0.48**	**1.15 ± 1.85**	**2.57 ± 0.68**	**2.14 ± 0.33**	**2.91 + 0.88**	**2.35 ± 1.79**
Cholesterol	38.50 ± 5.68	23.54 ± 13.93	18.28 ± 15.48	34.56 ± 15.32	18.03 ± 6.01	8.23 ± 9.22	40.41 ± 5.12	33.35 ± 6.94	33.18 ± 6.08	39.56 ± 6.99	37.65 ± 8.17	29.98 ± 16.25
Cholestanol	7.85 ± 1.93	3.86 ± 6.30	0.00 ± 0	1.67 ± 3.7	4.56 ± 6.48	0.00 ± 0	10.53 ± 1.96	5.59 ± 5.95	4.32 ± 3.76	4.01 ± 2.20	5.45 ± 2.60	4.06 ± 2.56
Cholestanone	**1.41 ± 0.58**	**0.36 ± 0.89**	**0.00 ± 0**	**0.00 ± 0**	**0.15 ± 0.39**	**0.00 ± 0**	**2.54 ± 1.02**	**0.31 ± 0.97**	**1.21 ± 1.06**	**1.56 ± 0.74**	**2.51 ± 1.31**	**1.96 ± 2.41**

652

653

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655

656   **Table 3**

657   Top five ranked models that examined the probability that presence of females in a specific

658   male's territory depends on the chemical composition of its femoral secretions. AICc=AIC

659   corrected for small sample sizes; ER=Evidence Ratio. The best model is shown in bold.

660

661

Probability of having females in male's own territory				
Variables included in the model	*AICc*	*Delta*	*Weight*	*ER*
11-Eicosenoic acid	**36.54**	**0**	**0.36**	**1.00**
11-Eicosenoic acid + Tetracosanoic acid	37.82	1.28	0.19	1.90
11-Eicosenoic acid + 9-Octadecenoic acid	38.36	0.54	0.14	1.31
11-Eicosenoic acid + Hexadecanoic acid	38.94	0.57	0.11	1.33
11-Eicosenoic acid + Tetracosanoic acid + Hexadecanoic acid	39.93	1	0.06	1.64

663 Figure caption:

664

665 **Fig. 1**

666 Map of the Galápagos Archipelago, showing the total number of femoral gland samples collected

667 per island, and the number of samples considered for statistical analysis in brackets. Island

668 abbreviations: GEN, Genovesa; MAR, Marchena; PIN, Pinta; SAN, Santiago; CRUZ, Santa

669 Cruz; SRL, San Cristóbal (La Lobería); SRPC, San Cristóbal (Punta Pitt); SFE, Santa Fe; ESP,

670 Española; FL, Floreana; IS, Isabela; FDA, Fernandina.

671

672 **Fig. 2**

673 a) Amount (percentage) of each chemical compound for all the sampled populations of marine

674 iguanas. Each colour represents one chemical compound.

675 b) Chemical composition plot, showing two-dimensional non-metric Multidimensional Scaling

676 (nMDS), which is based on the Bray-Curtis Similarity indices as calculated with *metaMDS* (in R;

677 using the *vegan* package). The closer the data point, the more similar the compound. Ellipses

678 represent CI=95%.

679

680 **Fig. 3**

681 Plot showing the relation between male body condition and the amount of a) 11-eicosenoic acid

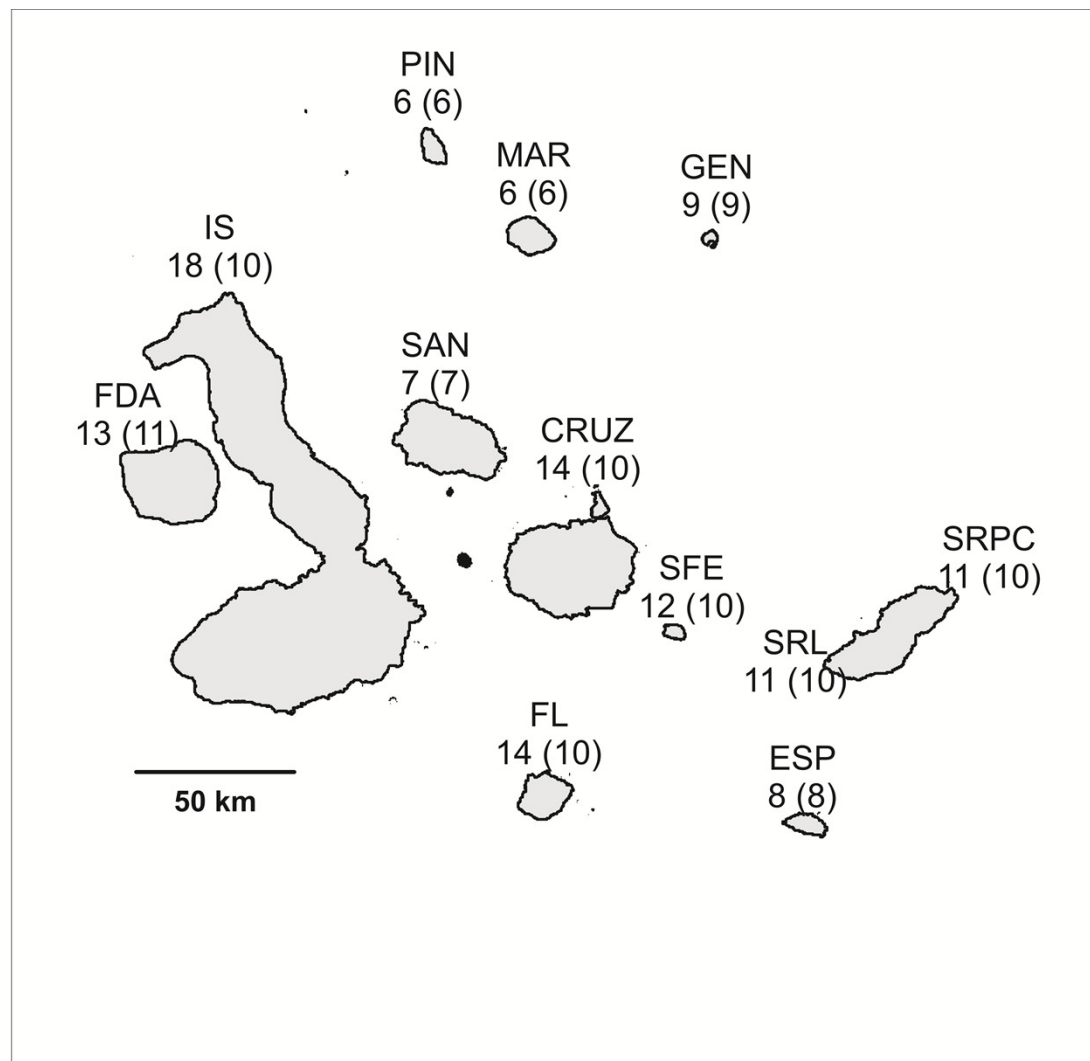682 and b) tetracosanoic acid. In grey 95 % CI.

683

684 **Fig. 4**

685 Plot showing the probability of the presence of females in male territories compared to the

686 amount of 11-eicosenoic acid. In grey 95 % CI.

687

688 **Fig. 5**

689 Box plot showing the head bobbing rate (Median $\pm$ Q1 and Q3) as a function of the presence or

690 absence of 11-eicosenoic acid.

692 **Fig. 1**

PIN
6 (6)

MAR
6 (6)

GEN
9 (9)

IS
18 (10)

SAN
7 (7)

FDA
13 (11)

CRUZ
14 (10)

SRPC
11 (10)

SFE
12 (10)

SRL
11 (10)

FL
14 (10)

ESP
8 (8)

50 km

693

695 **Fig. 2**

696 a)

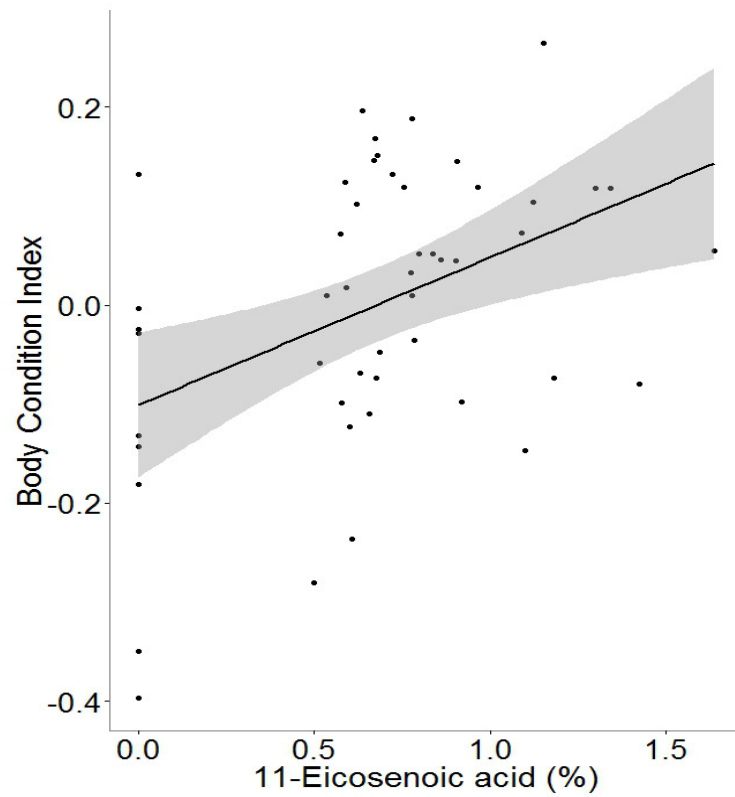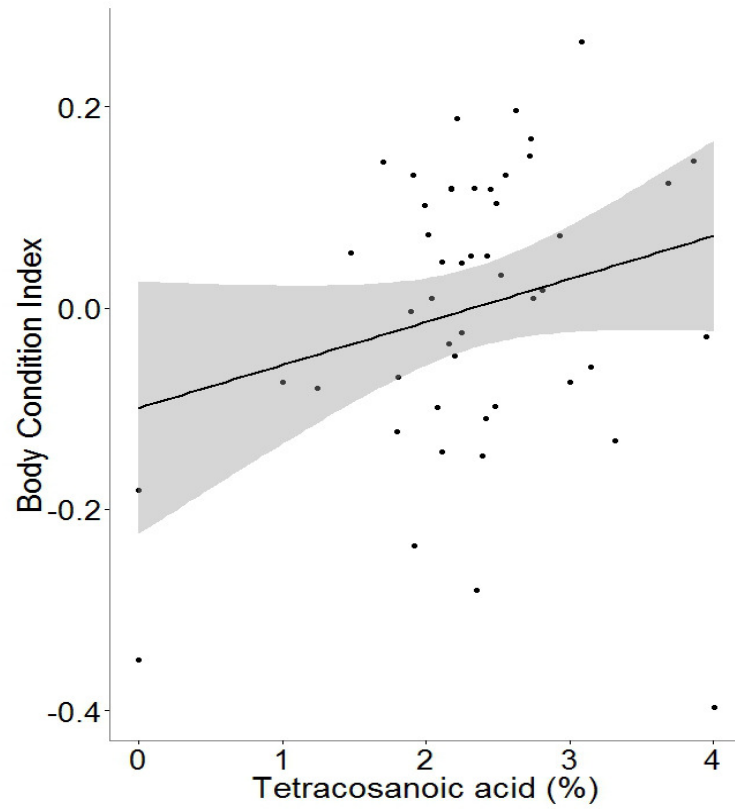

697
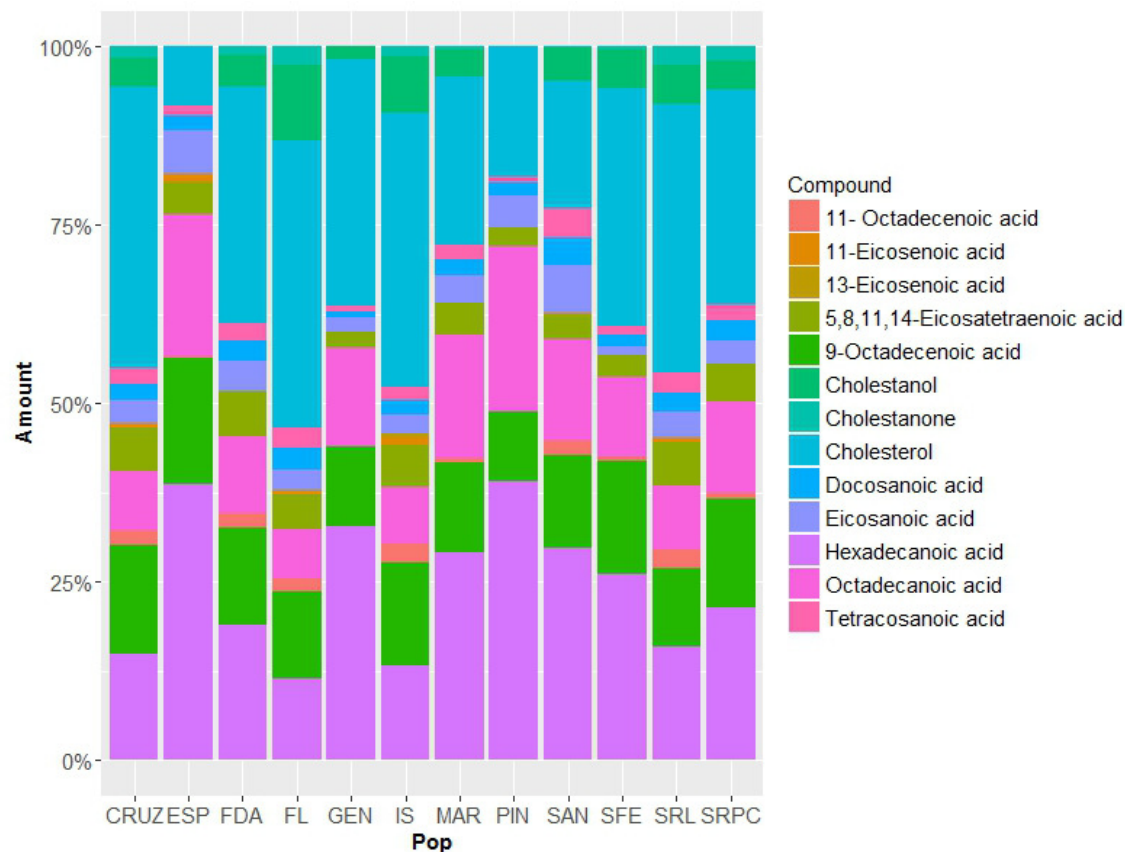
698 b)
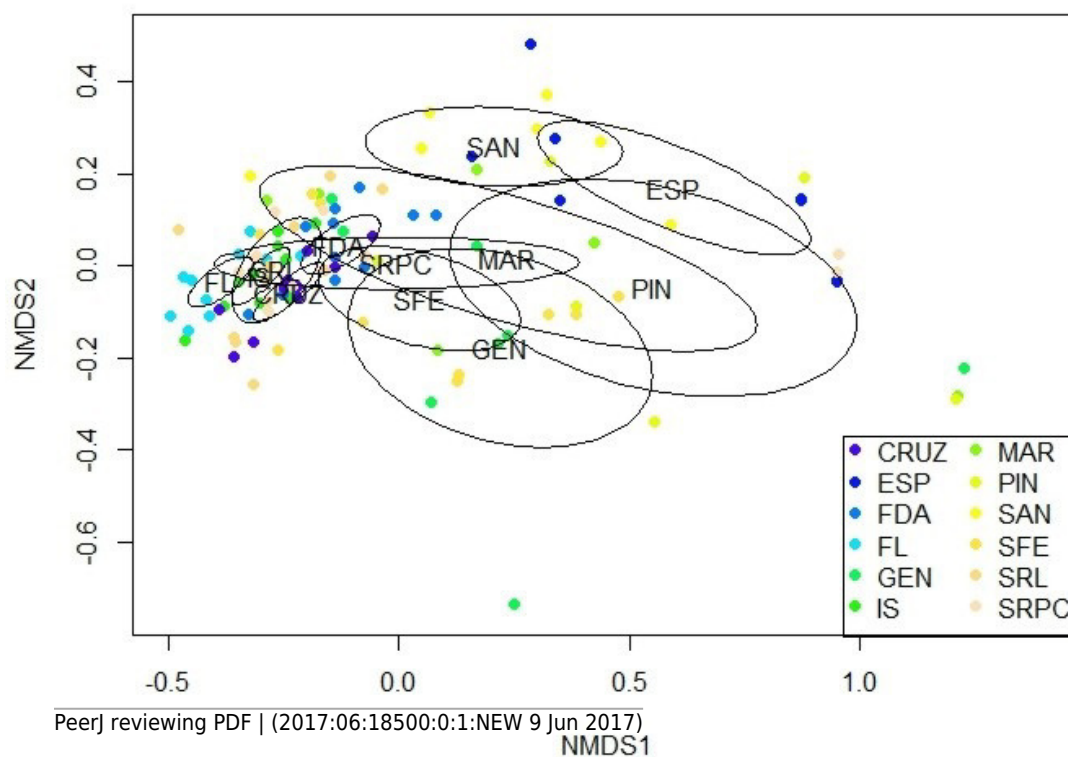
700 **Fig. 3**

701 a)

702

703 **b)**

704

705

706   **Fig. 4**
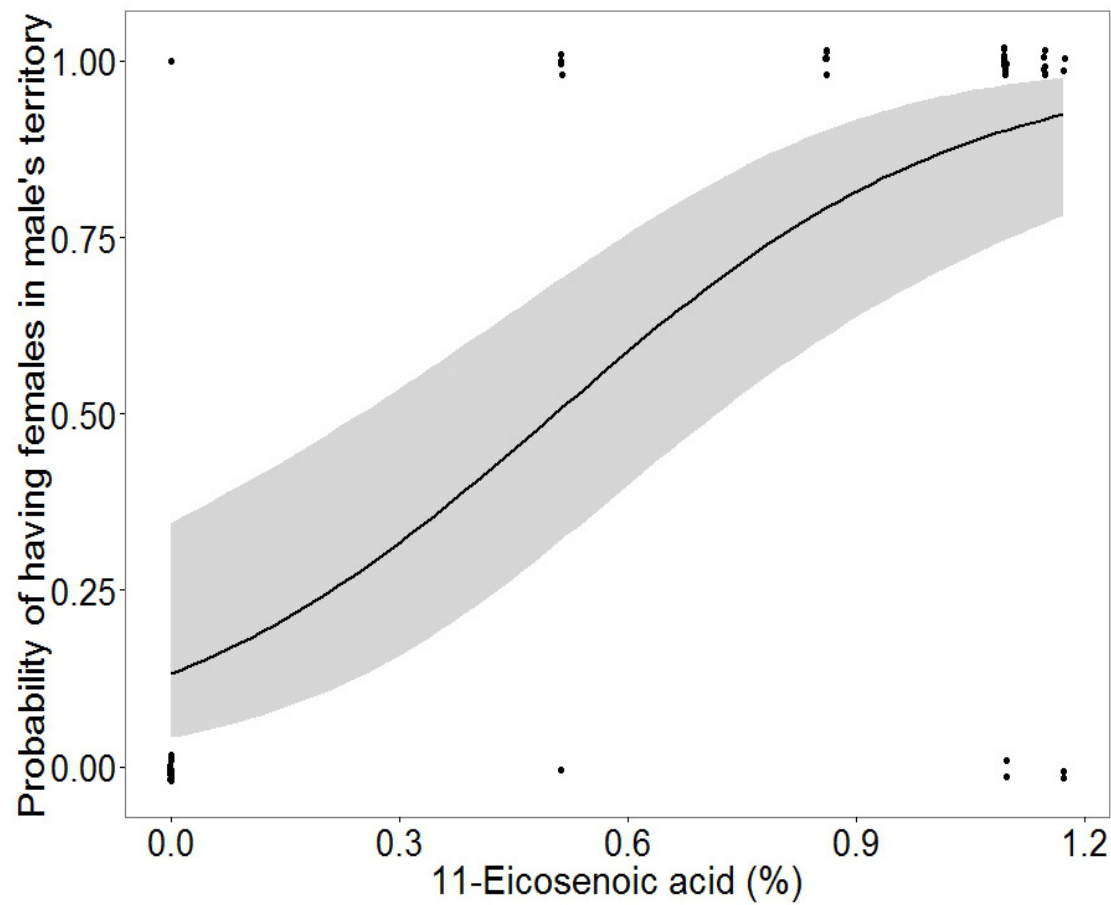
707
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709   **Fig. 5**

710
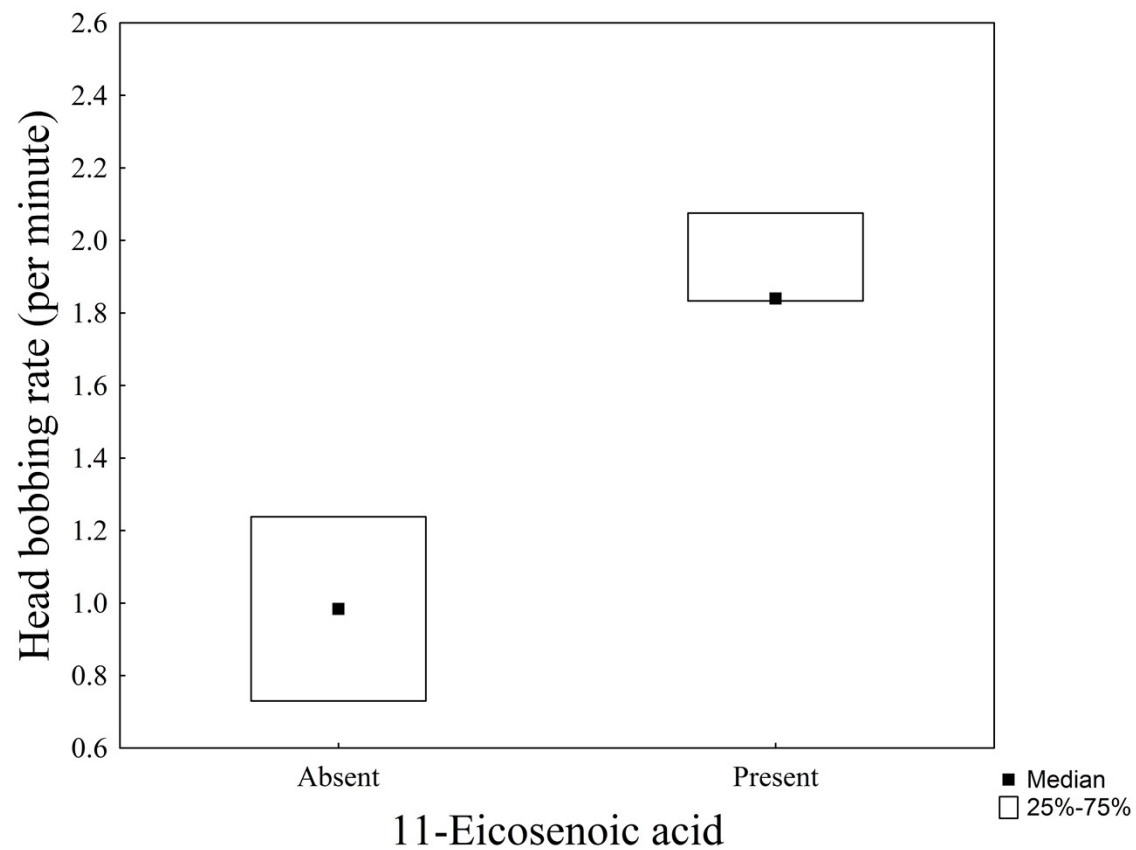
711

713 SUPPLEMENTARY MATERIAL

714

715 **Table S1**

716 Sampled populations of *A. cristatus* and their coordinates. "La Lobería" (SRL) and "Punta Pitt"

717 (SRPC) are considered different populations in recognition of their genetic divergence

718 (MacLeod et al. 2015).

719

Island	Coordinates	Abbreviation
Genovesa	N 0.31065, W 89.97349	GEN
Marchena	N 00.30051, W 090.50774	MAR
Pinta	N 00. 54340, W 090.73948	PIN
Santiago	S 00.24215, W 090.86495	SAN
Santa Cruz	S 00. 74180, W 090. 30732	CRUZ
San Cristóbal (La Lobería)	S 00.92214, W 089.62125	SRL
San Cristóbal (Punta Pitt)	S 00.71432, W 089.24174	SRPC
Española	S 01.39502, W 089.62029	ESP
Floreana	S 01.31968, W 090.50911	FL
Santa Fe	S 00.82581, W 090.02861	SFE
Fernandina	S 00.44264 W 091.38947	FDA

| Isabela | S 00.78524 | W 091.42460 | IS |

720

722

723 **Table S2**

724 Results of the principal component analysis (PCA), showing the factor loadings of each principal

725 component (PC) for the lipophilic compounds. PCAs were run prior to the development of the

726 models to explore lipid functionality in intraspecific communication of marine iguanas, in order

727 to investigate the multicollinearity among independent variables. Values in bold show the higher

728 values of coefficient loadings that indicate those chemical compounds that were selected as

729 independent variables for subsequent model development.

730

	PC1	PC2	PC3	PC4
Hexadecanoic acid	0.23	**0.90**	0.11	0.04
9-Octadecenoic acid	0.25	0.31	0.12	**-0.79**
11-Octadecenoic acid	0.27	-0.01	0.50	0.53
Octadecanoic acid	0.23	0.80	-0.31	0.14
5,8,11,14-Eicosatetraenoic acid	0.25	0.47	-0.21	-0.37
11-Eicosenoic acid	0.07	-0.04	**0.89**	-0.05
13-Eicosenoic acid	0.04	0.17	0.88	0.05
Eicosanoic acid	0.23	0.39	0.06	0.76
Docosanoic acid	-0.87	-0.04	-0.07	0.02
Tetracosanoic acid	**-0.89**	-0.01	-0.01	0.02
Cholesterol	0.17	-0.88	-0.28	0.15
Cholestanol	-0.78	-0.29	0.08	-0.14
Cholestanone	-0.73	-0.14	-0.33	0.04

731

733 **Table S3**

734 PERMANOVA pairwise comparisons of lipid profiles across populations (n=107). Significant

735 differences are highlighted in bold.

736

Island pairwise comparison	F Model	R^2	P value	P adjusted
IS Vs MAR	7.74	0.36	0.00	**0.01**
IS Vs PIN	19.54	0.58	0.00	**0.00**
IS Vs GEN	7.31	0.30	0.00	**0.00**
IS Vs SAN	28.60	0.66	0.00	**0.00**
IS Vs ESP	47.95	0.75	0.00	**0.00**
IS Vs FL	2.71	0.13	0.08	0.12
IS Vs SFE	8.11	0.31	0.00	**0.01**
IS Vs FDA	7.10	0.27	0.00	**0.01**
IS vs CRUZ	1.72	0.09	0.17	0.21
IS Vs SRL	1.70	0.09	0.16	0.19
IS Vs SRPC	2.98	0.14	0.09	0.12
MAR Vs PIN	0.80	0.07	0.40	0.43
MAR Vs GEN	0.89	0.06	0.43	0.45
MAR Vs SAN	0.44	0.04	0.76	0.76
MAR Vs ESP	2.71	0.18	0.08	0.12
MAR Vs FL	10.04	0.42	0.00	**0.01**
MAR vs SFE	1.57	0.10	0.19	0.22
MAR Vs FDA	3.37	0.18	0.03	0.05
MAR Vs CRUZ	7.00	0.33	0.00	**0.00**
MAR vs SRL	5.13	0.27	0.00	**0.01**
MAR vs SRPC	0.81	0.05	0.36	0.39
PIN vs GEN	2.23	0.15	0.11	0.15
PIN vs SAN	1.73	0.14	0.13	0.17
PIN vs ESP	1.33	0.10	0.26	0.29
PIN vs FL	23.27	0.62	0.00	**0.00**

PIN vs SFE	6.00	0.30	0.01	**0.02**
PIN vs FDA	11.93	0.44	0.00	**0.00**
PIN vs CRUZ	17.70	0.56	0.00	**0.00**
PIN vs SRL	13.70	0.49	0.00	**0.01**
PIN vs SRPC	3.81	0.21	0.05	0.08
GEN vs SAN	2.98	0.18	0.04	0.07
GEN vs ESP	7.24	0.33	0.00	**0.01**
GEN vs FL	9.35	0.35	0.00	**0.00**
GEN vs SFE	1.03	0.06	0.41	0.44
GEN vs FDA	3.79	0.17	0.00	**0.01**
GEN vs CRUZ	5.94	0.26	0.00	**0.00**
GEN vs SRL	4.78	0.22	0.00	**0.00**
GEN vs SRPC	1.51	0.08	0.19	0.23
SAN vs ESP	3.79	0.23	0.05	0.07
SAN vs FL	36.44	0.71	0.00	**0.00**
SAN vs SFE	5.90	0.28	0.01	**0.02**
SAN vs FDA	13.21	0.45	0.00	**0.00**
SAN vs CRUZ	25.86	0.63	0.00	**0.00**
SAN vs SRL	16.43	0.52	0.00	**0.00**
SAN vs SRPC	2.34	0.14	0.12	0.16
ESP vs FL	57.68	0.78	0.00	**0.00**
ESP vs SFE	15.60	0.49	0.00	**0.00**
ESP vs FDA	30.65	0.64	0.00	**0.00**
ESP vs CRUZ	43.97	0.73	0.00	**0.00**
ESP vs SRL	33.73	0.68	0.00	**0.00**
ESP vs SRPC	8.38	0.34	0.02	**0.03**
FL vs SFE	12.42	0.41	0.00	**0.00**
FL vs FDA	14.58	0.43	0.00	**0.01**
FL vs CRUZ	6.33	0.26	0.01	**0.01**
FL vs SRL	3.42	0.16	0.03	0.05

FL vs SRPC	4.84	0.21	0.01	**0.01**
SFE vs FDA	2.96	0.13	0.05	0.08
SFE vs CRUZ	6.59	0.27	0.00	**0.01**
SFE vs SRL	5.03	0.22	0.01	**0.02**
SFE vs SRPC	0.66	0.04	0.47	0.49
FDA vs CRUZ	4.98	0.21	0.02	**0.04**
FDA vs SRL	2.24	0.11	0.09	0.12
FDA vs SRPC	0.45	0.02	0.50	0.51
CRUZ vs SRL	1.32	0.07	0.24	0.28
CRUZ vs SRPC	2.48	0.12	0.13	0.17
SRL vs SRPC	1.84	0.09	0.25	0.28

737

738

739   **Table S4**

740   Descriptive statistics showing male behavioural data and chemical profile. Average head

741   bobbing rate (HB) was calculated per minute (see METHODS AND MATERIALS for details).

742   The "Females" column represents the number of times a male was accompanied by females

743   divided by the total number of observations. Chemical composition (%) shows the most

744   influential lipophilic components: HA = Hexadecanoic acid; TA = Tetracosanoic acid; 9OA = 9-

745   Octadecenoic acid, 11EA = 11-Eicosenoic acid. ID: Male identity.

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	Behavioral variables		Chemical composition (%)			
ID	HB rate	Females	HA	TA	11EA	9OA
1	1.24	0/7	19.95	0	0	14.45
3	2.34	9/9	13.06	0.77	1.09	19.95
10	2.07	5/6	11.69	2.47	0.51	12.23
12	1.6	2/4	13.03	1.16	1.17	23.70
13	0.73	0/5	13.43	2.05	0	13.89
392	NA	1/6	14.44	1.93	0	15.47
393	1.8	7/7	10.45	2.57	1.15	13.07
396	NA	5/5	16.57	2.41	0.86	16.74
397	2.4	4/6	11.19	2.48	1.09	13.22