

*Cryptic diversity in a fig wasp community-  
morphologically differentiated species are  
sympatric but cryptic species are  
parapatric*

Article

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1 **Article title:** Cryptic diversity in a fig wasp community – functionally differentiated species are  
2 sympatric but cryptic species are allopatric

3

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11

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14

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18

19 **Running title:** Cryptic diversity predicts species coexistence

20

21 **Abstract**

22 A key debate in ecology centres on the relative importance of niche and neutral processes in  
23 determining patterns of community assembly with particular focus on whether ecologically  
24 similar species with similar functional traits are able to coexist. Meanwhile, molecular studies are  
25 increasingly revealing morphologically indistinguishable cryptic species with presumably similar  
26 ecological roles. Determining the geographic distribution of such cryptic species provides  
27 opportunities to contrast predictions of niche versus neutral models. Discovery of sympatric  
28 cryptic species increases alpha diversity and supports neutral models, while documentation of  
29 allopatric/parapatric cryptic species increases beta diversity and supports niche models. We tested  
30 these predictions using morphological and molecular data, coupled with environmental niche  
31 modelling analyses, of a fig wasp community along its 2700 km latitudinal range. Molecular  
32 methods increased previous species diversity estimates from eight to eleven species, revealing  
33 morphologically cryptic species in each of the four wasp genera studied. Congeneric species pairs  
34 that were differentiated by a key morphological functional trait (ovipositor length) coexisted  
35 sympatrically over large areas. In contrast, morphologically similar species, with similar  
36 ovipositor lengths, typically showed parapatric ranges with very little overlap. Despite parapatric  
37 ranges, environmental niche models of cryptic congeneric pairs indicate large regions of potential  
38 sympatry, suggesting that competitive processes are important in determining the distributions of  
39 ecologically similar species. Niche processes appear to structure this insect community and  
40 cryptic diversity may typically contribute mostly to beta rather than alpha diversity.

41

## 42 **Introduction**

43 Ecologists have long sought to explain patterns of biodiversity and community assembly. A  
44 prominent recent debate has centred on the relative importance of ‘neutral’ (Hubbell 2001) versus  
45 ‘niche’ (e.g. Chesson 2000, Chase and Leibold 2003) processes that influence both species  
46 diversification and coexistence (e.g. Matthews and Whittaker 2014). In neutral models, observed  
47 patterns of species abundance and coexistence are stochastic outcomes unlinked to ecological  
48 functional traits. In contrast, diversity under niche models results from competitive and adaptive  
49 processes that lead to more stable patterns of community assembly. Importantly, niche models  
50 predict that localised coexistence of competing species should be limited by their ecological  
51 similarity (MacArthur and Levins 1967), while neutral models do not, providing us with testable  
52 alternative hypotheses.

53 Over the last 10-15 years, molecular investigation of biodiversity has increased rapidly  
54 and revealed extensive cryptic species diversity (Kress et al. 2015). Cryptic species exist when  
55 several reproductively isolated groups are genetically distinguishable within a single formally  
56 described species, or undescribed but recognisable “morphospecies”. Such cryptic species are  
57 likely to be ecologically very similar, due to lack of divergence in morphological functional traits  
58 and close relatedness. Consequently, cryptic species should coexist often in sympatry if neutral  
59 community assembly predominates, but rarely if niche processes are more important. This  
60 provides an excellent framework in which to test neutral versus niche processes. To date, we  
61 know little about how cryptic diversity is structured locally and distributed geographically.

62 However, two recent studies do support the idea that cryptic or near-identical species may  
63 competitively exclude each other. For example, Voda et al. (2015) found that pairs of cryptic

64 butterfly species in the western Mediterranean are less likely to co-occur than non-cryptic  
65 congeners, suggesting that ecological similarity limits local coexistence. Similarly, DNA  
66 barcoding showed that morphospecies of rolled-leaf beetles consist of species-complexes  
67 adjacently distributed along altitudinal gradients (Garcia-Robledo et al. 2016). However, neither  
68 study specifically investigated whether the identified cryptic species display any functional trait  
69 divergence or have ecologically diversified according to resource requirements.

70 Furthermore, identifying non-sympatric distributions of ecologically similar species does  
71 not necessarily indicate that niche processes are determining patterns of co-existence. For  
72 example, it is believed that the majority of speciation events are not driven by niche divergence  
73 but rather by genetic differentiation between geographically isolated allopatric populations  
74 (Coyne and Orr 2004), often followed by secondary contact that might leave sister species co-  
75 occurring in sympatry (e.g. Pigot et al. 2016). However, specific conditions have been proposed  
76 that should be typically viewed as supporting a hypothesis of competitive exclusion among  
77 ecologically similar, closely related species (Anderson et al. 2002, Gutierrez et al. 2014). These  
78 include focal species showing parapatric ranges with narrow contact zones, and the use of  
79 ecological niche models (ENMs) to identify regions of potential sympatry coupled with  
80 numerical inequities in species abundance across identified regions (Anderson et al. 2002,  
81 Darwell et al. 2016).

82 Testing whether pairs/sets of cryptic species are typically sympatric or allopatric also has  
83 important implications for food web structure. Sympatric cryptic species contribute noise to our  
84 understanding of ecological food webs. For example, some recent studies have found that one  
85 supposed resource-generalist species actually comprises multiple resource-specialist cryptic  
86 species (Hebert et al. 2004, Smith et al. 2007). Meanwhile, at a community level, Smith et al.

87 (2011) found that DNA barcoding of the 100+ arthropod enemies of the spruce budworm not  
88 only increased measures of species richness but also reduced estimates of food web connectance  
89 (May 1973). However, studies highlighting coexisting cryptic species often focus on only one or  
90 a few neighbouring sites (e.g. Molbo et al. 2003, Wellborn and Cothran 2004, Montero-Pau and  
91 Serra 2011, Smith et al. 2011) and local patterns may not be representative of interactions across  
92 species' geographic ranges.

93         The implications of cryptic species coexistence patterns also ramify to the  
94 macroecological level of regional biodiversity patterns. Essentially, sympatric cryptic species  
95 contribute to local diversity ('alpha diversity' – sensu Whittaker 1972), but allopatric (or  
96 parapatric) cryptic species contribute to geographic diversity across sites ('beta diversity').  
97 Testing for these alternative patterns helps to evaluate total ('gamma') diversity and clarifies  
98 which species actually interact at a local scale. This is key to understanding the interplay between  
99 biodiversity and ecological function, as well as the ecological and coevolutionary dynamics of  
100 species interactions (Paine 2002, Duffy et al. 2007, Smith et al. 2011, Rooney and McCann  
101 2012).

102         Exploring these issues first requires correct delimitation of species (Cristescu 2014),  
103 which is difficult for many invertebrates, because diversity is high and many species are  
104 undescribed. In addition, intraspecific phenotypic variation can be very high (Cook et al. 1997,  
105 Xiao et al. 2010, Puniamoorthy et al. 2012) and cryptic species further complicate the situation.  
106 Consequently, investigations of insect community ecology and biodiversity increasingly  
107 recognise the need to include barcoding and/or related molecular techniques alongside  
108 morphological and ecological data (Blaxter 2003, Hebert et al. 2003, Hebert and Gregory 2005,  
109 Acs et al. 2010). This is certainly true for communities of insect herbivores and their associated

110 parasitoid enemies that constitute perhaps 20% of global species diversity (Price 1980, May  
111 1990).

112         The multitrophic insect communities hosted by *Ficus* (Moraceae) fruits (figs) are a  
113 valuable emerging model for studies of insect community ecology and evolution (e.g. Hawkins  
114 and Compton 1992, Kerdelhue et al. 2000, Xiao et al. 2010, Segar et al. 2013, Segar et al. 2014).  
115 *Ficus* is a globally distributed, largely tropical, plant genus of >750 species, famous for its classic  
116 mutualism with fig- pollinating wasps (Chalcidoidea: Agaonidae). However, most of the insect  
117 species are non-pollinating fig wasps (NPFW) and either gall fig tissue (hereafter “gallers”) or  
118 parasitise other wasp larvae (hereafter “parasitoids”). These fig wasp communities are restricted  
119 to the well-defined resource of the fig fruit, involve insects that are almost all specific to a single  
120 *Ficus* species, and are therefore geographically defined by the range of the host plant.

121         The several thousand species of fig wasps globally belong to diverse chalcid wasp  
122 lineages (Rasplus et al. 1998, Cook and West 2005), but can be categorised into five functional  
123 groups (Segar et al. 2014): pollinators; small and large gallers; and small and large parasitoids. At  
124 the level of individual insects, pollinators are typically more common than non-pollinators, while  
125 gallers and parasitoids typically far outnumber large ones (Segar et al. 2014). Within these  
126 communities, ovipositor length is a key functional trait as it mediates the ability to lay eggs in  
127 resources (seeds or insect larvae) in different fig tissue layers (al-Beidh et al. 2012), or in figs at  
128 different stages of growth. In other words, ovipositor length is a key axis for niche differentiation  
129 (Weiblen and Bush 2002, Proffitt et al. 2007, Segar et al. 2013). As fig wasps show very high host  
130 plant specificity, communities on different fig species are largely independent (Cook and Segar  
131 2010), although some notable exceptions occur (McLeish et al. 2010, McLeish and van Noort  
132 2012). However, cryptic pollinator (Molbo et al. 2003, Haine et al. 2006, Darwell et al. 2014) and



133 non-pollinator species (Bouteiller-Reuter et al. 2009, Zhou et al. 2012) have been reported  
134 recently and widespread fig wasp communities provide opportunities to test the impact of cryptic  
135 species on community ecology and biodiversity patterns.

136 Here, we focus on two fig wasp functional groups (small gallers and parasitoids) hosted  
137 by a single fig species (*Ficus rubiginosa*) with a wide latitudinal range. Wasps in these functional  
138 groups comprise 85% of all non-pollinator wasps developing in *F. rubiginosa* figs (Segar et al.  
139 2014), so are both ecologically important and amenable to dense sampling. In addition, they have  
140 long external ovipositors - a key functional trait that can be measured and used to assess  
141 ecological divergence (e.g. Weiblen & Bush 2002). Our specific aims are to:

142 1) Use wide geographic sampling, combined with morphological and molecular taxonomy to  
143 establish the number of small galler and parasitoid wasp species hosted by *F. rubiginosa*.

144 2) Test if cryptic species that do not differ in key functional traits (ovipositor length and body  
145 size) are largely sympatric, representing hidden alpha diversity, or allopatric/parapatric (replacing  
146 each other across geographic sites), representing hidden beta diversity.

147 3) Test if closely related (congeneric) species that do differ in these key functional traits coexist  
148 locally or show geographical replacement.

149 4) Use ENMs to determine the geographic extent of potential sympatry for focal species pairs of  
150 interest.

151

152

153 **Methods**

154 *Study system*

155 The endemic Australian fig, *Ficus rubiginosa*, occupies a ca. 3000 km coastal belt that  
156 stretches from northern Queensland (tropical) to southern New South Wales (Mediterranean  
157 climate) in diverse habitats including eucalypt scrub, vine thicket and rainforest (Dixon et al.  
158 2001). In addition to its five genetically delimited pollinator species (Darwell et al. 2014),  
159 morphological and molecular investigation has identified at least 15 NPFW species, comprising  
160 11 genera from six families and subfamilies (Segar et al. 2014). The small galler and small  
161 parasitoid functional groups comprise species from four genera: *Sycoscapter*, *Philotrypesis*,  
162 *Watshamiella* (all Sycoryctinae) and *Eukobelea* (Sycophaginae). For the three sycoryctine  
163 genera, different ecological niches can be inferred. *Sycoscapter* (parasitoids) and *Philotrypesis*  
164 (inquilines) appear to be ecologically differentiated according to host attack strategy (Tzeng et al.  
165 2008, Zhai et al. 2008). Meanwhile, some *Watshamiella* species have been shown to be hyper-  
166 parasitic, reliant on other parasitoid wasp taxa to pierce fig fruit walls in order to gain oviposition  
167 access (Compton et al. 2009). Finally, the sycophagine *Eukobelea* is thought to be a flower galler  
168 and as such should not compete directly with sycoryctine parasitoids (Segar et al. 2014).

169 In three of the four genera, there are distinct morphospecies that differ substantially in  
170 either ovipositor length (*Sycoscapter* and *Watshamiella*) or colour (yellow and black  
171 *Philotrypesis*) (Segar et al. 2014). *Philotrypesis* morphospecies may be ecologically  
172 differentiated as some yellow (non-pigmented) fig wasps are known to disperse nocturnally  
173 (Warren et al. 2010). Further, in *Sycoscapter* there are two genetically described cryptic species  
174 within the “short” morphospecies (Bouteiller-Reuter et al. 2009).

175 *Field sampling*

176 Most sampling was conducted in Queensland during Apr-Sep 2009 along the eastern  
177 seaboard between Brisbane (26° 46S, 153°02E) and Dimbulah (17° 01S, 145°19E) in the  
178 Atherton Tablelands. Some inland sampling was carried out around Forty Mile Scrub (18° 06S,  
179 144°49E) and Chillagoe (17° 10S, 144°31E). Other sampling occurred in the Townsville and  
180 Brisbane regions in 2007-8, while sampling in New South Wales (NSW) has been conducted  
181 sporadically before 2008 and also in 2012-14.

182 For all taxa, we attempted to sample individuals from many sites across the host plant  
183 range and include only one individual per morphospecies per fig in our analyses. Overall,  
184 samples were taken from >500 fig syconia from 166 sites. Near-ripe figs were placed into  
185 hatching jars with mesh lids that allowed air flow, while preventing overheating and wasp escape.  
186 After 48h each fig and all its exited wasps were placed into 70% ethanol. Alternatively, figs were  
187 placed directly into alcohol and wasps were dissected out at a later date.

188

189 *Molecular methods*

190 We extracted DNA using a Chelex method (West et al. 1998) and then amplified one  
191 mitochondrial (cytb) and one nuclear (ITS2) marker. mtDNA is employed regularly in animal  
192 molecular species delimitation (e.g. barcoding), and nuclear ITS2 provides a complementary  
193 nuclear marker for species delimitation in Hymenoptera (Xiao et al. 2010). Sample sizes used for  
194 molecular analyses are detailed in Table 2. Sequencing was conducted on individuals from across  
195 the entire host plant range (see supplementary information for further details).

196 We first attempted to sequence all wasps using the CP1-CB2 cytb primer set (CP1 - GAT  
197 GAT GAA ATT TTG GAT C and CB2 - ATT ACA CCT CCT AAT TTA TTA GGA AT; Harry

198 et al. 1998), which amplifies a fragment of about 600 bp. However, this did not amplify all taxa,  
199 and so the shorter (ca. 400 bp) CB1-CB2 fragment was employed for these species (CB1 - TAT  
200 GTA CTA CCA TGA GGA CAA ATA TC; Jermin and Crozier 1994). The nuclear internal  
201 transcriber spacer 2 (ITS2) region was sequenced for all taxa using the primers ITS2F (ATT CCC  
202 GGA CCA CGC CTG GCT GA) and ITS2R (TCC TCC GCT TAT TGA TAT GC) (ITS2F -  
203 ATT CCC GGA CCA CGC CTG GCT GA and ITS2R - TCC TCC GCT TAT TGA TAT GC;  
204 White et al. 1990).

205 PCR amplification was conducted using a Techne Touchgene gradient machine with the  
206 following conditions: 1) CP1-CB2: 3 min at 94°C, 40 cycles of 30s at 92°C, 60s at 48°C, 1 min  
207 30s at 72°C, and 10 min at 72°C. 2) CB1-CB2: 3 min at 94°C, 30 cycles of 15 s at 95°C, 20 s at  
208 45°C, 30 s at 72°C, and 10 min at 72°C. 3) ITS2: 5 min at 94°C, 35 cycles of 30 s at 94°C, 40 s at  
209 55°C, 40 s at 72°C, and 10 min at 72°C. Subsequent ethanol purification and sequencing, by  
210 BigDye™ terminator cycling and a 3730xl DNA analyser, were conducted by Macrogen Inc.

211

### 212 *Sequence data analysis*

213 Chromatogram quality was assessed using Finch TV Version 1.4.0 and sequences edited  
214 and aligned using BioEdit (Hall 1999) with final adjustments by eye. Bayesian methods were  
215 used to construct phylogenies using MrBayes (Ronquist and Huelsenbeck 2003), after choosing  
216 the best model of nucleotide substitution for each gene with MrModeltest in PAUP\* (Swofford  
217 2002). Log-likelihood ratio tests selected the GTR+I+G model for both the nuclear and mtDNA  
218 datasets. ITS2 sequences were trimmed at either end because it is difficult to identify sequence  
219 start and end points due to the presence of indels (e.g. Li et al. 2010, Xiao et al. 2010).

220 Species were delimited as clearly defined congruent clades (with  $\geq 0.95$  p-values for at  
221 least one marker) from separate cytb and ITS2 phylogenies using the phylogenetic species  
222 concept (PSC; Eldredge and Cracraft 1980). To corroborate PSC species delimitation we  
223 investigated cytb data with jMOTU software (Jones et al. 2011). This software produces a  
224 frequency distribution of pairwise genetic distances, and an inflection point in this distribution  
225 represents the “barcoding gap” between species, and thus identifies molecular operational  
226 taxonomic units (MOTUs). Assessment of pairwise Kimura-2-parameter (K2P; Kimura 1980)  
227 genetic distances between putative congeneric species was performed to further investigate PSC  
228 delimitation. For *Philotrypesis* ‘yellow’, sequencing of cytb in many individuals revealed two  
229 species. However, within each species there was almost no sequence variation, so only a few  
230 individuals were sequenced for ITS2, which supported cytb analyses by revealing two species.

231

### 232 *Morphological analyses*

233 We used ANCOVA to explore morphological differentiation between genetically  
234 delimited congeneric species focusing on two key functional traits – ovipositor length, which  
235 determines at what stage or what depth a wasp may lay eggs into the fig, and body size (using  
236 hind tibia length as an index). We refer to the ratio between these two traits as “relative  
237 ovipositor length” (ROL). These analyses were performed in R (R Core Team 2015). For further  
238 comparison of pairs of congeneric species, we also calculated a “disimilarity index”, where  $DI =$   
239  $(ROL_A - ROL_B) / ROL_A$  between species A with the higher ROL and congeneric species B with  
240 the lower ROL.

241

242

243 *Environmental niche models*

244 To evaluate signatures of competitive exclusion and identify regions of potential  
245 sympatry we filtered locality data for each molecularly delimited species to include the maximum  
246 number of locations that were at least 10 km apart (Anderson and Raza 2010, Boria et al. 2014).  
247 This reduces sampling biases from non-uniform sampling efforts. For environmental data, we  
248 used the 19 WorldClim bioclimatic variables at 30" resolution (=0.86 km<sup>2</sup> pixels) (Hijmans et al.  
249 2005; <<http://biogeoberkeley.edu/worldclim/worldclim.htm> >). The WorldClim data are derived  
250 from precipitation, temperature and seasonality records and are a standard dataset for baseline  
251 predictions of species' geographic distributions.

252 We used Maxent version 3.3.3k which uses the maximum entropy algorithm to calculate  
253 ENMs (Phillips et al. 2006, Phillips and Dudik 2008). To ensure the correct model was fitted and  
254 avoid model overfitting, we used the 'ENMeval' package (Muscarella et al. 2014) in R (R  
255 development team) to determine the optimal feature class (FC) and regularisation multiplier  
256 (RM) settings. FC and RM settings were obtained by choosing the settings that returned a  $\Delta AICc$   
257 score of zero (Muscarella et al. 2014). For each species, models were evaluated using only  
258 environmental data from the subject species' range by creating a mask variable delimited by the  
259 'minimum convex polygon' around all species' locations and a 0.5° buffer zone in the R package  
260 adehabitatHR (Calenge 2006). To partition occurrence localities into testing and training bins  
261 (folds) for  $k$ -fold cross-validation, we used the 'checkerboard1' option for species with greater  
262 than 25 localities (i.e. >10km apart) and the 'jackknife' option for species with between 15-25  
263 localities (Muscarella et al. 2014). Additionally, the 'ENMeval' package struggles to evaluate  
264 optimal models for species with less than 15 locality records (here *Sycosapter* 'short' southern

265 species; *Philotrypesis* ‘yellow’ species 1; *Philotrypesis* ‘black’ southern species; *Eukobelea* both  
266 species); for these species we used the default settings in Maxent (under these circumstances it is  
267 likely that the ENM models are over-fitted and therefore represent conservative estimates of  
268 species’ potential ranges; Muscarella et al. 2014). ENMs across the entire study region were run  
269 in Maxent with a 10% training threshold rule which rejects the lowest 10% of predicted values  
270 (Pearson et al. 2007). This allows the transformation of probabilistic ENMs into binary  
271 predictions of suitable vs. non-suitable climatic conditions at each pixel. These ENMs were then  
272 overlaid for particular species pairs of interest.

273

## 274 **Results**

### 275 *Sequencing results*

276 Sequences were obtained from 307 and 194 individuals for cytb and ITS2, respectively.  
277 We found no evidence of pseudogenes or heteroplasmy (see Table 1; Supplementary Information  
278 for further details). According to our species delimitations, mitochondrial K2P pairwise distances  
279 range from 0-7.24% within species and 2.75-16.00% between different species in the same  
280 genus. The corresponding values for ITS2 data are 0-11.25% and 0.93-48.90% respectively. The  
281 high ITS2 values reflect large indels that contribute greatly to distances between species, whereas  
282 the aligned nucleotide regions of these species are far less divergent.

283

### 284 *Species delimitation*

285 Our PSC and jMOTU analyses strongly support the existence of 11 small galler and  
286 parasitoid species associated with *Ficus rubiginosa*. The previous figure was eight species and

287 our analysis adds three cryptic species in the genera *Philotrypesis* and *Eukobelea* (Table 2). All  
288 individuals sequenced for both genes were unambiguously placed into congruent cytb and ITS2  
289 clades (Figure S1) and node support values for all posited species were high ( $p \geq 0.95$ ) for at least  
290 one marker. Similarly, PSC species delimitation was congruent with MOTU identification for  
291 cytb data (Figure S2). The congruence of results across nuclear and mtDNA markers for all  
292 individuals sequenced supports reproductive isolation of our identified species and provides no  
293 evidence for hybridisation. Critically, this includes all sympatric congeneric individuals from  
294 different species. In addition, where we recognise congeneric morphospecies (e.g. *Watshamiella*  
295 ‘long’ and ‘short’) that differ in functional traits, these morphospecies are also congruent with  
296 molecular species divisions (see below).

297

#### 298 *Relationships between sympatric co-occurrence and degree of morphological similarity*

299 Sympatric congeneric species (i.e. *Sycoscapter* ‘long’ and ‘short’, two *Philotrypesis*  
300 ‘yellow’ and *Watshamiella* ‘long’ and ‘short’; Figure 1, Table 3) generally have significantly  
301 different ROLs; i.e. they are functionally differentiated. In contrast, congeneric species with  
302 ROLs described by the same simple regression model are essentially parapatric. Consequently,  
303 dissimilarity indices (DI) are consistently higher for congeneric species that are sympatric (0.190-  
304 0.327) and lower (0.005-0.072) for those that are allopatric (Table 3).

305 There are also some more nuanced aspects of these patterns, but these do not compromise  
306 the strong general patterns. First, both the *Sycoscapter* ‘short’ and *Eukobelea* congeneric pairs  
307 occupy largely parapatric northern and southern ranges, but have small contact zones around  
308 Brisbane in the centre of the host plant range. In addition, the ‘southern’ species of each pair also  
309 has a disjunct small northern refuge at Forty Mile Scrub. Also, the two virtually parapatric



310 *Philotrypesis* ‘black’ species (one ‘northern’ individual was found in New South Wales – figure  
311 1d) actually have significantly differentiated ROLs. However, their DI (0.072) is still very low,  
312 suggesting little functional differentiation compared to widespread sympatric pairs that have DI  
313 >0.19. Typically, ovipositor length in congeneric pairs with statistically differentiated ROLs is  
314 ~1mm compared to ~0.1mm in *Philotrypesis* ‘black’ (Figure 1d; indicating that the  
315 discriminative statistical power of the ANCOVA test is high).

316

### 317 *Species distribution models*

318 For morphologically indistinguishable (i.e. cryptic) congeneric species pairs, SDMs  
319 typically show large regions of potential sympatry indicated by overlapping models (Figure 1).  
320 For *Sycosapter* ‘short’ (Figure 1b), the ‘southern’ species is predicted to be climatically adapted  
321 to the entire east coast of Australia including the extensive regions of Queensland where it is not  
322 found. For *Philotrypesis* ‘black’ (Figure 1d), the ‘northern’ species is expected to be found across  
323 the entirety of the east coast including the southern regions where it is typically excluded. For  
324 *Eukobelea* (Figure 1f), ‘the southern’ species appears climatically adapted to the entire east coast  
325 including the northern regions from which it is excluded. Finally, for morphologically  
326 distinguishable, sympatric congeners, ENMs predict wide-ranging regions of species overlap  
327 (Figures 1a,c,e). In summary, ENM analyses show that the ranges of cryptic species are far  
328 smaller than expected based on abiotic factors, suggesting a major role for biotic interactions.

329

330

331 **Discussion**

332 As molecular investigation into the scale and structure of global biodiversity continues we will  
333 begin to form a more nuanced picture of the patterns and ecological significance of cryptic  
334 diversity (Smith et al. 2007, Smith et al. 2011). A fundamental issue is to understand the major  
335 processes that determine community assembly and the geographic relationships between pairs of  
336 cryptic species can provide important evidence on the relative impact of niche versus neutral  
337 processes in community assembly. In particular, we can test if ecologically similar cryptic species  
338 coexist or exclude each other at community and geographic scales (e.g. Voda et al. 2015). Our  
339 study of a multitrophic insect community that harbours extensive cryptic diversity offers a  
340 comprehensive view of a complex community across a wide geographic range and uses the  
341 uncovered diversity to test the key predictions about coexistence.

342

343 *Cryptic species, functional trait divergence and local coexistence*

344 Our molecular species delimitation strongly supports the existence of two small galler and  
345 nine small parasitoid species. This is a marked increase over a previous estimate of eight species  
346 in total, which already recognised two cryptic species in *Sycoscapter* (Segar et al. 2014). It also  
347 means that all four genera are now known to include morphologically similar but genetically  
348 distinct species, emphasising that cryptic diversity is a significant component of total diversity.

349 Niche theory posits that competing species should only coexist locally in sympatry if they  
350 diverge along some ecological trait axes or exhibit differential responses to one or more of  
351 resource availability, temporal or spatial heterogeneity, or predation. In contrast, neutral models  
352 predict communities that are assembled more stochastically and that ecologically similar species

353 will often coexist. Within three (*Sycoscapter*, *Philotrypesis* and *Watshamiella*) of our four study  
354 genera we found co-occurring species with marked differences in a key functional trait -  
355 ovipositor length (Figure 1a,c,e). This correlates with niche differentiation because wasps with  
356 different ovipositor lengths can lay eggs into different tissue layers of in figs or at different stages  
357 of fig development (Proffitt et al. 2007, Segar et al. 2014).

358 In contrast, cryptic congeneric species that lack this (or other obvious) trait divergence  
359 tend not to coexist locally and, though widely distributed, are predominantly allopatric (Figure  
360 1b,d,f). For example, the two *Sycoscapter* ‘short’ species, *Philotrypesis* ‘black’ and *Eukobelea*  
361 each comprise two species clearly identified by molecular taxonomy, but with low DIs and no or  
362 only subtle differences in ROLs. In *Philotrypesis* ‘black’, ANCOVA analyses statistically  
363 distinguished the species-specific ROLs; however, the low DI coupled with a visual inspection of  
364 data showing overlapping clouds of points suggests that their ROLs represent functionally similar  
365 ecological roles. For these three species pairs, the lack of differentiated ROLs and DIs suggest  
366 similar attack strategies on figs that, through limiting similarity, would hinder local coexistence.

367 In the case of *Philotrypesis* ‘yellow’ there is some nuance to the overall picture. Although  
368 both species co-occur widely in Queensland, species 1 appears absent from southern Queensland  
369 and northern NSW. However, this could potentially be a sampling artefact as collection effort  
370 was not as intense in these regions. Moreover, we have only found a small number of  
371 *Philotrypesis* ‘yellow’ wasps in the furthest north parts of New South Wales (Figure 1c),  
372 suggesting that these yellow species are predominantly tropical and do not extend to the higher  
373 latitudes of southern NSW. We also note that the intermediate sympatry/allopatry of the two  
374 *Philotrypesis* ‘yellow’ mirrors their intermediate dissimilarity indices (DI) that lie between the  
375 two extremes for allopatric and sympatric congeneric pairs.

376

377 *Ecological processes determining species coexistence*

378         Although the allopatric/parapatric geographic distributions of our cryptic congeneric  
379 NPFW species support a hypothesis of limiting similarity hindering local coexistence, these  
380 patterns are also consistent with a model of allopatric speciation with subsequent secondary  
381 contact (Coyne and Orr 2004). However, as these species' northern and southern ranges typically  
382 display narrow contact zones around Brisbane (excluding a single incursion into southern regions  
383 by a 'northern' *Philotrypesis* 'black' individual and the consistently anomalous Forty Mile Scrub  
384 region – see later Discussion), the latter hypothesis rests on the supposition that we are viewing  
385 the point in evolutionary history when these cryptic pairs, unlike the morphologically  
386 distinguishable congeners in this community, are undergoing secondary reconnection before  
387 developing more sympatric ranges. These patterns, along with our ENMs indicating large areas of  
388 potential sympatry with one of a pair of species largely absent, fulfil the required conditions  
389 proposed by Anderson et al (2002) to indicate competitive exclusion among ecologically similar,  
390 closely related species. Moreover, recent findings from this community showing that *Sycoscapter*  
391 'long' forms a single unbroken population across the range of *F. rubiginosa* (Sutton et al. 2016)  
392 suggests that the geographic ranges of these NPFWs are most likely determined by adaptive or  
393 competitive processes rather than being the result of sedentary range expansion. Additionally,  
394 although patterns of allopatric speciation followed by secondary contact may be common (e.g.  
395 Pigot et al. 2016), such studies do not typically test whether secondarily coexisting species are in  
396 direct competition for resources. For the small NPFW community occupying *F. rubiginosa*, we  
397 can confidently infer that all species are restricted to targeting the uniform resources entombed in  
398 the fig syconium as recent work indicates these species are all host specific (Darwell 2013).

399

400 *Do cryptic species increase alpha or beta diversity?*

401 Our findings suggest that detection of cryptic species increases beta diversity (geographic  
402 turnover of species) with little effect on alpha diversity in any given site or region. This implies  
403 that local alpha diversity may change little across the host plant range, but more detailed  
404 quantitative studies are needed to test this hypothesis. If the link between cryptic and beta  
405 diversity proves general across other taxa, features such as local food web dynamics and  
406 ecological functioning and stability (Rooney and McCann 2012) may be similar across ranges  
407 despite considerable cryptic diversity. Assessing patterns of beta diversity is a key research area  
408 in ecology (e.g. Graham and Fine 2008) and we show here the need to combine comprehensive  
409 geographic sampling with molecular revelation of cryptic species to reveal true biodiversity  
410 patterns. Moreover, our findings that functionally equivalent congeneric species do not typically  
411 co-occur suggests that niche-based models of community assembly better explain species  
412 coexistence among the small NPFWs of *F. rubiginosa* compared to predictions originating from  
413 neutral theory (Hubbell 2001).

414

415 *The importance of widespread geographic sampling*

416 Although some empirical studies (Molbo et al. 2003, Wellborn and Cothran 2004,  
417 Montero-Pau and Serra 2011) suggest that ecologically similar cryptic species may coexist,  
418 extrapolation from studies with limited geographic sampling is risky. For example, in both  
419 *Sycoscapter* ‘short’ and *Eukobelea*, two cryptic species co-occur around Brisbane. Indeed, this is  
420 where the two cryptic *Sycoscapter* ‘short’ species were first detected (Bouteiller-Reuter et al.  
421 2009) in a single population and at similar frequencies (Cook et al. 2015). We might therefore

422 predict that they co-occur widely. However, the Brisbane region represents a tiny fraction of their  
423 overall ranges (as revealed by this study) and our comprehensive sampling reveals a northern and  
424 a southern species that are widely parapatric but have a narrow overlap zone in the middle of the  
425 host plant range. This could be a case of allopatric speciation with limited secondary contact  
426 following range expansion. As such, one species may eventually outcompete the other in this  
427 zone or localised coexistence may continue as competitive exclusion is never realised due to  
428 ongoing immigration from neighbouring populations (e.g. Darwell et al. 2014). A recent  
429 population genetic study showed that *Sycoscapter* ‘long’ is effectively one large population  
430 throughout the host plant range, with only weak isolation-by-distance (Sutton et al. 2016). This  
431 suggests substantial dispersal and wide gene flow in at least some *Sycoscapter* wasps, as also  
432 reported for many fig-pollinating wasps, which would impede the formation of allopatric species  
433 pairs on the same host plant.

434         Similar north-south clinal patterns of cryptic diversification have been noted in the  
435 pollinators of *F. rubiginosa* (Darwell et al. 2014). The Burdekin gap, located just south of  
436 Townsville, is a well-documented major biogeographical barrier (Joseph and Moritz 1994). In  
437 fig-wasps, one of the pollinator species associated with *F. rubiginosa* is found almost exclusively  
438 around Townsville, whilst this study support a similar scenario for one of the yellow  
439 *Philotrypesis* species. This region has been noted as a transitional zone for species displacement  
440 in *Drosophila* (Schiffer et al. 2004, van Heerwaarden et al. 2009). The McPherson range around  
441 Brisbane is another recognised major biogeographical barrier (Edwards and Melville 2010), and  
442 *Sycoscapter* ‘short’, *Philotrypesis* ‘black’ and *Eukobelea* seem to divide here. At a gross level  
443 this Queensland-NSW split equates to a tropical-Mediterranean biome division straddling the  
444 geographic range of this fig species and may explain many species’ boundaries in this study. Of

445 further intrigue is that the north-south split in *Sycoscapter* ‘short’ and *Eukobelea* is not absolute.  
446 In both genera, the ‘southern’ species also occurs at one site (Forty Mile Scrub) in inland  
447 northern Queensland; a single ‘southern’ *Sycoscapter* was also found nearby (~100km away) in  
448 the Atherton Tablelands. The habitat at Forty Mile Scrub is remnant vine thicket, which was  
449 previously widespread and is distinctive from the eucalypt scrub habitat where the other samples  
450 were collected. Furthermore, the *F. rubiginosa* trees there are hemiepiphytic ‘stranglers’ rather  
451 than the more common lithophytic rock-dwelling forms, and have notably larger figs (CTD, *pers.*  
452 *ob.*). Thus, Forty Mile Scrub may favour otherwise southern-adapted species due to idiosyncratic  
453 ecological circumstances and adds to the overall impression that alpha and beta diversity patterns  
454 for small NPFWs inhabiting *F. rubiginosa* are driven by adaptive responses to a variety of  
455 ecological and climatic factors which together support a dominant role for niche processes in  
456 determining community assembly.

457

458 **Table 1. Summary characteristics of cytb and ITS2 sequence data.** Pairwise intraspecific and interspecific  
 459 distances are given using Kimura's two parameter algorithm (K2P). Sequence length is given in nucleotide base  
 460 pairs.  
 461

Genus	cytb			ITS2		
	Intraspecific K2P (%)	Interspecific K2P (%)	Sequence length	Intraspecific K2P (%)	Interspecific K2P (%)	Sequence length
<i>Sycoscapter</i>	0-4.13	6.25-14.29	351	0-4.63	2.58-8.66	303-380
<i>Philotrypesis</i>	0-7.24	2.75-16.00	394	0-6.67	2.24-12.78	320-358
<i>Watshamiella</i>	0-2.06	4.03-7.77	692	0-3.73	0.93-5.67	300-348
<i>Eukobelea</i>	0-3.93	6.96-10.12	663	0-11.25	21.44-48.90	520-565

462  
 463  
 464  
 465 **Table 2. Species diversity estimates for small non-pollinating chalcid wasp taxa associated with *F. rubiginosa***  
 466 **as discriminated by molecular phylogenetic methods before and after current study.** Known morphologically  
 467 determinant characters indicated in parentheses (s-short ovipositor, l-long ovipositor, y-yellow, b-black; §-congeneric  
 468 species distinguishable by ovipositor-hind tibia allometry); Sample sizes shown for each delimited species.  
 469

Wasp taxon	Previous findings	Current data	Sample size (N)
<i>Sycoscapter</i>	3 (s,s,l§)	3 (s,s,l§)	31, 32, 17
<i>Philotrypesis</i>	2 (y,b)	4 (y§,y§,b,b)	44, 77, 21, 15
<i>Watshamiella</i>	2 (s§,l§)	2 (s§,l§)	27, 46
<i>Eukobelea</i>	1	2	25, 23
<b>Total</b>	<b>8</b>	<b>11</b>	<b>360</b>

470  
 471  
 472 **Table 3. ANCOVA results, dissimilarity indices and relationship to congeneric co-occurrence in small NPFW**  
 473 **species found on *F. rubiginosa*.** ANCOVA: the minimum adequate model method of Crawley (2007) is employed;  
 474 ov~tib indicates a model with no factorial parameters; ov~tib+spp indicates a model with separate intercepts and a  
 475 single slope; ov~tib\*spp indicates a model with separate intercepts and slopes. P-values indicate statistical support of  
 476 the favoured model over the hierarchically simpler model. Dissimilarity indices range between 0.005-0.072 for  
 477 allopatric species pairs and 0.190-0.327 for species pairs found in sympatry. See figure 1 for ANCOVA plots.  
 478

	<i>Sycoscapter</i> long and short	<i>Sycoscapter</i> short	<i>Philotrypesis</i> black	<i>Philotrypesis</i> yellow	<i>Watshamiella</i> long and short	<i>Eukobelea</i>
Sympatric	Yes	No	No	Yes	Yes	No
Dissimilarity Index	0.324	0.024	0.072	0.190	0.327	0.005
Minimum model	ov~tib*spp	ov~tib	ov~tib+spp	ov~tib+spp	ov~tib+spp	ov~tib
P-value	<2e-16	1.42e-09	1.395e-12	<2e-16	<2e-16	7.48e-11
Adjusted R <sup>2</sup>	R <sup>2</sup> =0.944	R <sup>2</sup> =0.520	R <sup>2</sup> =0.848	R <sup>2</sup> =0.928	R <sup>2</sup> =0.955	R <sup>2</sup> =0.668
F-statistic	F <sub>3,83</sub> =482.6	F <sub>1,49</sub> =55.25	F <sub>2,28</sub> =84.39	F <sub>2,76</sub> =553.2	F <sub>2,70</sub> =781.2	F <sub>1,38</sub> =79.51

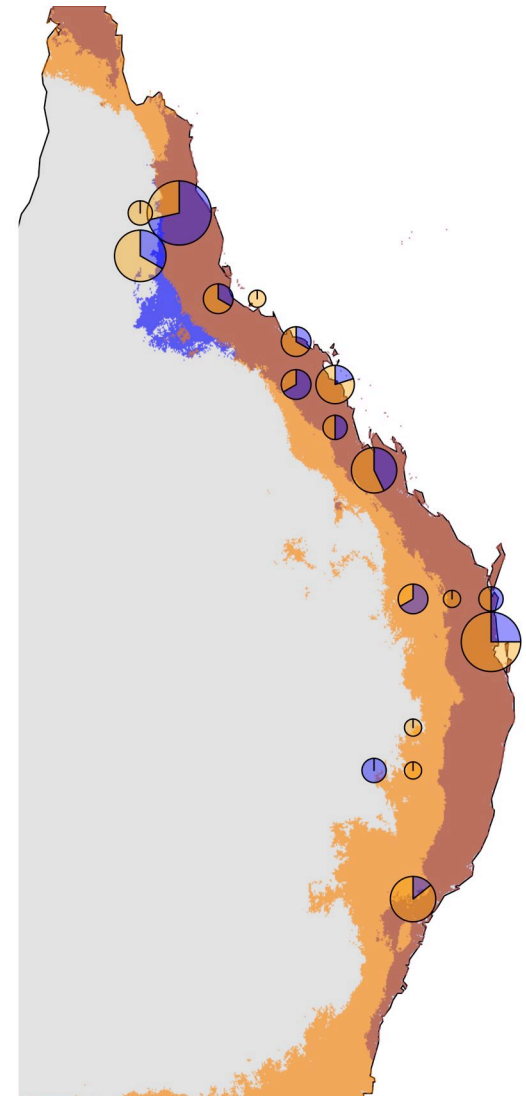
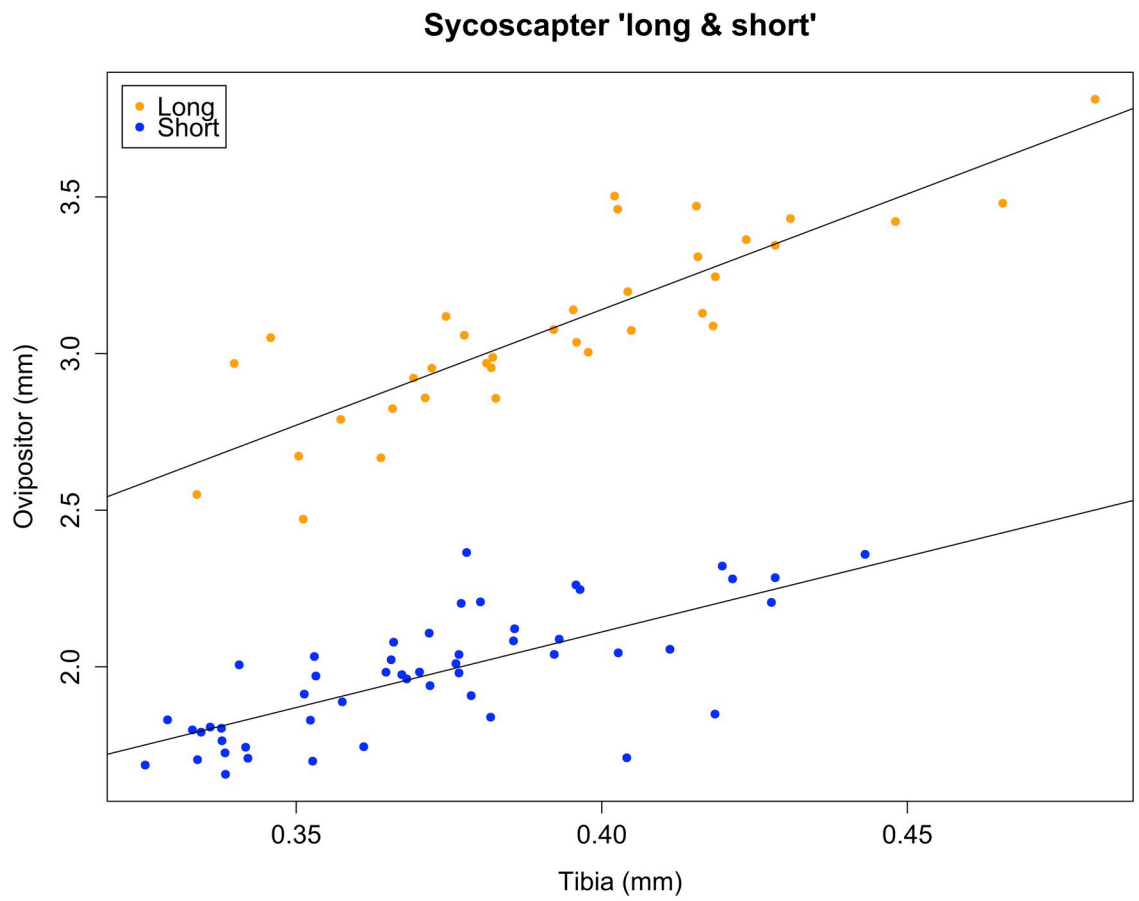
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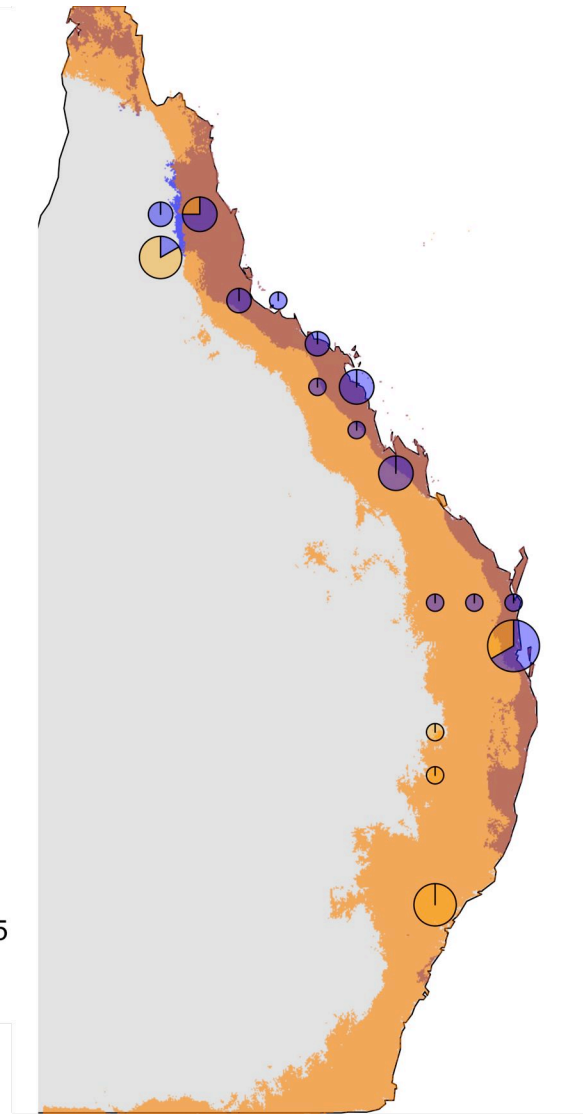
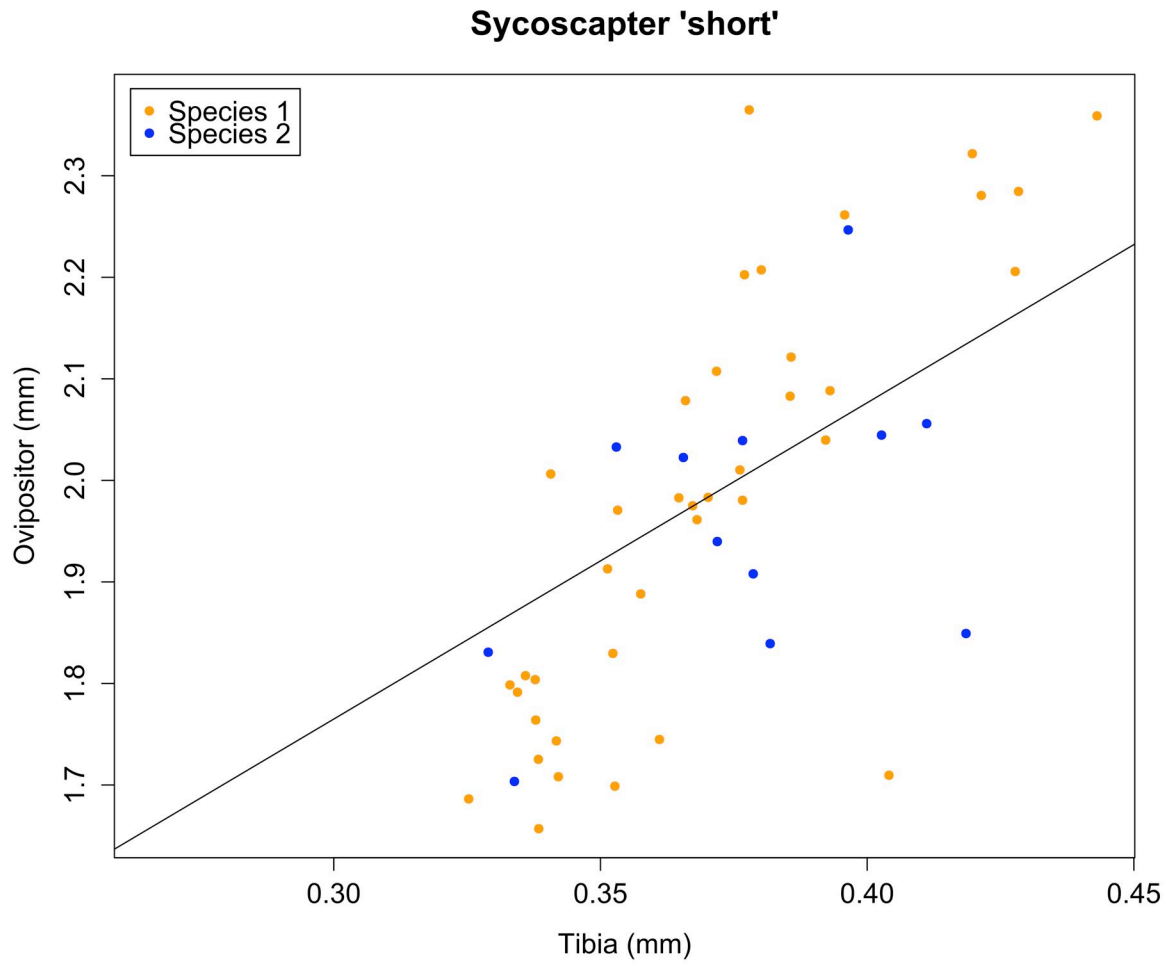
480 **Figure legend**

481 **Figure 1. ANCOVA plots for ovipositor – hind tibia allometries for six congeneric species**  
482 **pair comparisons (left-hand panel) and associated species abundance distribution maps**  
483 **plotted on top of overlaid species distribution models (ENMs) (right-hand panel).** Figure 1a  
484 shows comparisons of *Sycoscapter* ‘long’ versus both ‘short’ species combined and figure 1b  
485 shows comparisons of both *Sycoscapter* ‘short’ species only; figures 1c and 1d show  
486 comparisons of *Philotrypesis* species pair colour morphs thought to be nocturnal (yellow) and  
487 diurnal (black) dispersers; figures 1e and 1f show comparisons of *Watshamiella* (‘long’ and  
488 ‘short’ ovipositor) and *Eukobelea* congeneric pairs. Individual ENMs for species pairs follow the  
489 same blue and orange colour species coding; thus, overlapping regions of potential sympatry are  
490 coloured brown.  
491

492 Figure 1a – *Sycosapter* 'long & short'



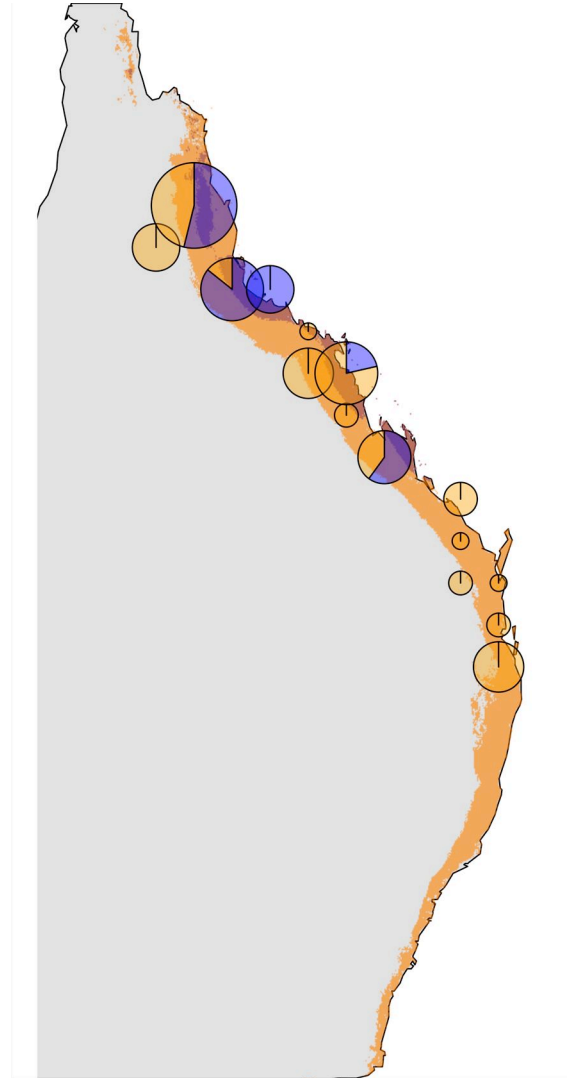
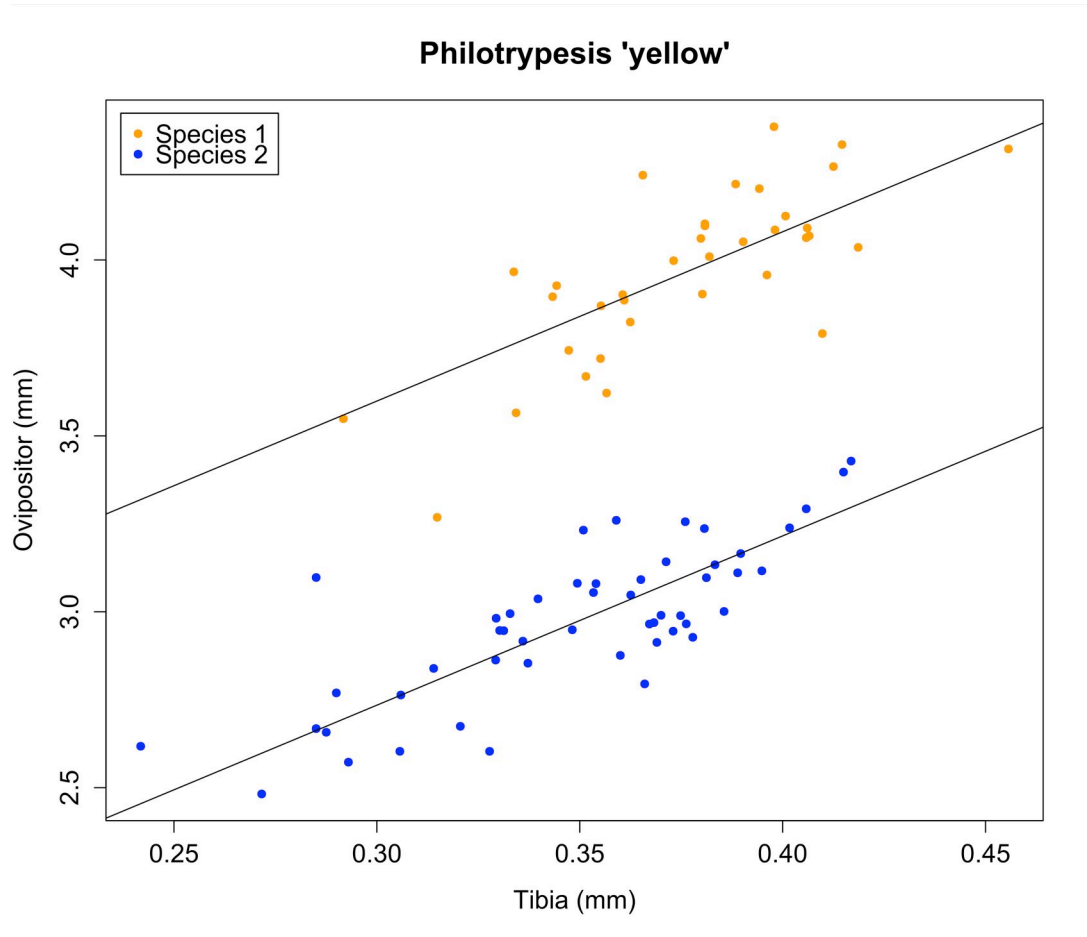
495 Figure 1b – *Sycoscapter* ‘short’



496

497

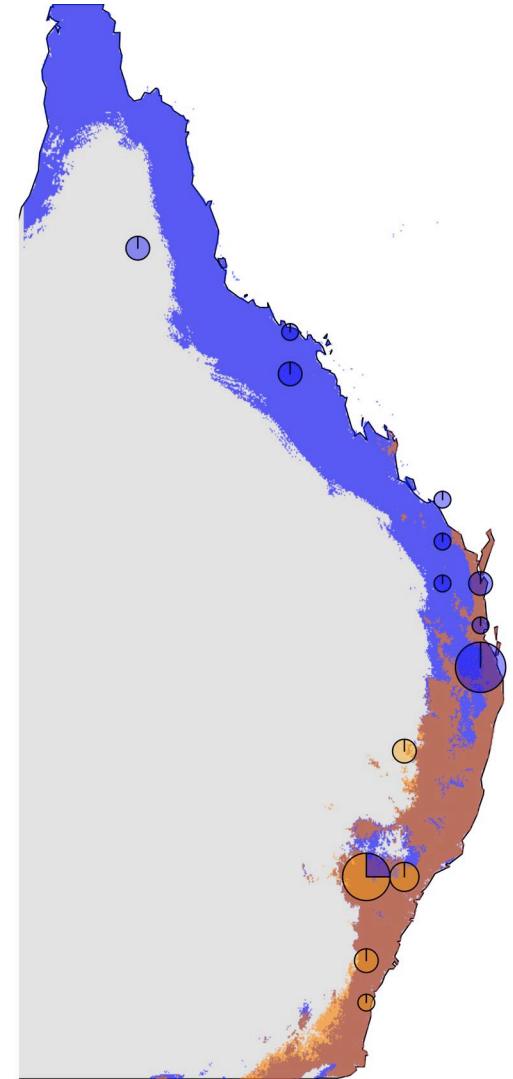
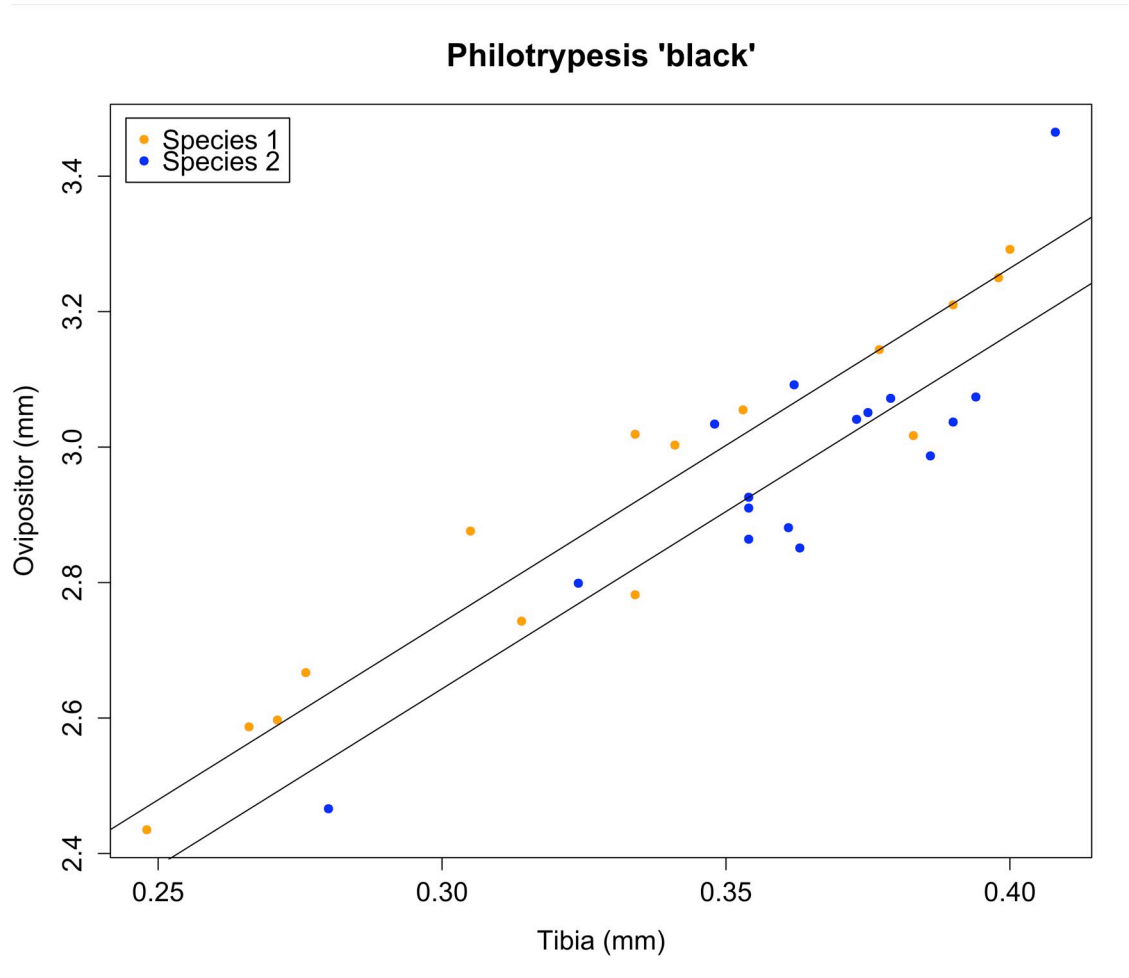
498 Figure 1c – *Philotrypesis* 'yellow'



499

500

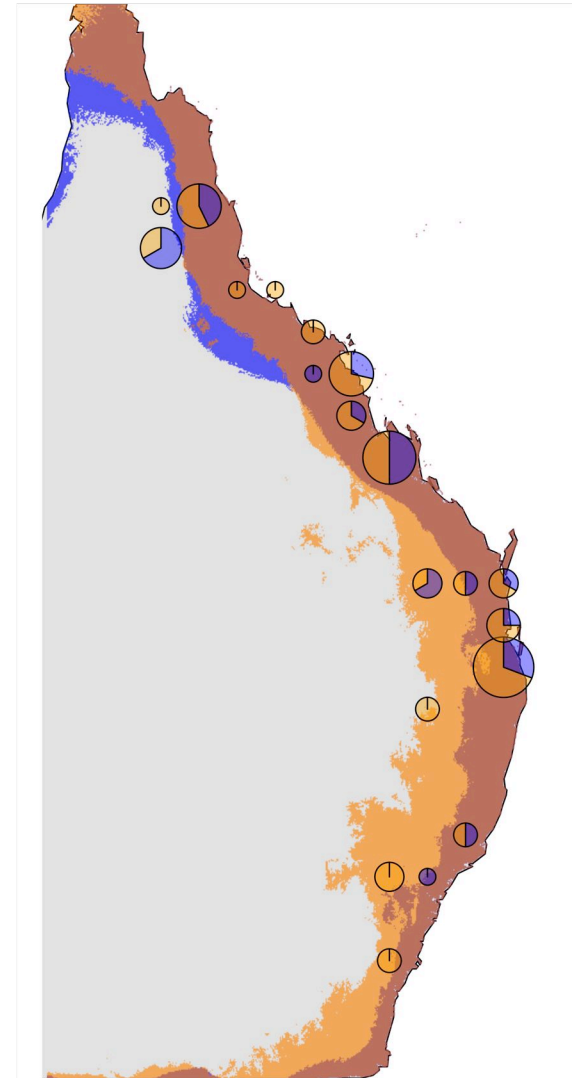
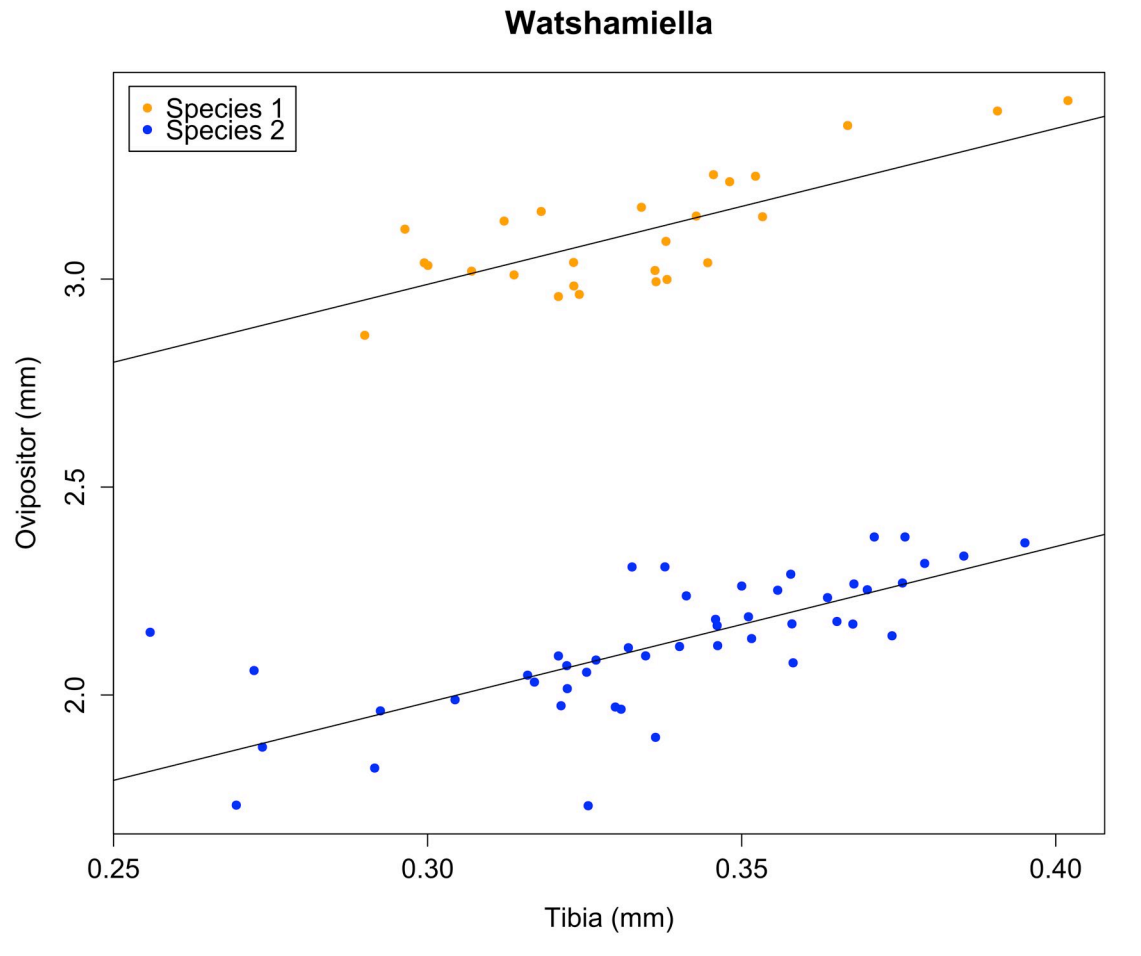
501 Figure 1d – *Philotrypesis* 'black'



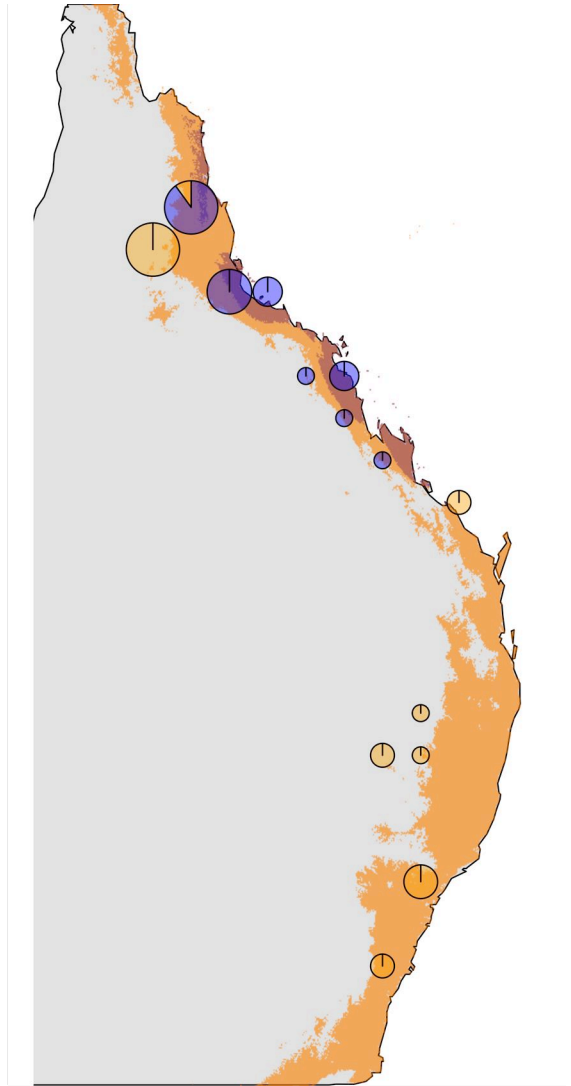
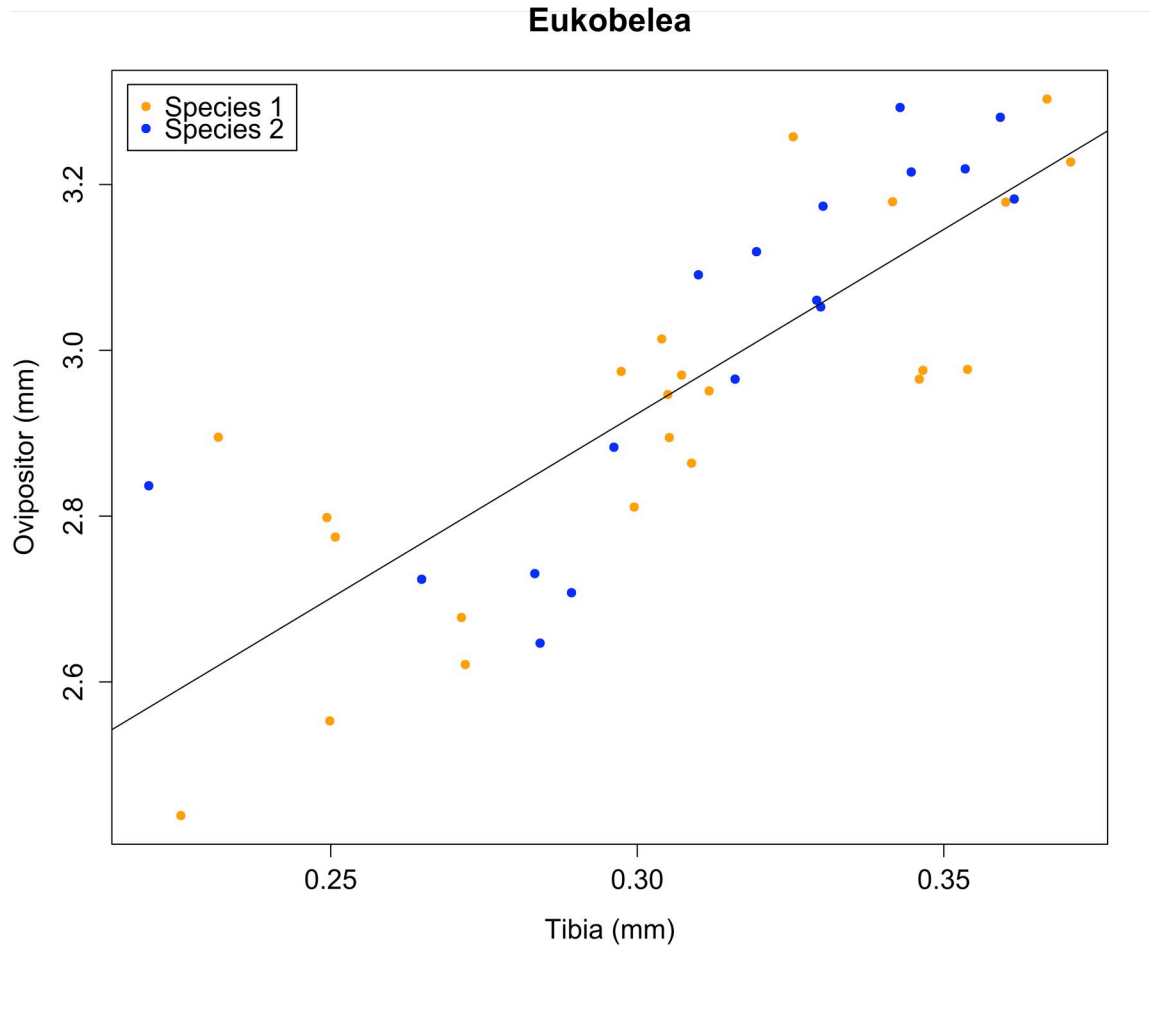
502

503

504 Figure 1e – *Watshamiella*



505



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512

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716 **Data accessibility:** We confirm that, should the manuscript be accepted, the data supporting the  
717 results will be archived in an appropriate public repository such as Dryad or Figshare and the  
718 data DOI will be included at the end of the article. Sequence data will be archived on Genbank.

719

720 **Statement of authorship:** CTD and JMC both designed the study and conducted field  
721 collections. CTD wrote the first draft of the manuscript, and all authors contributed substantially  
722 to revisions. CTD performed all taxonomic, morphological and molecular lab work,  
723 morphometric and statistical analyses, and molecular species delimitation.

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