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

Article

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

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Running title: Cryptic diversity predicts species coexistence

Abstract

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

Introduction

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

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

However, two recent studies do support the idea that cryptic or near-identical species may 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

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

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

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

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

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

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

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

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

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

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

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

PCR amplification was conducted using a Techne Touchgene gradient machine with the following conditions: 1) CP1-CB2: 3 min at 94°C, 40 cycles of 30s at 92°C, 60s at 48°C, 1 min 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 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 55°C, 40 s at 72°C, and 10 min at 72°C. Subsequent ethanol purification and sequencing, by BigDyeTM terminator cycling and a 3730xl DNA analyser, were conducted by Macrogen Inc.

Sequence data analysis

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

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

Morphological analyses

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

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^2 pixels) (Hijmans et al.
249 2005; <http://biogeo.berkeley.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 ΔAICc
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 0.5º 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 *Sycoscapter* '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

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

Relationships between sympatric co-occurrence and degree of morphological similarity

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

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

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

Species distribution models

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

Discussion

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

Cryptic species, functional trait divergence and local coexistence

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

Niche theory posits that competing species should only coexist locally in sympatry if they diverge along some ecological trait axes or exhibit differential responses to one or more of resource availability, temporal or spatial heterogeneity, or predation. In contrast, neutral models 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 (Proffit 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

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

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

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

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

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

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

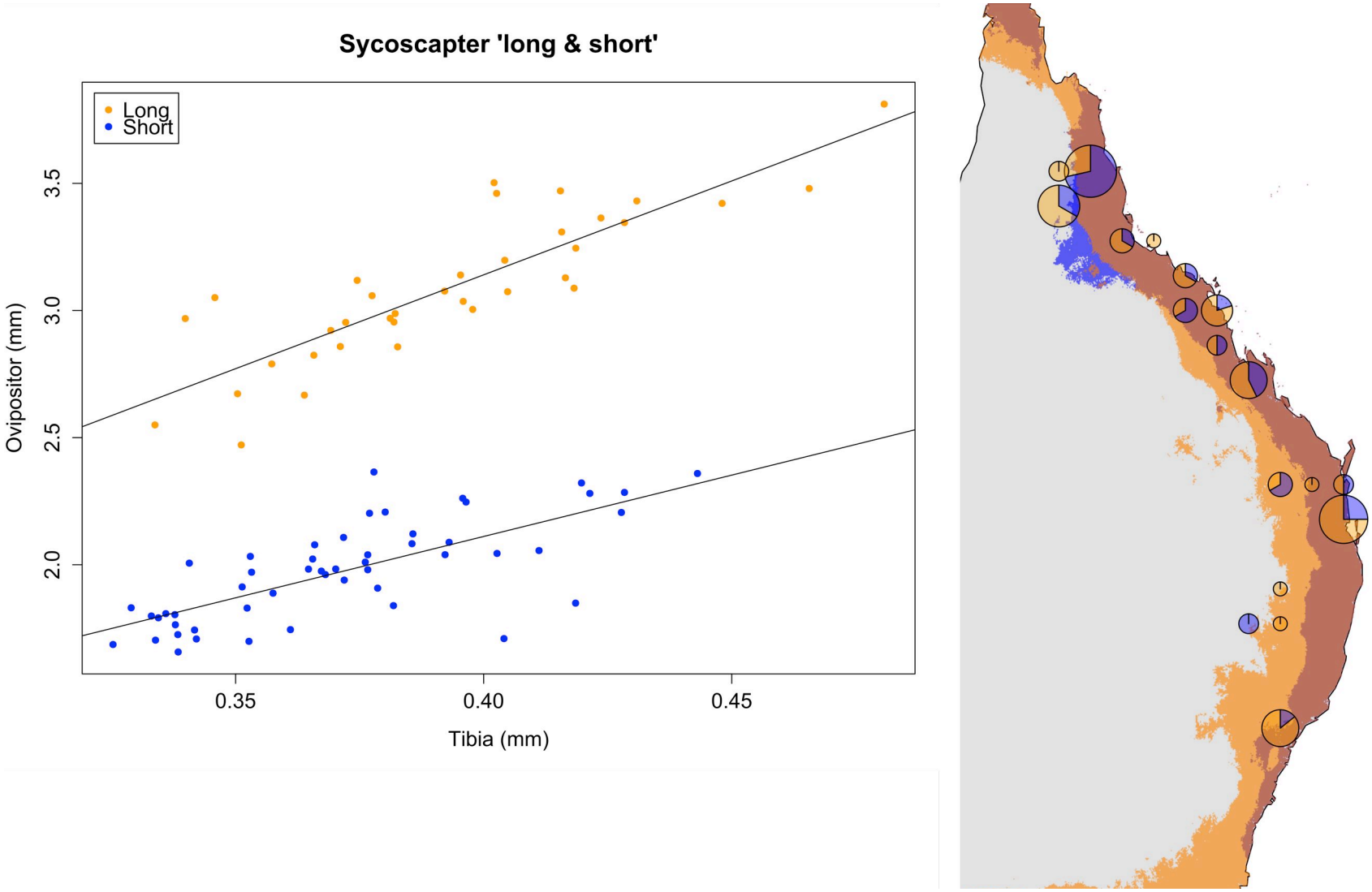
Table 3. ANCOVA results, dissimilarity indices and relationship to congeneric co-occurrence in small NPFW species found on *F. rubiginosa*. ANCOVA: the minimum adequate model method of Crawley (2007) is employed; ov~tib indicates a model with no factorial parameters; ov~tib+spp indicates a model with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

	Sycoscapter **long and short**	***Sycoscapter*** **short**	***Philotrypesis*** **black**	***Philotrypesis*** **yellow**	***Watshamiella*** **long and short**	***Eukobelea***
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^2	R^2=0.944	R^2=0.520	R^2=0.848	R^2=0.928	R^2=0.955	R^2=0.668
F-statistic	$F_{3,83}$=482.6	$F_{1,49}$=55.25	$F_{2,28}$=84.39	$F_{2,76}$=553.2	$F_{2,70}$=781.2	$F_{1,38}$=79.51

Figure legend

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

492 Figure 1a – *Sycoscapter* ‘long & short’



493

Figure 1b – *Sycoscapter* 'short'

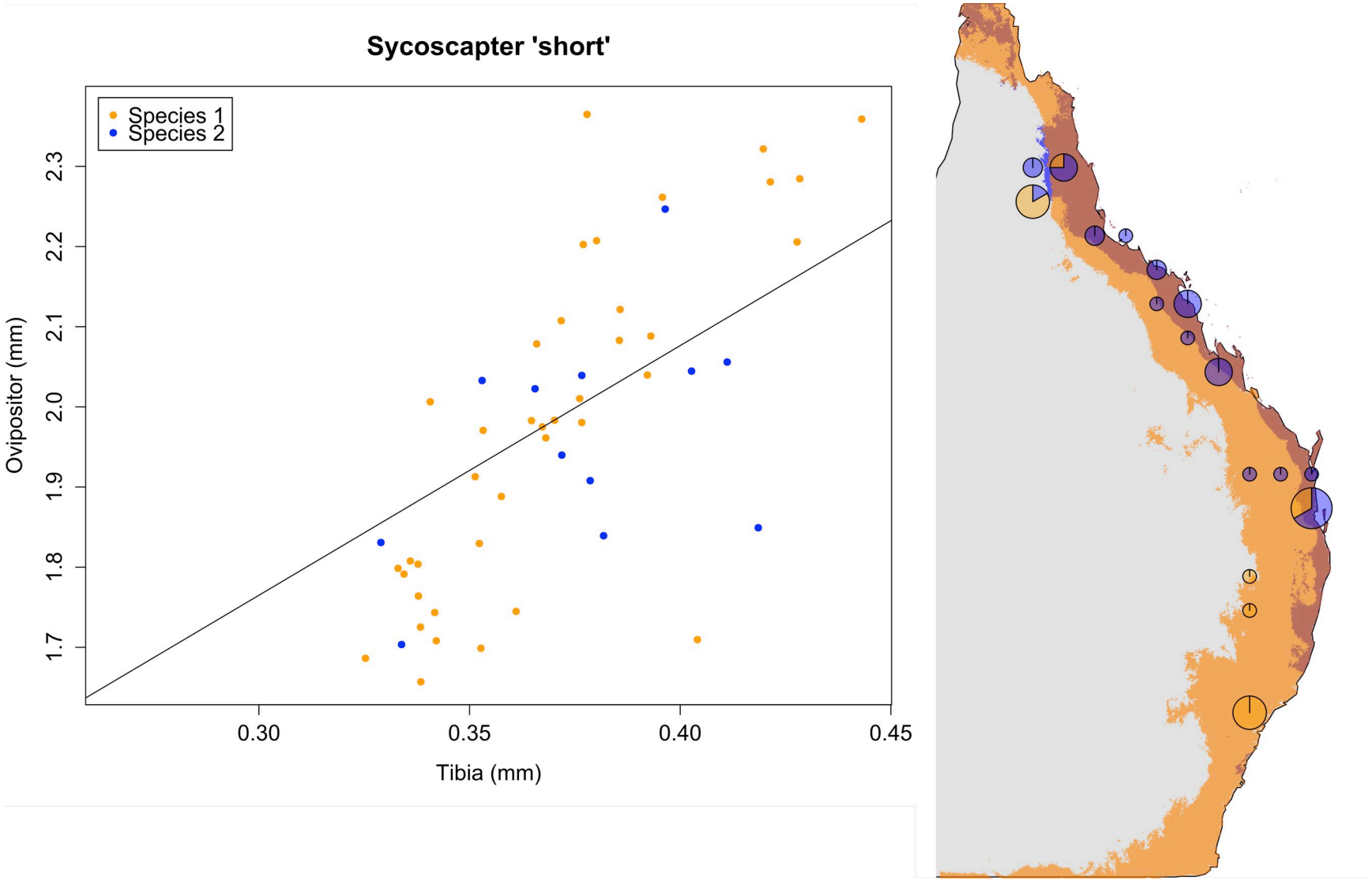
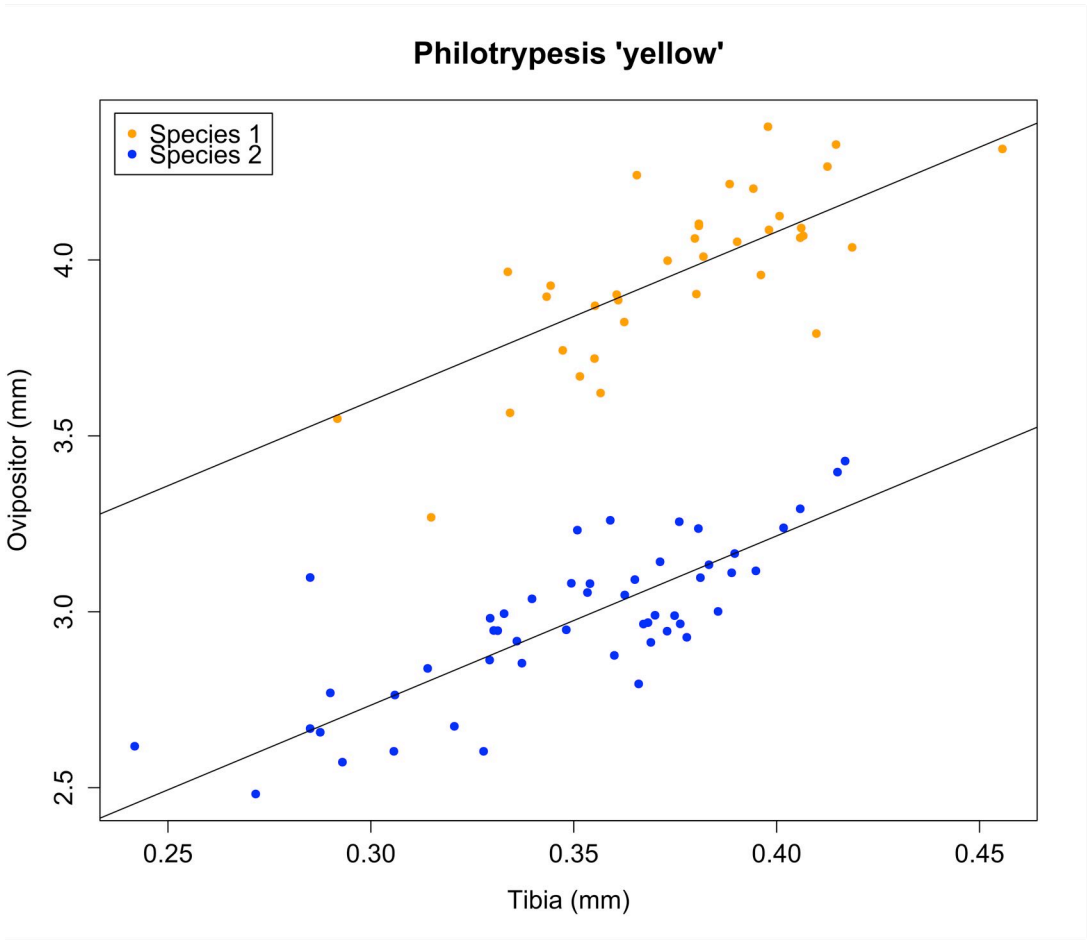
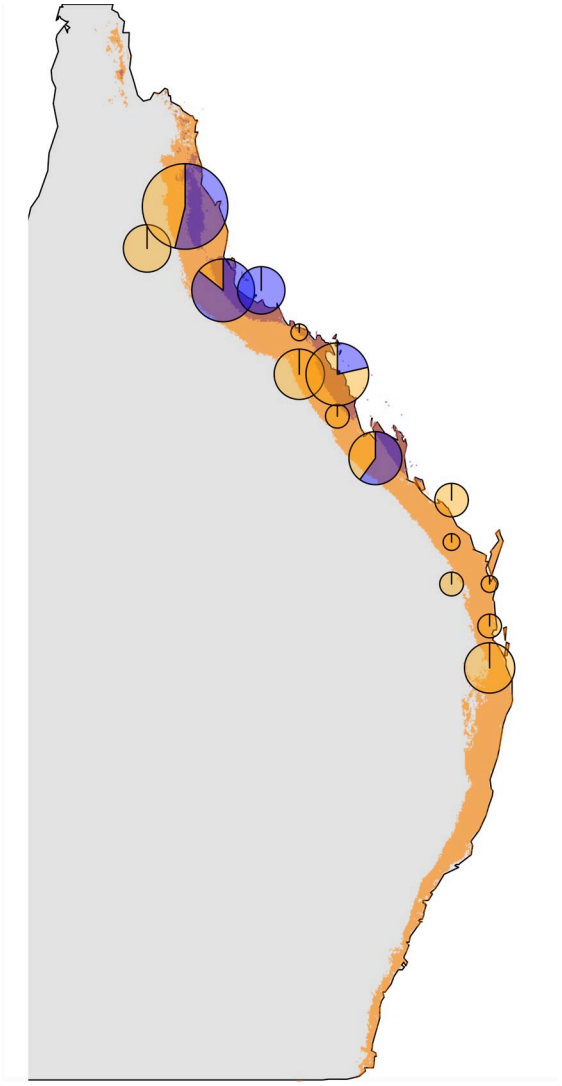
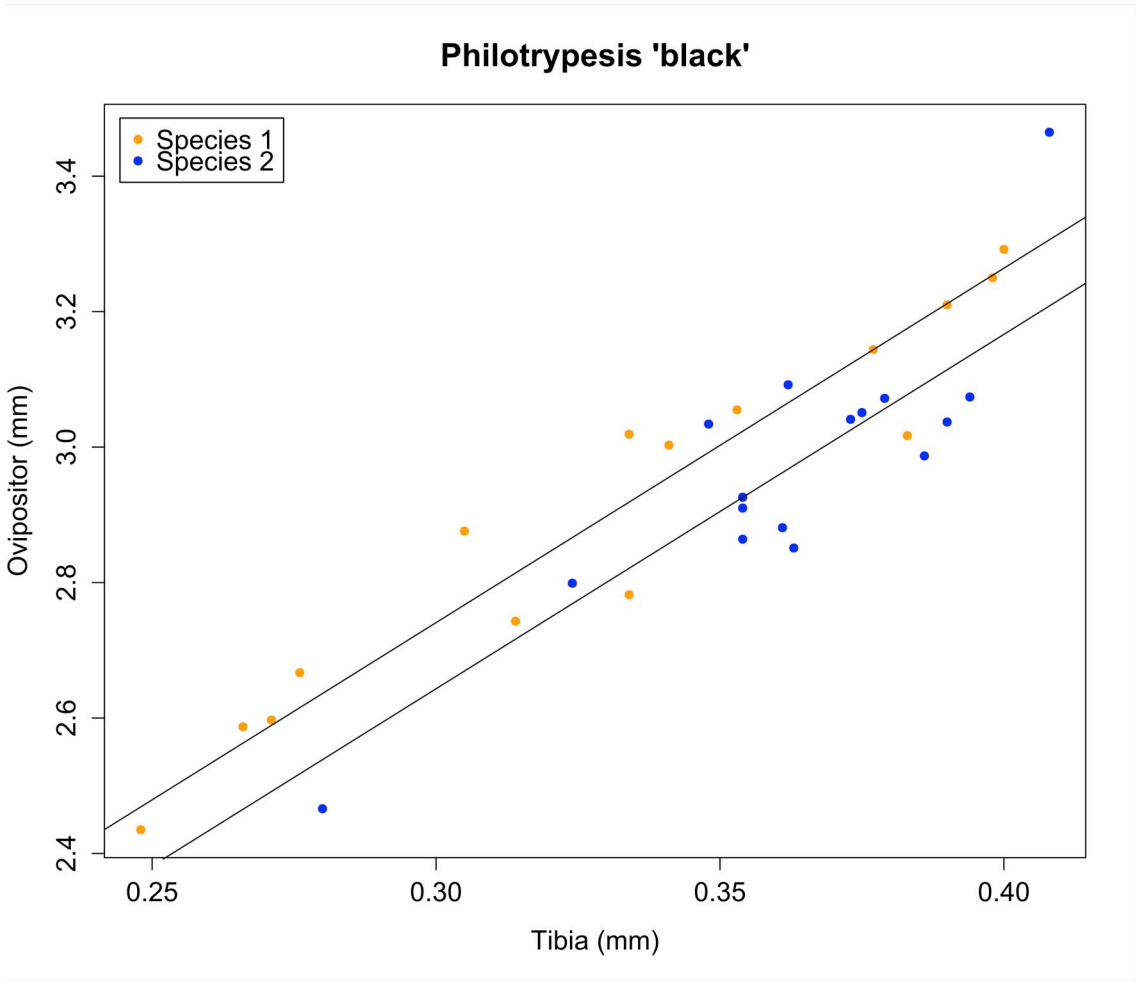


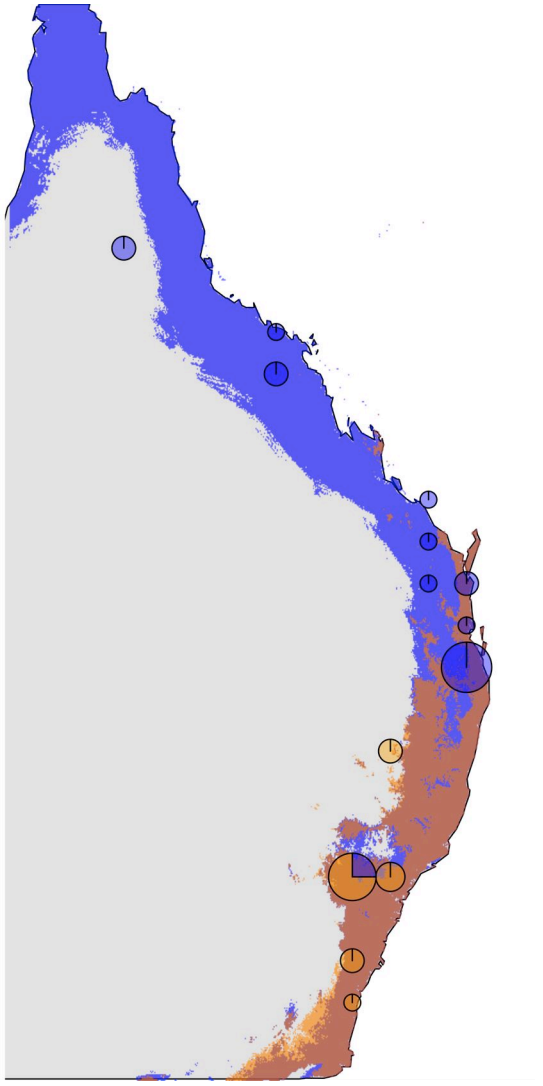
Figure 1c – *Philotrypesis* ‘yellow’





501 Figure 1d – *Philotrypesis* ‘black’

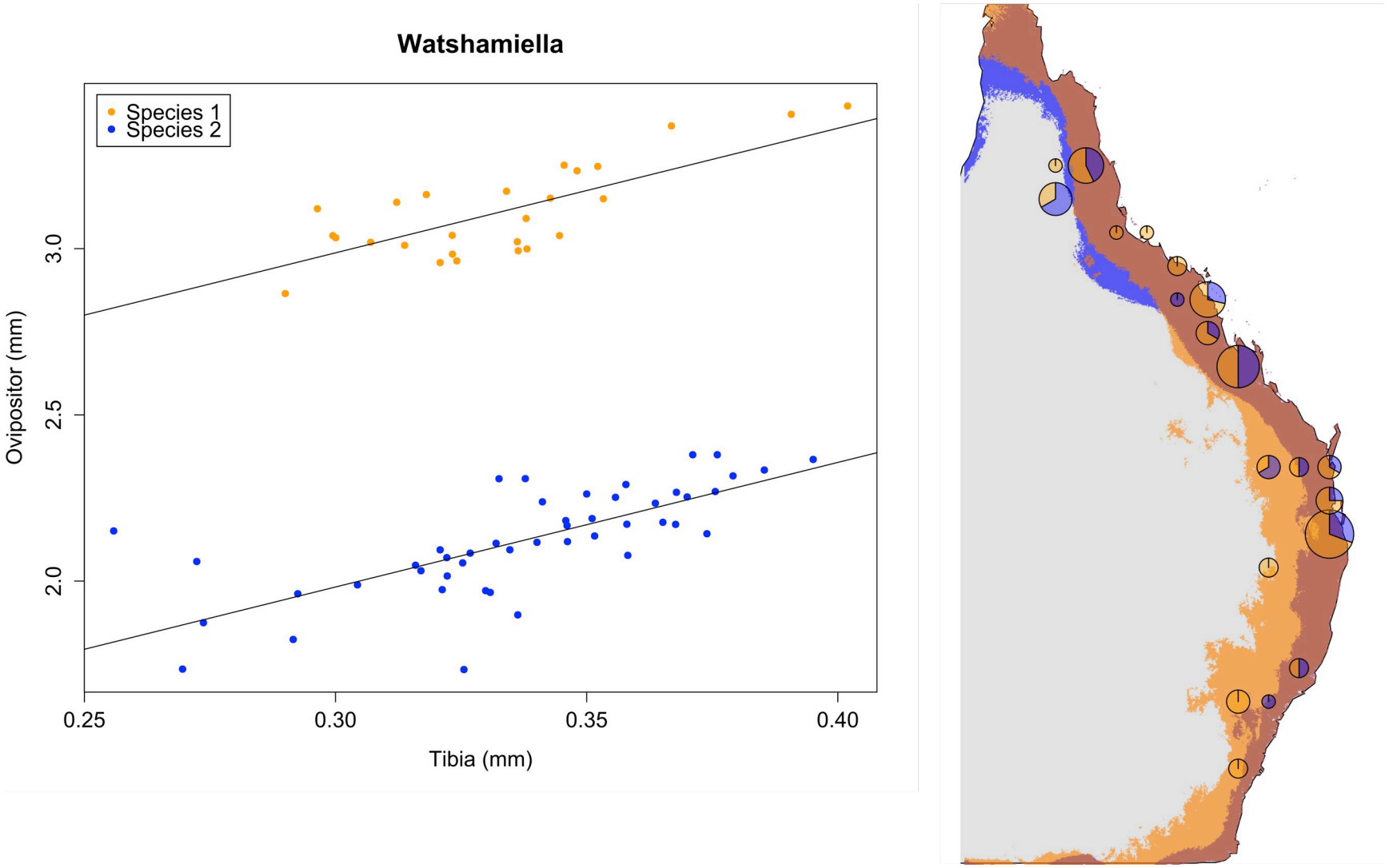




502

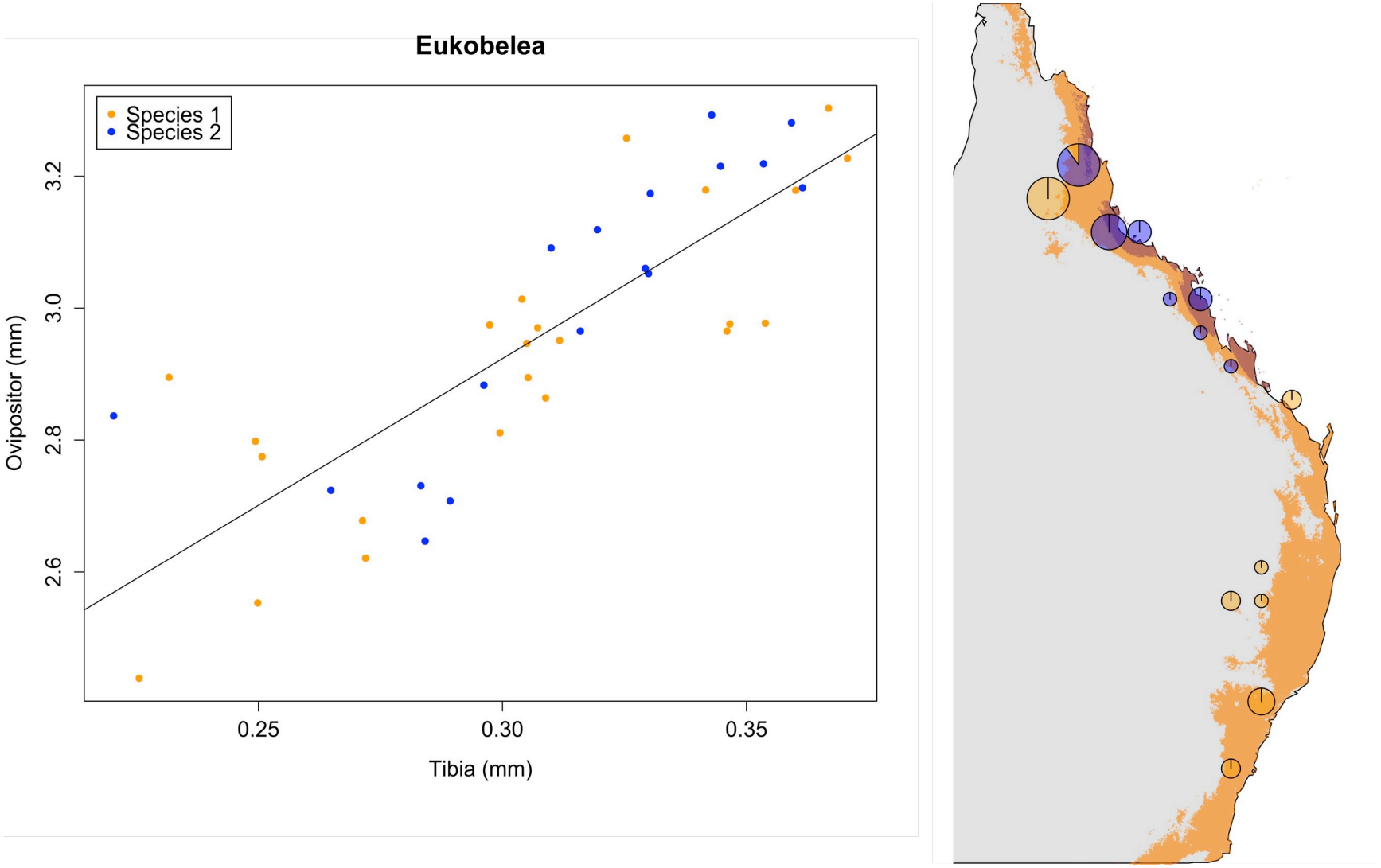
503

504 Figure 1e – *Watshamiella*



505

506 Figure 1f – *Eukobelea*



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

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