A molecular phylogeny of forktail damselflies (genus *Ischnura*) reveals a dynamic macroevolutionary history of female colour polymorphisms

Rachel Blow[1], Beatriz Willink[2], and Erik I. Svensson[3*]

[1] Department of Zoology, University of Cambridge, Cambridge, UK

[2] School of Biology, University of Costa Rica, San José, Costa Rica 11501-2060

[3] Evolutionary Ecology Unit, Department of Biology, Lund University, SE-223 62 Lund, Sweden

*Corresponding author email: erik.svensson@biol.lu.se

Keywords: ancestral state reconstruction, geographic range, morphic speciation, sexual conflict, StarBEAST2, trans-species polymorphism.

Abstract

Colour polymorphisms are popular study systems among biologists interested in evolutionary dynamics, genomics, sexual selection and sexual conflict. In many damselflies, such as in the globally distributed genus *Ischnura* (forktails), female colour polymorphisms occur in some species. Female-polymorphic species contain two or three female morphs, one of which is male-coloured (androchrome or male mimic) and co-exists with sexually dimorphic (heterochrome) females. These female colour polymorphisms are considered to be maintained by frequency-dependent sexual conflict, but their macroevolutionary histories are unknown, due to the lack of a robust molecular phylogeny. Here, we present the first time-calibrated phylogeny of *Ischnura*, using a multispecies coalescent approach (StarBEAST2), incorporating both molecular data and fossil information of 41 extant species (55% of the genus). We estimate the age of *Ischnura* to be between 13.8 and 23.4 millions of years, i.e. Miocene. We infer the ancestral state of this genus as female monomorphism with heterochrome females, with multiple gains and losses of polymorphisms, evidence of trans-species polymorphisms and a significant positive relationship between polymorphism incidence and current geographic range size. Our study provides a robust phylogenetic framework for future research on the dynamic macroevolutionary history of this clade with its extraordinary diversity of sex-limited female polymorphisms.

Introduction

The evolutionary processes shaping within- and between-population genetic diversity are classical research themes in population genetics and evolutionary biology that go back to the early days of the Modern Synthesis (Crow and Kimura, 1970; Dobzhansky, 1970; Fisher, 1930; Haldane, 1949, 1932; Hartl and Clark, 1997; Provine, 1986; Wright, 1932, 1931). Before the development of molecular tools to quantify genetic variation in natural populations, ecological geneticists and evolutionary biologists often studied discrete and heritable phenotypic polymorphisms governed by one or a few loci (Dobzhansky, 1970; Ford, 1975; Provine, 1986; Schemske and Bierzychudek, 2001; Svensson, 2017; Turelli et al., 2001). Since these early days, genetic colour polymorphisms became popular study systems to address questions about the maintenance of genetic and phenotypic variation (Gordon et al., 2015; Llaurens et al., 2017; Mitchell-Olds et al., 2007; Svensson, 2017; Svensson et al., 2009) and the molecular basis behind suites of correlated traits (Andrade et al., 2019; Gangoso et al., 2011; McKinnon and Pierotti, 2010; Sinervo and Svensson, 2002; Willink et al., 2020; Woronik et al., 2019). Colour polymorphisms are widespread among many animal taxa and their genetic basis is often due to multiple segregating alleles at a single locus, with different degrees of dominance in relation to each other (Svensson et al., 2009; Wellenreuther et al., 2014). Such colour polymorphisms provide excellent opportunities to link the microevolutionary processes that maintain genetic variation within populations (Le Rouzic et al., 2015; Svensson, 2017; Takahashi et al., 2014) to macroevolutionary dynamics of discrete and heritable traits (Jamie and Meier, 2020; Willink et al., 2019).

In many colour polymorphic animals, different male morphs are favoured under different ecological and social conditions and the male morphs are often maintained by balancing natural

62 or sexual selection, such as negative frequency-dependent selection or overdominance (Blows
63 et al., 2003; Formica and Tuttle, 2009; Hughes et al., 2013; Lank et al., 1995; Sinervo and
64 Lively, 1996; Tuttle et al., 2016; Wellenreuther et al., 2014). In contrast to many previous
65 studies on male colour polymorphisms, there are still relatively few studies on female colour
66 polymorphisms and other heritable female polymorphisms (Iversen et al., 2019; Karlsson et al.,
67 2013; Svensson et al., 2009). Whereas male colour morphs are often associated with different
68 reproductive strategies and sexual competition over access to females, female colour
69 polymorphisms have been linked to avoidance of male mating harassment and as female
70 defenses in sexual conflict (Lee et al., 2019; Neff and Svensson, 2013; Willink et al., 2019).
71 For instance, in the insect order Odonata (dragonflies and damselflies), female colour
72 polymorphisms occur in several genera and many species (Fincke et al., 2005). Female colour
73 polymorphisms are particularly common in the globally distributed genus *Ischnura* (Gering,
74 2017; Gosden and Svensson, 2009; Sirot and Brockmann, 2001; Takahashi and Watanabe,
75 2010) (Fig. 1) within the superfamily Coenagrionoidea, which includes pond damselflies and
76 their relatives (Dijkstra et al., 2014).
77
78 Within the damselfly genus *Ischnura,* there are both female-monomorphic and female-
79 polymorphic species (Fincke et al., 2005; Sanchez-Guillen et al., 2011; Willink et al., 2019).
80 Of the approximately 75 species in the genus *Ischnura*, c.a. 40% are female-monomorphic,
81 having only one distinct female phenotype, whilst another 40% have two, and some species
82 even have three female morphs (Willink *et al.* 2019). The female-morph status of the remaining
83 20% of species is currently unknown (Willink *et al.* 2019). In female-polymorphic species of
84 *Ischnura*, females are either phenotypically similar in colouration and sometimes also body
85 shape to males (referred to here as androchrome females, or male mimics) (Gosden and
86 Svensson, 2009), or they are phenotypically different from males (referred to here as

87 heterochrome females). These colour polymorphisms are limited in their expression to females,
88 and males are monomorphic (Svensson et al., 2020, 2009a). Such female colour polymorphisms
89 are particularly suited for phylogenetic comparative studies of the macroevolution of discrete
90 and heritable morphs.
91

92 The classical explanation for the evolutionary origin and adaptive function of female colour
93 polymorphisms in *Ischnura,* and other damselflies is the "male-mimicry hypothesis"
94 (Robertson 1985; Cordero 1989). Male-mimics are thought to have evolved as a response to
95 selection from sexual conflict over mating, which favoured visual features that made such male-
96 like females less recognizable as potential mates to males (Cordero et al., 1998; Gosden and
97 Svensson, 2009; Willink et al., 2019; Xu et al., 2014). The mimicry hypothesis predicts that the
98 common ancestor of polymorphic damselflies was a monomorphic species with heterochrome
99 females, which was invaded after a novel male mimicking female phenotype emerged that
100 subsequently increased in frequency due to a frequency-dependent fitness advantage of male
101 mimicry. An alternative, though not mutually exclusive hypothesis for the evolution and
102 maintenance of such female polymorphisms are flexible search images in males (Fincke, 2004;
103 Miller and Fincke, 1999). Under this hypothesis, males are expected to switch mating
104 preferences between morphs, depending on their frequency, resulting in increased male
105 preferences for currently common morphs (Van Gossum et al., 2001). The most common
106 female morph is then expected to be disproportionally harassed, resulting in negative
107 frequency-dependent selection (NFDS) and the stable maintenance of the polymorphism across
108 many generations (Gosden and Svensson, 2009; Iserbyt et al., 2013, 2011; Le Rouzic et al.,
109 2015; Svensson et al., 2005; Takahashi et al., 2014, 2010). Unlike a pure version of the male-
110 mimicry hypothesis, female colour polymorphisms in *Ischnura* could under this latter scenario
111 have evolved from an ancestral state of either heterochrome or androchrome females and with

female monomorphism. We re-emphasize that these two hypotheses are not mutually exclusive, and both can operate simultaneously, with a crucial role for NFDS maintaining female polymorphisms under both scenarios. Regardless of mechanism, NFDS is therefore thought to be the main force maintaining such female polymorphisms (Le Rouzic et al., 2015; Svensson et al., 2005; Takahashi et al., 2010).

Female-monomorphic species of *Ischnura* have received less attention compared to female-polymorphic taxa, and consequently we know very little about why there is such a striking variation in the presence or absence of female-limited polymorphisms in different subclades within this genus. One explanation could be that female-monomorphic lineages reflect an ancestral state with low intensity of sexual conflict. If so, female polymorphisms may have subsequently evolved in lineages experiencing novel selective regimes of more intense sexual conflict. This could occur, for instance, due to a rapid increase in population density or transition to a new mating system. Alternatively, female-monomorphic lineages could reflect transitions from an ancestral state of female-limited polymorphism, via founder effects or genetic drift, or through the selective fixation of one of the alternative previously sympatric morphs (Corl et al., 2010; West-Eberhard, 1986). Such speciation by morph loss has been termed “morphic speciation” (Corl et al., 2010; West-Eberhard, 1986). The morphic speciation hypothesis explicitly predicts that monomorphic taxa should primarily be located at the terminal tips on a phylogeny, with polymorphism being the ancestral state (Corl et al., 2010). Regardless of the generality and validity of the morphic speciation hypothesis, our knowledge about the relationship between polymorphism andspeciation is limited and there are only a few phylogenetic comparative studies in this area (Hugall and Stuart-Fox, 2012; McLean and Stuart-Fox, 2014; Schwander and Leimar, 2011). Recently, it has also been emphasized that

136 some colour polymorphisms can be maintained across speciation events resulting in
137 transspecies polymorphisms (Jamie and Meier, 2020).
138
139 There have been a few previous comparative studies in the genus *Ischnura,* aiming to
140 understand the ecological correlates of colour polymorphisms and their evolutionary origin.
141 These previous studies suggested that female-limited colour polymorphisms are more common
142 in species with female polyandry, than in species with (putative) female monandry, consistent
143 with polymorphism being promoted by sexual conflict (Mattern and Van Gossum, 2008;
144 Robinson and Allgeyer, 1996). However, these previous comparative studies suffer from
145 several limitations, as they did not take into account the shared evolutionary history of related
146 species (Robinson and Allgeyer, 1996) or they used highly sensitive methods to infer ancestral
147 character states such as maximum parsimony (Mattern and Van Gossum, 2008). The use of
148 maximum parsimony is particularly problematic as it provides poor estimations of tree
149 topologies (Wright and Hillis, 2014) and unreliable inferences of ancestral character states,
150 especially for rapidly evolving characters like behavioural traits (Cunningham et al., 1998;
151 Schluter et al., 1997). For these reasons, inferences of ancestral character states should
152 preferably come from Bayesian or Maximum Likelihood (ML) approaches (Cunningham et al.,
153 1998; Schluter et al., 1997; Wright and Hillis, 2014). A previous study claimed, based on the
154 results from from maximum parsimony, that female colour polymorphism was the ancestral
155 state in *Ischnura* (Mattern and Van Gossum, 2008). Such an inferred polymorphic ancestral
156 state in *Ischnura* is, however, unlikely to be robust given the problems with maximum
157 parsimony, and this hypothesis would therefore need to be re-evaluated, using updated
158 phylogenetic information and more reliable modern comparative methods.
159

160 Here, we present novel phylogenetic and comparative data and analyses from the genus
161 *Ischnura* aimed to shed light on the origin of female colour polymorphisms, and we also
162 estimated the age and studied the evolutionary history of this genus. We use a multispecies
163 coalescent model to simultaneously estimate a posterior distribution of species tree topologies
164 and divergence times for 55% of species in the genus *Ischnura.* We then investigate the
165 macroevolutionary history of female-limited polymorphisms by reconstructing the ancestral
166 state of this trait. We proceed by examining whether the presence of female-limited colour
167 polymorphisms are associated with large geographic range sizes, a factor that is likely to reflect
168 population size and the potential for morphic speciation by neutral processes. A positive
169 association between the presence of female colour polymorphisms and geographic range would
170 be expected in both of the following scenarios: 1) if morphs are lost by neutral or selective
171 processes after a reduction of population (and range) size or 2) if the effects of female colour
172 polymorphisms on population fitness allow female-polymorphic species to sustain larger
173 populations over a greater geographic range.

174

175 Our study contributes to our understanding the macroevolutionary history of this genus, the
176 origin of female colour polymorphism and the demographic factors promoting the maintenance
177 of these polymorphisms. In presenting these results, we hope to stimulate future empirical
178 investigations on these and other damselfly clades, and we discuss some promising hypotheses
179 for follow-up studies. We note that many damselfly groups, although popular in much
180 ecological and evolutionary research (Cordoba-Aguilar, 2008) are still largely unknown with
181 respect to phylogenetic relationships and evolutionary history, although some recent progress
182 has been made, particularly within the family Coenagrionidae (Beatty et al., 2017; Callahan and
183 McPeek, 2016; Dijkstra et al., 2014; Swaegers et al., 2014; Torres-Cambas et al., 2019; Vega-
184 Sánchez et al., 2019).

185

186 **1. Materials and Methods**

187

188 *2.1 Data collection*

189 We have gathered DNA-sequence data, phenotypic data and geographic range size data for
190 41 species of *Ischnura*. Two of the taxa in this dataset are nominally in the genus *Pacificagrion*,
191 however recent molecular data shows they are nested within the *Ischnura* clade (Willink et al.
192 2019). Likewise, two other taxa, including a yet undescribed species, were classified in the
193 genus *Amorphostigma* (Marinov et al., 2015). However, Dijkstra et al. (2014) subsumed
194 *Amorphostigma* into *Ischnura*, and recent molecular data support this change (Willink et al.
195 2019). Here, we use the generic names *Ischnura* and *Pacificagrion*, according to the "World
196 Odonata List" (https://www.pugetsound.edu/academics/academic-resources/slater-
197 museum/biodiversity-resources/dragonflies/world-odonata-list2/), which maintains a
198 taxonomic classification of Odonata with currently valid names.

199

200 We obtained sequences for five molecular markers including nuclear (H3: 28.86 kb, PMRT:
201 51.68 kb, 28S: 67.91 kb) and mitochondrial regions (COI: 57.72 kb, 16S: 47.64 kb) from 147
202 specimens, of which 64 were sequenced for this study (Table S1). Genomic DNA was extracted
203 from 1-2 legs using a Qiagen DNeasy tissue kit and following the manufacturer's instructions,
204 except that we increased the amount of proteinase K and incubation time for museum samples
205 collected before 2005, in order to obtain a higher DNA yield. Polymerase chain reaction (PCR)
206 primers and amplification programs used for the molecular markers were the same as in Willink
207 et al. (2019). Amplification products were purified using the Affymetrix ExoSAP-IT reagent
208 (Cat. No. 78200) and sequenced on an ABI 3100 capillary sequencer at Lund University. These
209 novel sequence data were complemented with already-published sequences (Table S1),

210 downloaded from the National Centre for Biotechnology Information
211 (https://www.ncbi.nlm.nih.gov/genbank/). There were no indels in the sequences of two
212 molecular markers (COI and H3), which were aligned using MUSCLE (Edgar, 2004). To
213 reduce gap overmatching in ribosomal sequences (16S and 28S) and intronic regions (present
214 in PMRT), we used the phylogenetically informed algorithm PRANK (Löytynoja and
215 Goldman, 2008, 2005) to align these data.
216
217 Data on female colour morph status (i.e. female-monomorphic, or female-polymorphic) has
218 been recently compiled for *Ischnura* damselflies, based on published literature, field
219 observations, examination of museum specimens and online resources (Willink et al. 2019).
220 We complemented these data with information on whether female-polymorphic species had
221 two or three morphs (i.e. female-dimorphic or female-trimorphic) (Table S2). Here, species
222 with a single female morph were classified as female-monomorphic with androchrome females,
223 if they had only one female morph with colouration and patterning similar to that of conspecific
224 males, or as female-monomorphic with heterochrome females, if they had only one female
225 morph with colouration and patterning differing from the conspecific males. If a species
226 contained two distinct female morphs (always one androchrome and one heterochrome), we
227 classified it as female-dimorphic. Finally, species that had three morphs (which were always
228 one androchrome and two heterochrome female morphs), were classified as female-trimorphic.
229
230 We complemented these colour-state data with coarse-grained estimates of geographic range
231 size (Table S2). Because accurate geographic distribution data is lacking for many species, we
232 estimated geographic range using occurrence data from administrative regions, mainly
233 countries, and where available, states or provinces (Table S2). Presence data was translated to
234 geographic range size (km^2) by adding the areas of all the countries or other administrative

235 regions where a species is known to occur (Table S2), as has been done previously in
236 comparative studies where geographic range is taken as a proxy for population size (Ross et al.,
237 2013). The areas of administrative regions were extracted from the GADM database of global
238 administrative areas v. 3.6 (Global Administrative Areas, 2018).
239

240 1.2 *Time-calibrated species tree*

241 We analysed sequence data under a multispecies coalescent model using StarBEAST2 (Ogilvie
242 et al., 2017). As the model assumes free recombination amongst unlinked loci, we linked the
243 tree models between mitochondrial sequences. We placed a birth-death prior, conditional on
244 the root, on the species tree to allow for extinction, set the gene ploidy to 0.5 for mitochondrial
245 sequences and 2.0 for nuclear sequences and allowed population-size to be analytically
246 integrated to avoid over-parameterization. We used a relaxed uncorrelated log normal (UCLN)
247 clock per locus to allow for variation in evolutionary rates between lineages. Substitution
248 models for each locus were inferred during the analysis using the package "bModelTest"
249 (Bouckaert and Drummond, 2017) to allow for site model uncertainty. We placed a lognormal
250 prior with a mean of 0.0115 and standard deviation of 1.0 on each relaxed mitochondrial clock
251 and a broader lognormal prior with a mean of 0.0115 and standard deviation of 2.0 on each
252 relaxed nuclear clock to centre clock rates on the suggested insect mitochondrial divergence
253 rate of 2.3% per million years (Brower, 1994) whilst allowing for clock rate uncertainty,
254 particularly in the nuclear loci.
255

256 Data for *Ischnura* fossils were downloaded from the Paleobiology database (www.pbdb.org)
257 on 14th May 2018. There were three fossils classified as either in or closely related to the genus
258 *Ischnura*. Two of these fossils were relatively young (7.2 - 5.3 mya), each one consisting of a
259 single wing with missing fragments (Gentilini and Bagli, 2004). The third fossil was preserved

260 in Dominican amber (20.44 – 13.82 mya) and was identified and described as *Ischnura velteni*
261 based on a combination of wing venation and morphological traits in other body parts (Bechly,
262 2000). The combination of these characters suggests that *I. velteni* belongs to the extant genus
263 *Ischnura* (Bechly, 2000), and we therefore considered it the most appropriate crown fossil with
264 which to calibrate the root age of the *Ischnura* tree. We placed an exponentially distributed
265 prior on the root age of the species tree with an offset of 13.8 to ensure the most recent common
266 ancestor (MRCA) of *Ischnura* could not have appeared after the minimum age of this known
267 member of the genus. The mean of the exponential was drawn from a gamma hyperprior with
268 a shape parameter $\alpha = 5.0$ and an inverse scale parameter $\beta = 2.0$. This hyperprior specification
269 centred the root age prior distribution around 10 mya prior to the offset, allowing a moderately
270 high level of uncertainty in the age difference between the fossil and the MRCA of *Ischnura.*
271
272 We ran two separate Markov Chain Monte Carlo (MCMC) algorithms for 200 million iterations
273 with a sampling rate of 20,000. We confirmed that independent chains were stationary and had
274 converged for these and all subsequent analyses in R v.3.6.1 (R Core Team 2019) using the
275 "coda" package (Plummer et al., 2006). We combined posterior trees using LogCombiner
276 (available as part of BEAST v2.4.7)(Bouckaeret al., 2014), simultaneously discarding 20% of
277 the posterior as burnin, and summarized them into a maximum clade credibility (MCC) tree
278 using TreeAnnotator (available as part of BEAST v2.4.7). The MCC tree and all further
279 phylogenetic plots were annotated in R v.3.6.1 (R Core Team 2019) using the packages "ape"
280 (Paradis and Schliep, 2019), "ggtree" (Yu et al., 2017) and "ggplot2" (Wickham, 2016).
281
282 1.3 *Reconstruction of ancestral character states of female colour polymorphism*
283 We used the Multistate-method in BayesTraits version 3.0 (Pagel et al., 2004) to infer female-
284 colour character states at ancestral nodes, given this new *Ischnura* phylogeny and the female-

colour character states of extant species. To account for phylogenetic uncertainty, we ran the model using a sample of 1000 trees from the combined species-tree posterior of the two independent runs. Additionally, we ran the model using reversible jump MCMC to account for uncertainty in the number of model parameters. In this model, the number of different transition rate parameters and their values are sampled in proportion to their posterior probability. Consequently, in each posterior sample a number of transition rates are set to zero, and the non-zero rates belong to one or more categories. We placed an exponential prior on all rate parameters, with the mean seeded from a uniform hyperprior bounded between 1 and 100. We ran two separate chains for 10 million iterations, sampling every 1000 with 20% posterior burnin. Trees were scaled to an average branch length of 0.01 to prevent various rates from becoming too small. Although our main focus is on ancestral state reconstructions, we present the posterior distributions of transition rate parameters in the Supporting Material (Fig. S1).

1.4 *Linking female colour polymorphism to geographic range size*

We investigated if the occurrence of female colour polymorphisms could be influenced by demographic changes by using geographic range size of extant species as a proxy for population size. Geographic range size is also a biologically interesting variable in the light of recent work showing that female colour polymorphisms can increase population fitness (Takahashi et al., 2014). e used a Bayesian phylogenetic mixed-effect model (BPMM)(Hadfield and Nakagawa, 2010) implemented in the package MCMCglmm v. 2.29 (Hadfield, 2010) to test for such phylogenetic correlation. Female-morph status was treated as a binary response variable, with species being classified as either female-monomorphic or female-polymorphic. Since the occurrences of female-trimorphic species and female-monomorphic species with androchrome females were low, we classified these species-states as being female-polymorphic and female-monomorphic, respectively. We thus investigated if the probability of a species being female-

310 polymorphic were significantly and positively related to geographic range size (a continuous
311 predictor variable).
312
313 We accounted for phylogenetic uncertainty by using a random sample of 2100 species-trees
314 generated in StarBEAST2. To estimate the phylogenetic random effect, each tree was sampled
315 for 10000 iterations, of which only the last iteration was saved to the posterior, and the first 100
316 were also discarded as burnin (O'Connor et al., 2018). We used a Knonecker prior (mu=0, V=
317 σ2units + π2/3) for the fixed effects as it is relatively flat when a logit link function is used
318 (Hadfield, 2015). We used a parameter-expanded χ^2 distribution (V=1 nu=1000, alpha.mu=0,
319 alpha.V = 1) for the prior on the phylogenetic variance components (Villemereuil et al., 2013).
320 The residual variance was fixed to 1, as it cannot be identified for binary response models. As
321 a statistical test of whether the probability of female colour polymorphism increases with range
322 size, we report the fraction of the posterior distribution in which increasing range size does not
323 result in an increasing probability of female colour polymorphism. The results of this analysis
324 were visualized by plotting the expected probability of female colour polymorphism against
325 estimated geographic range size.
326
327 **2. Results**
328
329 3.1 *Divergence time estimation*
330 Our time-calibrated phylogeny suggests that the age of the MRCA of the genus *Ischnura* lies
331 in a 95% highest posterior density (HPD) interval between 13.8 and 23.4 mya, i.e. during the
332 early Miocene (Fig. 2). A comparison of the posterior root age in our model to a model ran
333 without molecular data suggests the sequence data were sufficiently informative to narrow this
334 posterior distribution (Fig. 3), although age estimates had quite broad error margins (Fig. 2).

335 Nonetheless, divergence dating revealed several interesting features, such as the very recent
336 divergence of two of the most intensely studied polymorphic species in Europe (*I. elegans* and
337 *I. graellsii*) (Fig. 2). Our phylogenetic analysis also showed that the species that feature in most
338 evolutionary and ecological studies (*I. elegans*, *I. graellsii*, *I. ramburii* and *I. senegalensis*) are
339 part of awell supported clade (Clade II excluding *I. capreolus* and *I. barberi;* Fig. 2), with a
340 common ancestor less than 10 mya (Fig. 2). We found that all species from Australia and the
341 South Pacific islands, except *I. heterosticta,* form a single clade (Clade I; Fig. 2). Similarly, we
342 recovered a strongly supported Palearctic clade (Clade III; Fig. 2) and strongly supported clades
343 distributed primarily in the Neotropical (*I. prognata* to *I. cruzi*) and Nearctic (*I. verticalis* to *I.*
344 *kellicotti*) regions (Clade IV; Fig. 2).

345

346 2.2 *Ancestral state reconstruction of female colour polymorphism*

347 Out of the 41 species included in this study, 13 were female-monomorphic with heterochrome
348 females (**category 1**), 22 were female-dimorphic (**category 2**), three were female-trimorphic
349 (**category 3**) and finally three were female-monomorphic with androchrome females (**category**
350 **4**) (Fig. 4). The MRCA of *Ischnura* was reconstructed as being female-monomorphic with
351 heterochrome females, i.e. the ancestor was sexually dimorphic, with an mean posterior
352 probability (PP) of 81% (Fig. 4). Clade I (Fig. 2), which includes most taxa from the Pacific
353 islands was also inferred as having a heterochrome female ancestor (PP = 81%; Fig. 4). All
354 female-trimorphic species shared a female-trimorphic common ancestor (PP = 100%; Fig.
355 4).These taxa are included in Clade II, for which the most probable ancestral state if female
356 dimorphism (PP = 73%; Fig. 4).

357

358 Two evolutionary transitions occurred with high posterior probability during the diversification
359 of *Ischnura*, according to the RJ MCMC analysis. The transition rate from female colour

360 dimorphism (**category 2**) to female colour monomorphism with androchrome females
361 (**category 4**) (i.e. the evolutionary loss of the heterochrome female morph) was above zero in
362 99.7% of the posterior (Fig. S1). Similarly, the transition from female colour monomorphism
363 with heterochrome females (**category 1**) to female colour dimorphism (**category 2**) (i.e. the
364 evolutionary gain of an androchrome female morph) occurred with non-zero rate in 94% of the
365 posterior (Fig. S1). In contrast, the least probable transition, from female colour monomorphism
366 with heterochrome females (**category 1**) to female colour trimorphism (**category 3**) occurred
367 in only 7% of the posterior (Fig. S1). The extant data on female-colour states and the posterior
368 distribution of *Ischnura* time trees are therefore consistent with multiple evolutionary histories
369 and different combinations of other character-state transitions being present or absent.
370

371 3.3 *Geographic range size and female-colour polymorphism*

372 Current geographic range sizes varied from 4.3 x 10^7 Km^2 in the largely subtropical species *I.*
373 *senegalensis*, which is distributed from sub-Saharan Africa to Japan, to 222 Km^2 to species
374 endemic to American Samoa (Table S2). We found that all island-endemic species are female-
375 monomorphic, except for *I. foylei*, which is female-dimorphic and only known from the Jambi
376 province in Sumatra, Indonesia (Table S2). In contrast, the three species distributed across the
377 longitudinal range of the Old World, *I. senegalensis*, *I. pumilio* and *I. elegans,* are either female-
378 dimorphic or -trimorphic. The probability of a species being female-polymorphic increased
379 significantly with its geographic range size (PMCMC = 0.003; Fig. 5). The probability of
380 female colour polymorphism increased by ~ 5% (posterior mean = 0.051, 95% HPD interval =
381 0.008 – 0.990) with an increase of 1 x10^6 Km^2 in the geographic range size.
382
383
384

385 **4. Discussion**

386 Here, we have presented the first molecular time-calibrated phylogeny for the damselfly genus
387 *Ischnura*, an insect group that has been subject to much interest in evolutionary and ecological
388 research, particularly sexual selection, sexual conflict and female colour polymorphism (Fig.
389 1). The general branching patterns in this new phylogeny are largely and qualitatively in
390 agreement with previous molecular phylogeniescovering a subset of the taxa included here
391 (Chippindale et al., 1999; Willink, 2018; Willink et al., 2019). Both dragonflies (suborder
392 Anisoptera) and damselflies (suborder Zygoptera) have long been popular study organisms in
393 ecology, evolution and conservation biology (Cordoba-Aguilar, 2008). However, time-
394 calibrated molecular phylogenies that could facilitate phylogenetic comparative studies in these
395 two insect groups are still relatively few, although recently the situation has been improving
396 (Callahan and McPeek, 2016; Damm et al., 2010; Letsch et al., 2016; Waller and Svensson,
397 2017).

398

399 Using a UCLN molecular clock and fossil calibration, we inferred the MRCA of *Ischnura* as
400 living between 13.8 and 23.4 mya, that is, the early Miocene. This is earlier than the MRCA of
401 the closely related damselfly genus *Enallagma*, which has an estimated age of about 10 my
402 (mid- to late Miocene), and where many speciation events seem to have been driven by glacial
403 cycles during the Pleistocene (Callahan and McPeek, 2016). An early Miocene origin of
404 *Ischnura* is also slightly earlier than the inferred origin of the closely related Palearctic
405 damselfly genus *Coenagrion,* during the later part of Miocene 15 mya (Swaegers et al., 2014)
406 and the two genera *Melanosobasis* and *Nesobasis* in Fiji originating during late-middle
407 Miocence (8-12 mya)(Beatty et al., 2017). We emphasize that our age estimate for the MRCA
408 of *Ischnura* rests on the assumption that *I. velteni* is a crown fossil (Bechly 2000). Future studies

409 based on alternative dating strategies will be important to validate this inferred early Miocene
410 origin of *Ischnura*.

411

412 According to our phylogeny and ancestral state reconstruction, all of the well-studied species
413 in *Ischnura*, *I. senegalensis*, *I. graellsii, I. elegans* and *I. ramburii* (Gering, 2017; Robertson,
414 1985; Sirot et al., 2003; L.K. Sirot and Brockmann, 2001) are part of a single clade, likely
415 descended from a female-dimorphic ancestor (Fig. 4), that lived less than 10 mya (Figs. 2,4).
416 While the genus *Ischnura* has increasingly attracted attention in ecological an
417 microevolutionary studies, there is much untapped potential in understanding its
418 macroevolutionary history and repeated transitions between female colour states. Our ancestral
419 state reconstruction suggests that the ancestor of the genus *Ischnura* was female-monomorphic
420 with heterochrome females, although we caution that the support for this inference was 81%
421 (Fig. 4). Such a sexually dimorphic ancestor would imply multiple evolutionary transitions to
422 obtain female-dimorphism, through the evolutionary emergence of a male-mimic. This
423 evolutionary transition occurred with non-zero rate in 94% of the posterior distribution of
424 transition rate estimates in our RJ MCMC analysis (Fig. S1). This suggests that female colour
425 polymorphism, with heterochrome and androchrome females coexisting within species, has
426 evolved convergently multiple times in this genus, presumably as an evolutionary response to
427 increased intensity of sexual conflict. Specifically, the emergence of a novel male-mimic might
428 have have been possible if it gained an initial fitness benefit due to advantages in intersexual
429 mimicry through reduced male mating harassment (Robertson, 1985; Svensson et al., 2009;
430 Willink et al., 2019). Such male-mimics might have subsequently increased to high frequencies
431 in several populations and species, and later became maintained as stable polymorphisms by
432 frequency- and/or density dependent sexual selection caused by male mating harassment

433 (Gosden and Svensson, 2009; Le Rouzic et al., 2015; Svensson et al., 2005; Takahashi et al.,
434 2014, 2010).

435

436 We further note that the female-trimorphic clade, containing the well-studied European species
437 *I. elegans* and *I. graellsii,* most likely evolved from a female-dimorphic ancestor (Fig. 4). The
438 evolutionary emergence of a third female morph in an already female-polymorphic system
439 might indicate that sexual conflict through male mating harassment was particularly intense in
440 this clade, which would fit with available empirical evidence from previous field and
441 experimental studies (Cordero, 1992; Cordero et al., 1998; Gosden and Svensson, 2009;
442 Takahashi et al., 2014; Willink et al., 2019). In support of this, *I. graellsii* and *I. elegans* have
443 some of the longest copulation durations of all investigated species so far and mate for several
444 hours (Robinson and Allgeyer, 1996). The long copulation duration in these two female-
445 trimorphic species contrasts with the much shorter copulations and lower mating rates of some
446 of the North American monomorphic female species, some of which have also been suggested
447 to be monandrous (Robinson and Allgeyer, 1996). Thus, high mating rates, high degree of
448 density-dependent male mating harassment (Gosden and Svensson, 2009) and long copulation
449 duration (Robinson and Allgeyer, 1996) in the female-trimorphic clade containing *I. elegans*
450 and *I. graellsii* might all have contributed to the evolution and maintenance of a new morph in
451 these species. Models of sexual conflict suggest that an evolutionary outcome is the emergence
452 of multiple genetic clusters of both males and females (Gavrilets and Waxman, 2002; Hayashi
453 et al., 2007). There is now empirical evidence for female phenotypic polymorphisms that might
454 have evolved as a response to sexual conflict in a small but growing number of taxa (Iversen
455 et al., 2019; Karlsson et al., 2013; Lee et al., 2019; Moon and Kamath, 2019; Reinhardt et al.,
456 2007; Svensson et al., 2005).

457

458 The results of the present study run counter to the conclusions in a previous comparative study,
459 where the authors used maximum parsimony, and claimed that the ancestral state in *Ischnura*
460 was female polymorphism, rather than female monomorphism (Mattern and Van Gossum,
461 2008). However, this previous parsimony-based study did not take into account phylogenetic
462 uncertainty in the inference of ancestral character states and did not include some of tropical
463 taxa, the majority of which are female-monomorphic in our dataset (Table S2). Applying
464 statistical models of character evolution that take in to account phylogenetic uncertainty and
465 aim for a complete or at least unbiased taxonomic sampling, as we have done here, are important
466 steps to produce robust ancestral state estimations that appropriately represent uncertainty
467 (Cunningham et al., 1998; Heath et al., 2008; Losos, 2011; Pagel et al., 2004; Revell, 2013).
468 This is particularly important when inferring the ancestral states of rapidly evolving characters,
469 such as behavioural traits and the female colour polymorphisms studied here, which show a
470 dynamic macroevolutionary history with several gains and losses across the phylogeny (Fig. 4).
471
472 Polymorphisms have been suggested to promote speciation if different morphs diverge
473 phenotypically in sympatry, followed by selective or random loss in allopatry, resulting in the
474 fixation of alternative morphs in different populations (Jamie and Meier, 2020; West-Eberhard,
475 1986). This process – termed "morphic speciation" – predicts that monomorphic taxa are
476 commonly found at the terminal tips on a phylogeny (Corl et al., 2010; West-Eberhard, 1986).
477 There is some evidence for such a process among populations of colour polymorphic side-
478 blotched lizards (*Uta stansburiana*)(Corl et al., 2010). Our results do not lend support for
479 widespread morphic speciation, but are consistent with losses of heterochrome females in
480 female-dimorphic taxa, as this evolutionary transition occurred in 99.7% of the posterior in the
481 RJ MCMC analysis. The most likely candidate for such potential morphic speciation is the
482 common ancestor between *I. verticalis* and *I. posita*. These taxa are part of a North American

483 clade that diversified rapidly during the Pleistocene (*I. damula* to *I. cervula;* Fig. 2). Such rapid
484 speciation and diversification could have resulted from repeated glacial cycles, as in the closely
485 related genus *Enallagma* that contains many sympatric young species in North America
486 (Callahan and McPeek, 2016; McPeek and Gavrilets, 2006). Such glacial cycles and repeated
487 Pleistocene bottlenecks could have led to morph fixation of a male-like female androchrome
488 morph in the ancestor of *I. posita* during a period of allopatry (Fig. 4). *I. posita* is currently a
489 widely distributed species, ranging from Nova Scotia in Canada to Central America (Paulson,
490 2012; Table S2). If morph loss in the ancestor of *I. posita* resulted from genetic drift during a
491 period of allopatry and population contraction, it must have then been followed by rapid and
492 recent range expansion. While this hypothesis remains to be investigated for *I. posita,* closely
493 related and young sympatric taxa in *Enallagma* are thought to be the result of rapid speciation
494 and range expansion after glacial retreat in the Quaternary (McPeek and Gavrilets, 2006;
495 Turgeon et al., 2005).

496

497 Another potential outcome of morphic speciation leading to fixation of male-like androchrome
498 females is *I. kellicotti*, although it is less clear whether the lineage leading to *I. kellicotti* had a
499 female-polymorphic ancestor (Fig. 4). Nevertheless, the potential for morphic speciation in *I.*
500 *kellicotti* is interesting because females display an interesting pattern of ontogenetic colour
501 changes. Sexually immature females start with a bright orange colouration that turns blue and
502 male-like in mature females, and develops increasing pruinescence with age thereafter
503 (Paulson, 2009, 2012). If used as a signal of reproductive availability (cf. Willink et al. 2019),
504 the evolution of distinct developmental colour phases may have reduced male mating
505 harassment and facilitated morphic speciation. We also note that in several relatively distantly
506 related species of *Ischnura*, immature females express orange colouration prior to reproductive
507 maturity (e. g. in *I. ramburii, I. pumilio* and *I. senegalensis*; Fig. 1E-G) which could be such a

508 signal of reproductive unsuitability. Alternatively, an evolutionary transition in habitat and
509 mating system could have led to morph loss and morphic speciation in the ancestor of *I.*
510 *kellicotti*. Individuals of *I. kelicotti* ("Lilypad forktail") are typically found sitting on lilypad
511 leaves in ponds (Paulson, 2012, 2009), consistent with a switch from non-territorial male-male
512 scrambling competition over females with high mating harassment, to a new mating system of
513 partial male territoriality with a lower degree of mating harassment. Such relaxed pressure from
514 sexual conflict may have lead to the loss of female polymorphism. We propose that a
515 combination of "phylogenetic natural history" (Uyeda et al., 2018) and more detailed
516 knowledge about the molecular and genetic basis of female colour development (M. Takahashi
517 et al., 2018; Willink et al., 2020) could shed light on this issue and increase our general
518 understanding of other macroevolutionary events in the genus *Ischnura*.

519

520 The presence of female colour polymorphism was significantly and positively correlated with
521 geographic range size (Fig. 5), which could come about in at least three different, but non-
522 mutually exclusive, ways. First, species with large ranges are also likely to have large effective
523 population sizes and this will decrease the effects of genetic drift and morph fixation. It is
524 interesting to note here that several of the female-monomorphic taxa are endemic to small
525 islands in the Pacific Ocean (Table S2). Second, and certainly not mutually exclusive, species
526 with larger ranges and associated larger population sizes, might experience stronger sexual
527 conflict due to higher population densities, consistent with more intense male mating
528 harassment under such conditions (Gosden and Svensson, 2009). Finally, female-polymorphic
529 species might also be able exploit a broader range of niches and thereby also achieve higher
530 population mean fitness compared to female-monomorphic species, with positive effects on
531 both population size and geographic range size (Forsman, 2016; Svensson, 2017; Takahashi et
532 al., 2014; Y. Takahashi et al., 2018; Takahashi and Noriyuki, 2019; West-Eberhard, 1986).

533 Although the data in this study does not allow us to firmly distinguish between these non-
534 mutually exclusive explanations for the positive relationship between the incidence of colour
535 polymorphism and geographic range size (Fig. 5), these different scenarios clearly deserved
536 attention in future studies. A positive relationship between geographic range size and presence
537 of colour polymorphism in *Ischnura* and other related damselflies was reported in a recent
538 study, although without considering phylogenetic relatedness among species, as no molecular
539 phylogeny was available at that time for *Ischnura* (Takahashi and Noriyuki, 2019).

540

541 Interestingly, the least supported nodes of our MCC tree are in Clade II and the North American
542 clade within Clade IV (*I. kellicotti* to *I. verticalis*; Fig. 2), which are both dominated by female-
543 polymorphic taxa (Fig. 4). These subsets of taxa were not particularly underrepresented in our
544 dataset (Table S1). There are at least two potential biological explanations for this pattern. First,
545 interspecific reproductive interference may be boosted by sexual conflict over mating rates
546 (McPeek and Gavrilets, 2006; Svensson et al., 2009) and might therefore be more common
547 between female-polymorphic species. This could result in more frequent hybridization and
548 introgression in polymorphic clades (Okude et al., 2020; Sanchez-Guillen et al., 2005; Sánchez-
549 Guillén et al., 2011). Second, faster speciation when population sizes are large can result in
550 deep coalescence among lineages and thus increase gene tree discordance (Maddison, 1997).If
551 female-polymorphic species have larger population sizes as our results suggest (Fig. 5), and
552 colour-polymorphisms accelerate speciation (Hugall and Stuart-Fox, 2012), then the speciation
553 dynamics of female-polymorphic clades may render them particularly challenging for
554 phylogenetic inference. These alternative evolutionary scenarios are clearly worthy of further
555 research.

556

557 Finally, in one, and potentially two, of the female-polymorphic clades, the female
558 polymorphism seem to have arisen early and was subsequently maintained across multiple
559 speciation events (Fig. 4). Therefore, the *Ischnura* female colour polymorphisms is an example
560 of trans-species polymorphisms (Jamie and Meier, 2020), when multiple allelic variants
561 become established in a population prior to speciation and are subsequently passed on to
562 descendant taxa across multiple speciation events (Klein, 1987). Other examples of trans-
563 species polymorphisms are rare, the most well-known being immune system polymorphisms
564 and blood group variation in great apes (Azevedo et al., 2015) and self-incompatibility allelic
565 systems in plants (Goldberg et al., 2010; Richman et al., 1996; Richman and Kohn, 2000).
566 However, such trans-species polymorphisms may be more common than the limited current
567 evidence suggests, particularly in colour polymorphic taxa (Corl et al., 2010; Jamie and Meier,
568 2020). In the damselfly genus *Ischnura*, some female-polymorphic species have higher
569 population-level fitness when female morph frequencies are even, compared to when they are
570 more biased (Takahashi *et al.* 2014) and in this study we also showed that polymorphic species
571 have larger range sizes than monomorphic species (Fig. 5). Although these female colour
572 polymorphisms are maintained by negative frequency-dependent selection at the individual
573 level (Le Rouzic et al., 2015; Svensson et al., 2005; Takahashi et al., 2010) the higher-level
574 population effects mentioned above might also indicate an additional potential role for species-
575 level selection and biased extinction of female-monomorphic taxa. Such higher level-effects
576 would be similar to how self-incompatability alleles are maintained in plant mating systems by
577 species selection (Goldberg et al., 2010).

578

579 **Conclusions**

580 The results in this study suggest that the damselfly genus *Ischnura* originated in the early
581 Miocene, with ancestral females being monomorphic and markedly distinct from males in their

582 colour pattern (heterochrome). The female colour polymorphisms in this genus arose on several
583 occasions through repeated origins of a male-mimic in different species. Our ancestral state
584 reconstruction also suggests that female colour polymorphisms have survived through multiple
585 speciation events in at least two clades, contrary to the expectation of speciation by selective or
586 random morph loss in allopatric populations (morphic speciation). However, we find at least
587 one potential loss of the female colour polymorphism, and specifically of heterochrome
588 females, that could have resulted in morphic speciation. The multiple transitions in female-
589 morph states across the phylogeny imply that the selective pressures from sexual conflict differ
590 betweentaxa, and that the strength of frequency-dependent conflict might be related to local
591 population sizes and densities, with consequences for geographic range size and potentially also
592 for extinction risk. This study provides a foundation for future micro- and macroevolutionary
593 studies on how selective and neutral processes have shaped the evolution of discrete female-
594 morphs in *Ischnura* damselflies, by providing a phylogeny for future comparative studies in
595 this genus.

596

597 **Acknowledgements**

598 We are grateful to Debora Goedert and Masahito Tsuboi for constructive criticisms of an early
599 draft of this manuscript. We wish to thank Tammy Ho, Chiara De Pasqual and Eva Friman for
600 assistance in obtaining molecular sequence data. We also wish to thank all the following
601 odonatologists who kindly provided samples for the phylogenetic analyses and shared their
602 observations on colour variation in *Ischnura* species: Sylvain Charlat, Peter and Elaine Cowan,
603 Nathalia von Ellenrieder, Sonia Ferreira, Kawsar Khan, Oleg Kosterin, Viktor Nilsson-Örtman,
604 Ryo Futahashi, Roselyn and David Sparrow, Oliver Flint, curator of Odonata in the National
605 Entomological Collection at the Smithsonian Institute, and Bill Mauffray, curator of Odonata
606 at the Florida State Collection of Arthropods. Specimens were collected with permits from the

Guyana Environmental Protection Agency (Reference No. 010715 BR001 and 012615 SP: 002), from Secretaría de Ambiente y Desarrollo Sustentable, Argentina (Ref No. 11084/16) and from the Cameroon Ministry of Forestry and Wildlife (Ref. No. 0000034). We acknowledge the logistic support from the Iwokrama International Centre for Rain Forest Conservation and Development, Guyana, the Karanambu Trust, Guyana, and the National Park Administration, Argentina. Funding for this study was provided by research grants from The Swedish Research Council (VR: grant no. 2016-03356), Carl Tryggers Foundation (CTS), Gyllenstiernska Krapperupstiftelsen (grant no. KR2018-0038), Lunds Djurskyddsfond, The Royal Physiographic Society in Lund, Stiftelsen Olle Enqvist Byggmästare and Stina Werners Foundation to E.I.S. and from The Royal Physiographic Society of Lund, the Jörgen Lindströms Fund and "Lunds Djurskyddsfond" to B.W.

Data Availability

Novel sequence data will be made available on the National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov/genbank/). All raw data, command files and scripts are available at GitHub (https://rachelblow.github.io/IschnuraPolymorphismEvolutionaryHistory/) and Dryad Digital Repository (https://datadryad.org/stash) including convergence and stationarity checks of MCMC analyses.

Competing Interests

The authors declare that they have no competing interests.

References

Andrade, P., Pinho, C., Lanuza, G.P. i de, Afonso, S., Brejcha, J., Rubin, C.-J., Wallerman, O., Pereira, P., Sabatino, S.J., Bellati, A., Pellitteri-Rosa, D., Bosakova, Z., Bunikis, I., Carretero, M.A., Feiner, N., Marsik, P., Paupério, F., Salvi, D., Soler, L., While, G.M., Uller, T., Font, E., Andersson, L., Carneiro, M., 2019. Regulatory changes in pterin and carotenoid genes underlie balanced color polymorphisms in the wall lizard. Proc. Natl. Acad. Sci., USA 116, 5633–5642. https://doi.org/10.1073/pnas.1820320116

Azevedo, L., Serrano, C., Amorim, A., Cooper, D.N., 2015. Trans-species polymorphism in humans and the great apes is generally maintained by balancing selection that modulates the host immune response. Hum. Genomics 9, 21. https://doi.org/10.1186/s40246-015-0043-1

Beatty, C.D., Herrera, M.S., Skevington, J.H., Rashed, A., Gossum, H.V., Kelso, S., Sherratt, T.N., 2017. Biogeography and systematics of endemic island damselflies: The Nesobasis and Melanesobasis (Odonata: Zygoptera) of Fiji. Ecol. Evol. 7, 7117–7129. https://doi.org/10.1002/ece3.3175

Bechly, G., 2000. A new fossil damselfly species (Insecta: Odonata: Zygoptera: Coenagrionidae: Ischnurinae) from Dominican Amber. Stuttgarter Beitr. Naturk. Ser. B. 299, 1–9.

Blows, M.W., Brooks, R., Kraft, P.G., 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. Evolution 57, 1622–1630.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLOS Comput. Biol. 10, e1003537. https://doi.org/10.1371/journal.pcbi.1003537

655 Bouckaert, R.R., Drummond, A.J., 2017. bModelTest: Bayesian phylogenetic site model
656 averaging and model comparison. BMC Evol. Biol. 17, 42.
657 https://doi.org/10.1186/s12862-017-0890-6
658 Brower, A.V., 1994. Rapid morphological radiation and convergence among races of the
659 butterfly Heliconius erato inferred from patterns of mitochondrial DNA evolution. Proc.
660 Natl. Acad. Sci., USA 91, 6491–6495.
661 Callahan, M.S., McPeek, M.A., 2016. Multi-locus phylogeny and divergence time estimates of
662 Enallagma damselflies (Odonata: Coenagrionidae). Mol. Phylogenet. Evol. 94, 182–
663 195. https://doi.org/10.1016/j.ympev.2015.08.013
664 Chippindale, P.T., Dave, V.K., Whitmore, D.H., Robinson, J.V., 1999. Phylogenetic
665 relationships of North American damselflies of the genus Ischnura (Odonata:
666 Zygoptera: Coenagrionidae) based on sequences of three mitochondrial genes. Mol.
667 Phylogenet. Evol. 11, 110–121.
668 Cordero, A., 1992. Density-dependent mating success and colour polymorphism in females of
669 the damselfly Ischnura graellsii (Odonata: Coenagrionidae). J. Anim. Ecol. 61, 769–
670 780.
671 Cordero, A., Carbone, S.S.., Utzeri, C., 1998. Mating opportunities and mating costs are
672 reduced in androchrome female damselflies, Ischnura elegans. Anim. Behav. 55, 185–
673 187.
674 Cordoba-Aguilar, A., 2008. Dragonflies and damselflies: model organisms for ecological and
675 evolutionary research. Oxford University Press, Oxford.
676 Corl, A., Davis, A.R., Kuchta, S.R., Sinervo, B., 2010. Selective loss of polymorphic mating
677 types is associated with rapid phenotypic evolution during morphic speciation. Proc.
678 Natl. Acad. Sci., USA 107, 4254–4259. https://doi.org/10.1073/pnas.0909480107

679 Crow, J.F., Kimura, M., 1970. An Introduction to population genetic theory. Harper & Row.,
680 New York.
681 Cunningham, C.W., Omland, K.E., Oakley, T.H., 1998. Reconstructing ancestral character
682 states: a critical reappraisal. Trends Ecol. Evol. 13, 361–366.
683 Damm, S., Dijkstra, K.D., Hadrys, H., 2010. Red drifters and dark residents: the phylogeny and
684 ecology of a Plio-Pleistocene dragonfly radiation reflects Africa's changing
685 environment (Odonata, Libellulidae, Trithemis). Mol. Phylogenet. Evol. 54, 870–882.
686 https://doi.org/10.1016/j.ympev.2009.12.006
687 Dijkstra, K.-D.B., Kalkman, V.J., Dow, R.A., Stokvis, F.R., Van Tol, J., 2014. Redefining the
688 damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata).
689 Syst. Entomol. 39, 68–96. https://doi.org/10.1111/syen.12035
690 Dobzhansky, T., 1970. Genetics of the evolutionary process. Columbia University Press, New
691 york.
692 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high
693 throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340
694 Fincke, O.M., 2004. Polymorphic signals of harassed female odonates and the males that learn
695 them support a novel frequency-dependent model. Anim. Behav. 67, 833–845.
696 Fincke, O.M., Jödicke, R., Paulson, D., Schultz, D.T., 2005. The evolution and frequency of
697 female color morphs in Holarctic Odonata: why are male-like morphs typically the
698 minority? Int. J. Odonatol. 8, 183–212.
699 Fisher, R.A., 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.
700 Ford, E.B., 1975. Ecological genetics. Chapman and Hall, London.
701 Formica, V.A., Tuttle, E.M., 2009. Examining the social landscapes of alternative
702 reproductive strategies. J. Evol. Biol. 22, 2395–2408. https://doi.org/10.1111/j.1420-
703 9101.2009.01855.x

Forsman, A., 2016. Is colour polymorphism advantageous to populations and species? Mol. Ecol. https://doi.org/10.1111/mec.13629

Gangoso, L., Grande, J.M., Ducrest, A.-L., Figuerola, J., Bortolotti, G.R., Andrés, J.A., Roulin, A., 2011. MC1R-dependent, melanin-based colour polymorphism is associated with cell-mediated response in the Eleonora's falcon. J. Evol. Biol. 24, 2055–2063. https://doi.org/10.1111/j.1420-9101.2011.02336.x

Gavrilets, S., Waxman, D., 2002. Sympatric speciation by sexual conflict. Proc. Natl. Acad. Sci., USA 99, 10533–10538.

Gentilini, G., Bagli, L., 2004. Fossil Zygoptera and Anisoptera from the Upper Miocene of Monte Castellaro (Pesaroo Marches, Central Italy). Quad. Studi Nat. Romagna 19, 17–44.

Gering, E.J., 2017. Male-mimicking females increase male-male interactions, and decrease male survival and condition in a female-polymorphic damselfly. Evolution 71, 1390–1396. https://doi.org/10.1111/evo.13221

Global Administrative Areas, 2018. GADM database of Global Administrative Areas, version 3.6. [online] URL: www.gadm.org.

Goldberg, E.E., Kohn, J.R., Lande, R., Robertson, K.A., Smith, S.A., Igić, B., 2010. Species Selection Maintains Self-Incompatibility. Science 330, 493–495. https://doi.org/10.1126/science.1194513

Gordon, S.P., Kokko, H., Rojas, B., Nokelainen, O., Mappes, J., 2015. Colour polymorphism torn apart by opposing positive frequency-dependent selection, yet maintained in space. J. Anim. Ecol. 84, 1555–1564. https://doi.org/10.1111/1365-2656.12416

Gosden, T.P., Svensson, E.I., 2009. Density-dependent male mating harassment, female resistance, and male mimicry. Am. Nat. 173, 709–721. https://doi.org/10.1086/598491

Hadfield, J.D., 2015. Increasing the efficiency of MCMC for hierarchical phylogenetic models of categorical traits using reduced mixed models. Methods Ecol. Evol. 6, 706–714. https://doi.org/10.1111/2041-210X.12354

Hadfield, J.D., 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. J. Stat. Softw. 33, 1–22. https://doi.org/10.18637/jss.v033.i02

Hadfield, J.D., Nakagawa, S., 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. J. Evol. Biol. 23, 494–508. https://doi.org/10.1111/j.1420-9101.2009.01915.x

Haldane, J.B.S., 1949. Disease and evolution. Ric. Sci. Suppl. A 19, 68–76.

Haldane, J.B.S., 1932. The causes of evolution. Longmans, Green & Co., London.

Hartl, D.L., Clark, A.G., 1997. Principles of population genetics. Sinauer Associates, Inc., Sunderland, MA.

Hayashi, T.I., Vose, M., Gavrilets, S., 2007. Genetic differentiation by sexual conflict. Evolution 61, 516–529.

Heath, T.A., Hedtke, S.M., Hillis*, D.M., 2008. Taxon sampling and the accuracy of phylogenetic analyses. J. Syst. Evol. 46, 239–257. https://doi.org/10.3724/SP.J.1002.2008.08016

Hugall, A.F., Stuart-Fox, D., 2012. Accelerated speciation in colour-polymorphic birds. Nature 485, 631–634. https://doi.org/10.1038/nature11050

Hughes, K.A., Houde, A.E., Price, A.C., Rodd, F.H., 2013. Mating advantage for rare males in wild guppy populations. Nature 503, 108–110. https://doi.org/10.1038/nature12717

751 Iserbyt, A., Bots, J., Van Dongen, S., Ting, J.J., Van Gossum, H., Sherratt, T.N., 2011.
752 Frequency-dependent variation in mimetic fidelity in an intraspecific mimicry system.
753 Proc. R. Soc. B-Biol. Sci. 278, 3116–3122. https://doi.org/10.1098/rspb.2011.0126
754 Iserbyt, A., Bots, J., Van, G.H., Sherratt, T.N., 2013. Negative frequency-dependent selection
755 or alternative reproductive tactics: maintenance of female polymorphism in natural
756 populations. BMC Evol. Biol. 13, 139. https://doi.org/10.1186/1471-2148-13-139
757 Iversen, L.L., Svensson, E.I., Christensen, S.T., Bergsten, J., Sand-Jensen, K., 2019. Sexual
758 conflict and intrasexual polymorphism promote assortative mating and halt population
759 differentiation. Proc. R. Soc. B-Biol. Sci. 286, 20190251.
760 https://doi.org/10.1098/rspb.2019.0251
761 Jamie, G.A., Meier, J.I., 2020. The Persistence of Polymorphisms across Species Radiations.
762 Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2020.04.007
763 Karlsson, G.K., Kovalev, A., Svensson, E.I., Gorb, S.N., 2013. Male clasping ability, female
764 polymorphism and sexual conflict: fine-scale elytral morphology as a sexually
765 antagonistic adaptation in female diving beetles. J. R. Soc. Interface 10, 20130409.
766 https://doi.org/10.1098/rsif.2013.0409
767 Klein, J., 1987. Origin of major histocompatibility complex polymorphism: The trans-species
768 hypothesis. Hum. Immunol. 19, 155–162. https://doi.org/10.1016/0198-
769 8859(87)90066-8
770 Lank, D.B., Smith, C.M., Hanotte, O., Burke, T., Cooke, F., 1995. Genetic Polymorphism for
771 Alternative Mating-Behavior in Lekking Male Ruff Philomachus-Pugnax. Nature 378,
772 59–62.
773 Le Rouzic, A., Hansen, T.F., Gosden, T.P., Svensson, E.I., 2015. Evolutionary time-series
774 analysis reveals the signature of frequency-dependent selection on a female mating
775 polymorphism. Am. Nat. 185E, E182–E196.

Lee, J.-W., Kim, H.-N., Yoo, S., Yoo, J.-C., 2019. Common cuckoo females may escape male sexual harassment by color polymorphism. Sci. Rep. 9, 1–9. https://doi.org/10.1038/s41598-019-44024-6

Letsch, H., Gottsberger, B., Ware, J.L., 2016. Not going with the flow: a comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. Mol. Ecol. 25, 1340–1353. https://doi.org/10.1111/mec.13562

Llaurens, V., Whibley, A., Joron, M., 2017. Genetic architecture and balancing selection: the life and death of differentiated variants. Mol. Ecol. 26, 2430–2448. https://doi.org/10.1111/mec.14051

Losos, J.B., 2011. Seeing the Forest for the Trees: The Limitations of Phylogenies in Comparative Biology. Am. Nat. 177, 709–727. https://doi.org/10.1086/660020

Löytynoja, A., Goldman, N., 2008. Phylogeny-Aware Gap Placement Prevents Errors in Sequence Alignment and Evolutionary Analysis. Science 320, 1632–1635. https://doi.org/10.1126/science.1158395

Löytynoja, A., Goldman, N., 2005. An algorithm for progressive multiple alignment of sequences with insertions. Proc. Natl. Acad. Sci., USA. 102, 10557–10562. https://doi.org/10.1073/pnas.0409137102

Maddison, W.P., 1997. Gene Trees in Species Trees. Syst. Biol. 46, 523–536. https://doi.org/10.1093/sysbio/46.3.523

Marinov, M., Schmaedick, M., Polhemus, D., Stirne, R.L., 2015. Faunistic and taxonomic investigations on the Odonata fauna of the Samoan archipelago with particular focus on taxonomic ambiguities in the “Ischnurine complex. J. Int. Dragonfly Fund 91, 1–56.

Mattern, M., Van Gossum, H., 2008. A phylogenetic perspective on absence and presence of a sex-limited polymorphism. Anim. Biol. 58, 257–273. https://doi.org/10.1163/157075608X328099

McKinnon, J.S., Pierotti, M.E., 2010. Colour polymorphism and correlated characters: genetic mechanisms and evolution. Mol. Ecol. 19, 5101–5125. https://doi.org/10.1111/j.1365-294X.2010.04846.x

McLean, C.A., Stuart-Fox, D., 2014. Geographic variation in animal colour polymorphisms and its role in speciation. Biol. Rev. 89, 860–873. https://doi.org/10.1111/brv.12083

McPeek, M.A., Gavrilets, S., 2006. The evolution of female mating preferences: Differentiation from species with promiscuous males can promote speciation. Evolution 60, 1967–1980.

Miller, M.N., Fincke, O.M., 1999. Cues for mate recognition and the effect of prior experience on mate recognition in Enallagma damselflies. J. Insect Behav. 12, 801–814.

Mitchell-Olds, T., Willis, J.H., Goldstein, D.B., 2007. Which evolutionary processes influence natural genetic variation for phenotypic traits? Nat. Rev. Genet. 8, 845–856. https://doi.org/10.1038/nrg2207

Moon, R.M., Kamath, A., 2019. Re-examining escape behaviour and habitat use as correlates of dorsal pattern variation in female brown anole lizards, Anolis sagrei (Squamata: Dactyloidae). Biol. J. Linn. Soc. 126, 783–795. https://doi.org/10.1093/biolinnean/blz006

Neff, B.D., Svensson, E.I., 2013. Polyandry and alternative mating tactics. Philos. Trans. R. Soc. Lond. Biol. Sci. 368, 20120045. https://doi.org/10.1098/rstb.2012.0045

O'Connor, E.A., Cornwallis, C.K., Hasselquist, D., Nilsson, J.-Å., Westerdahl, H., 2018. The evolution of immunity in relation to colonization and migration. Nat. Ecol. Evol. 2, 841–849. https://doi.org/10.1038/s41559-018-0509-3

824 Ogilvie, H.A., Bouckaert, R.R., Drummond, A.J., 2017. StarBEAST2 Brings Faster Species
825 Tree Inference and Accurate Estimates of Substitution Rates. Mol. Biol. Evol. 34, 2101–
826 2114. https://doi.org/10.1093/molbev/msx126
827 Okude, G., Fukatsu, T., Futahashi, R., 2020. Interspecific crossing between blue-tailed
828 damselflies Ischnura elegans and I. senegalensis in the laboratory. Entomol. Sci. 23,
829 165–172. https://doi.org/10.1111/ens.12408
830 Pagel, M., Meade, A., Barker, D., 2004. Bayesian Estimation of Ancestral Character States on
831 Phylogenies. Syst. Biol. 53, 673–684. https://doi.org/10.1080/10635150490522232
832 Paradis, E., Schliep, K., 2019. ape 5.0: an environment for modern phylogenetics and
833 evolutionary analyses in R. Bioinformatics 35, 526–528.
834 https://doi.org/10.1093/bioinformatics/bty633
835 Paulson, D., 2012. Dragonflies and Damselflies of the East. Princeton University Press, NJ.
836 Paulson, D., 2009. Dragonflies and Damselflies of the West, Princeton Field Guides. Princeton
837 University Press, Princeton, N.J.
838 Plummer, M., Best, N., Cowles, K., Vines, K., 2006. CODA: convergence diagnosis and output
839 analysis for MCMC. R News 6, 7–11.
840 Provine, W.B., 1986. Sewall Wright and evolutionary biology. University of Chicago Press,
841 Chicago.
842 Reinhardt, K., Harney, E., Naylor, R., Gorb, S., Siva-Jothy, M.T., 2007. Female-Limited
843 Polymorphism in the Copulatory Organ of a Traumatically Inseminating Insect. The
844 Am. Nat. 170, 931–935. https://doi.org/10.1086/522844
845 Revell, L.J., 2013. Two new graphical methods for mapping trait evolution on phylogenies.
846 Methods Ecol. Evol. 4, 754–759. https://doi.org/10.1111/2041-210X.12066

847 Richman, A.D., Kohn, J.R., 2000. Self-incompatability alleles from Physalis : Implications for
848 historical inference from balanced genetic polymorphisms. Proc. Natl. Acad. Sci., USA
849 96, 168–172.
850 Richman, A.D., Uyenoyama, M.C., Kohn, J.R., 1996. Allelic diversity and gene genealogy at
851 the self-incompatability locus in solanaceae. Science 273, 1212–1216.
852 Robertson, H.M., 1985. Female dimorphism and mating behaviour in a damselfly, Ischnura
853 ramburi: females mimicking males. Anim. Behav. 33, 805–809.
854 Robinson, J.V., Allgeyer, R., 1996. Covariation in life-history traits, demographics and
855 behaviour in ischnuran damselflies: The evolution of monandry. Biol. J. Linn. Soc. 58,
856 85–98.
857 Ross, L., Hardy, N.B., Okusu, A., Normark, B.B., 2013. Large Population Size Predicts the
858 Distribution of Asexuality in Scale Insects. Evolution 67, 196–206.
859 https://doi.org/10.1111/j.1558-5646.2012.01784.x
860 Sanchez-Guillen, R.A., Hansson, B., Wellenreuther, M., Svensson, E.I., Cordero-Rivera, A.,
861 2011. The influence of stochastic and selective forces in the population divergence of
862 female colour polymorphism in damselflies of the genus Ischnura. Heredity (Edinb.)
863 107, 513–522. https://doi.org/10.1038/hdy.2011.36
864 Sanchez-Guillen, R.A., Van Gossum, H., Rivera, A.C., 2005. Hybridization and the inheritance
865 of female colour polymorphism in two ischnurid damselflies (Odonata :
866 Coenagrionidae). Biol. J. Linn.Soc. 85, 471–481.
867 Sánchez-Guillén, R.A., Wellenreuther, M., Cordero-Rivera, A., Hansson, B., 2011.
868 Introgression and rapid species turnover in sympatric damselflies. BMC Evol. Biol. 11,
869 210. https://doi.org/10.1186/1471-2148-11-210
870 Schemske, D.W., Bierzychudek, P., 2001. Perspective: Evolution of flower color in the desert
871 annual Linanthus parryae: Wright revisited. Evolution 55, 1269–1282.

Schluter, D., Price, T., Mooers, A.O., Ludwig, D., 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51, 1699–1711.

Schwander, T., Leimar, O., 2011. Genes as leaders and followers in evolution. Trends Ecol. Evol. 26, 143–151. https://doi.org/10.1016/j.tree.2010.12.010

Sinervo, B., Lively, C.M., 1996. The rock-paper-scissors game and the evolution of alternative male strategies. Nature 380, 240–243.

Sinervo, B., Svensson, E., 2002. Correlational selection and the evolution of genomic architecture. Heredity 16, 948–955.

Sirot, Laura K., Brockmann, H.J., 2001. Costs of sexual interactions to females in rambur's forktail damselfly, Ischnura ramburi (Zygoptera: Coenagrionidae). Anim Behav. 61, 415–424. https://doi.org/10.1006/anbe.2000.1605

Sirot, L.K., Brockmann, H.J., 2001. Costs of sexual interaction to females in Rambur's forktail damselfly, Ischnura ramburi (Zygoptera: Odonata). Anim. Behav. 61, 415–424.

Sirot, L.K., Brockmann, H.J., Marinis, C., Muschett, G., 2003. Maintenance of a female-limited polymorphism in Ischnura ramburi (Zygoptera : Coenagrionidae). Anim. Behav. 66, 763–775.

Svensson, E.I., 2017. Back to basics: using colour polymorphisms to study evolutionary processes. Mol. Ecol. 26, 2204–2211. https://doi.org/10.1111/mec.14025

Svensson, E.I., Abbott, J., Hardling, R., 2005. Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. Am. Nat. 165, 567–576.

Svensson, E.I., Abbott, J.K., Gosden, T.P., Coreau, A., 2009. Female polymorphisms, sexual conflict and limits to speciation processes in animals. Evol. Ecol. 23, 93–108.

Svensson, E.I., Willink, B., Duryea, M.C., Lancaster, L.T., 2020. Temperature drives pre-reproductive selection and shapes the biogeography of a female polymorphism. Ecol. Lett. 23, 149–159. https://doi.org/10.1111/ele.13417

897 Swaegers, J., Janssens, S.B., Ferreira, S., Watts, P.C., Mergeay, J., McPeek, M.A., Stoks, R.,
898 2014. Ecological and evolutionary drivers of range size in Coenagrion damselflies. J.
899 Evol. Biol. 27, 2386–2395. https://doi.org/10.1111/jeb.12481
900 Takahashi, M., Takahashi, Y., Kawata, M., 2018. Candidate genes associated with color morphs
901 of female-limited polymorphisms of the damselfly Ischnura senegalensis. Heredity 122,
902 81–92. https://doi.org/10.1038/s41437-018-0076-z
903 Takahashi, Y., Kagawa, K., Svensson, E.I., Kawata, M., 2014. Evolution of increased
904 phenotypic diversity enhances population performance by reducing sexual harassment
905 in damselflies. Nat. Commun. 5, 4468. https://doi.org/10.1038/ncomms5468
906 Takahashi, Y., Noriyuki, S., 2019. Colour polymorphism influences species' range and
907 extinction risk. Biol. Lett. 15, 20190228. https://doi.org/10.1098/rsbl.2019.0228
908 Takahashi, Y., Tanaka, R., Yamamoto, D., Noriyuki, S., Kawata, M., 2018. Balanced genetic
909 diversity improves population fitness. Proc. R. Soc. B-Biol. Sci. 285, 20172045.
910 https://doi.org/10.1098/rspb.2017.2045
911 Takahashi, Y., Watanabe, M., 2010. Female reproductive success is affected by selective male
912 harassment in the damselfly Ischnura senegalensis. Anim Behav. 79, 211–216.
913 https://doi.org/10.1016/j.anbehav.2009.10.032
914 Takahashi, Y., Yoshimura, J., Morita, S., Watanabe, M., 2010. Negative frequency-dependent
915 selection in female color polymorphism of a damselfly. Evolution 64, 3620–3628.
916 https://doi.org/10.1111/j.1558-5646.2010.01083.x
917 Torres-Cambas, Y., Ferreira, S., Cordero-Rivera, A., Lorenzo-Carballa, M.O., 2019.
918 Mechanisms of allopatric speciation in an Antillean damselfly genus (Odonata,
919 Zygoptera): Vicariance or long-distance dispersal? Mol. Phylogenet. Evol. 137, 14–21.
920 https://doi.org/10.1016/j.ympev.2019.04.018

921 Turelli, M., Schemske, D.W., Bierzychudek, P., 2001. Stable two-allele polymorphisms
922 maintained by fluctuating fitnesses and seed banks: Protecting the blues in Linanthus
923 parryae. Evolution 55, 1283–1298.
924 Turgeon, J., Stoks, R., Thum, R.A., Brown, J.M., McPeek, M.A., 2005. Simultaneous
925 Quaternary radiations of three damselfly clades across the Holarctic. Am. Nat. 165, E78-
926 107. https://doi.org/10.1086/428682
927 Tuttle, E.M., Bergland, A.O., Korody, M.L., Brewer, M.S., Newhouse, D.J., Minx, P., Stager,
928 M., Betuel, A., Cheivron, Z.A., Warren, W.C., Gonser, R.A., Balakrishnan, C.N., 2016.
929 Divergence and functional degradation of a sex chromosome-like supergene. Curr. Biol.
930 26, 344–350.
931 Van Gossum, H., Stoks, R., De Bruyn, L., 2001. Reversible frequency-dependent switches in
932 male mate choice. Proc. R.Soc. Lond., B. 268, 83–85.
933 Vega-Sánchez, Y.M., Mendoza-Cuenca, L.F., González-Rodríguez, A., 2019. Complex
934 evolutionary history of the American Rubyspot damselfly, Hetaerina americana
935 (Odonata): Evidence of cryptic speciation. Mol. Phylogenet. Evol. 139, 106536.
936 https://doi.org/10.1016/j.ympev.2019.106536
937 Villemereuil, P. de, Gimenez, O., Doligez, B., 2013. Comparing parent–offspring regression
938 with frequentist and Bayesian animal models to estimate heritability in wild
939 populations: a simulation study for Gaussian and binary traits. Methods Ecol. Evol. 4,
940 260–275. https://doi.org/10.1111/2041-210X.12011
941 Waller, J.T., Svensson, E.I., 2017. Body size evolution in an old insect order: No evidence for
942 Cope's Rule in spite of fitness benefits of large size. Evolution 71, 2178–2193.
943 https://doi.org/10.1111/evo.13302

Wellenreuther, M., Svensson, E.I., Hansson, B., 2014. Sexual selection and genetic colour polymorphisms in animals. Mol. Ecol. 23, 5398–5414. https://doi.org/10.1111/mec.12935

West-Eberhard, M.J., 1986. Alternative adaptations, speciation, and phylogeny (A Review). Proc. Natl. Acad. Sci, USA 83, 1388–1392. https://doi.org/10.1073/pnas.83.5.1388

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, 2nd ed, Use R! Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4

Willink, B., 2018. The descent of damselflies and variation in relation to sex, PhD-thesis. Lund University (Sweden).

Willink, B., Duryea, M.C., Svensson, E.I., 2019. Macroevolutionary Origin and Adaptive Function of a Polymorphic Female Signal Involved in Sexual Conflict. Am. Nat. 194, 707–724. https://doi.org/10.1086/705294

Willink, B., Duryea, M.C., Wheat, C., Svensson, E.I., 2020. Changes in gene expression during female reproductive development in a color polymorphic insect. Evolution https://doi.org/10.1111/evo.13979

Woronik, A., Tunström, K., Perry, M.W., Neethiraj, R., Stefanescu, C., Celorio-Mancera, M. de la P., Brattström, O., Hill, J., Lehmann, P., Käkelä, R., Wheat, C.W., 2019. A transposable element insertion is associated with an alternative life history strategy. Nat Commun 10, 1–11. https://doi.org/10.1038/s41467-019-13596-2

Wright, A.M., Hillis, D.M., 2014. Bayesian Analysis Using a Simple Likelihood Model Outperforms Parsimony for Estimation of Phylogeny from Discrete Morphological Data. PLOS ONE 9, e109210. https://doi.org/10.1371/journal.pone.0109210

Wright, S., 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. Proc. Sixth Internat. Congr. Gen. 1, 356–366.

Wright, S., 1931. Evolution in Mendelian Populations. Genetics 16, 97–159.

Xu, M., Cerreta, A.L., Schultz, T.D., Fincke, O.M., 2014. Selective use of multiple cues by males reflects a decision rule for sex discrimination in a sexually mimetic damselfly. Anim. Behav. 92, 9–18. https://doi.org/10.1016/j.anbehav.2014.03.016

Yu, G., Smith, D.K., Zhu, H., Guan, Y., Lam, T.T.-Y., 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol. Evol- 8, 28–36. https://doi.org/10.1111/2041-210X.12628

991 **Figure Legends**

992

993 **Figure 1.** Some representative species of the damselfly genus *Ischnura*, from both the Old and
994 the New World, exemplifying the extensive phenotypic and colour variation within and
995 between species in this genus. All species in this panel are included in the molecular phylogeny
996 we present in this paper. **A.** An immature female *Ischnura capreolus*, revealing the yellow
997 colour females of this species exhibit prior to sexual maturation (Brazil). **B.** A sexually mature
998 female of *I. graellsii* of the infuscans-morph (Portugal). **C.** Sexually mature male *I. graellsii*
999 (Portugal). **D.** Sexually mature male *I. ramburii* (Texas, USA). **E-G:** Females in several both
1000 closely and distantly related species show an orange colour phase in females prior to sexual
1001 maturation. **E.** Immature female *I. ramburii* (Texas, USA). **F.** Immature female *I. pumilio*
1002 (Sweden). **G.** Immature female *I. senegalensis* (Japan). **H.** Mature male *I. senegalensis* (Japan).
1003 **I-K:** In addition to ontogenetic colour variation in females, some species of *Ischnura* also show
1004 heritable female colour polymorphism at the adult stage. Here are the three different female
1005 colour morphs in the trimorphic species *I. elegan*s (all photos from Sweden). **I.** Male *I. elegans*
1006 mating with an androchrome (A-female; male mimic) female morph. **J.** Male *I. elegans* mating
1007 with the *infuscans-obsoleta* (O-female) morph. **K.** Male *I. elegans* mating with the *infuscans*
1008 (I-female) morph. All photographs taken by E.I. Svensson.

1009

1010 **Figure 2.** Time calibrated *Ischnura* phylogeny of 41 species of damselflies from the genus
1011 *Ischnura* as estimated in StarBEAST2. Time axis represents millions of years from the present
1012 and grey bars at nodes indicate 95% HPD intervals for age estimates. Values at nodes indicate
1013 posterior probability of internal nodes.

1014

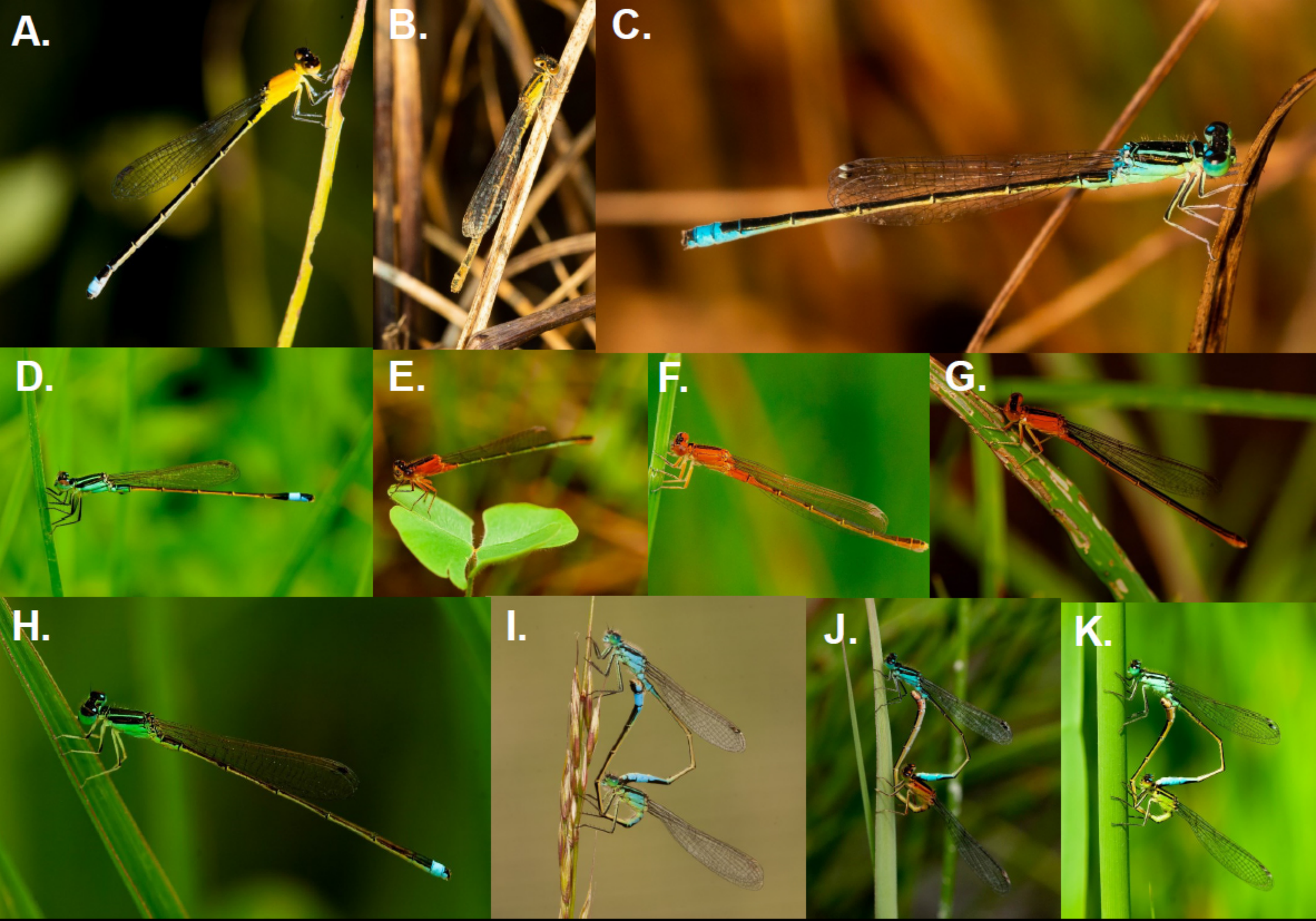
1015 **Figure 3.** Posterior divergence time estimation at the root node sampling from the prior only
1016 (gold) and sampling with data (grey). Age estimation followed an exponentially distributed
1017 prior with an offset of 13.8 my to prevent improbable age estimation (see Methods).

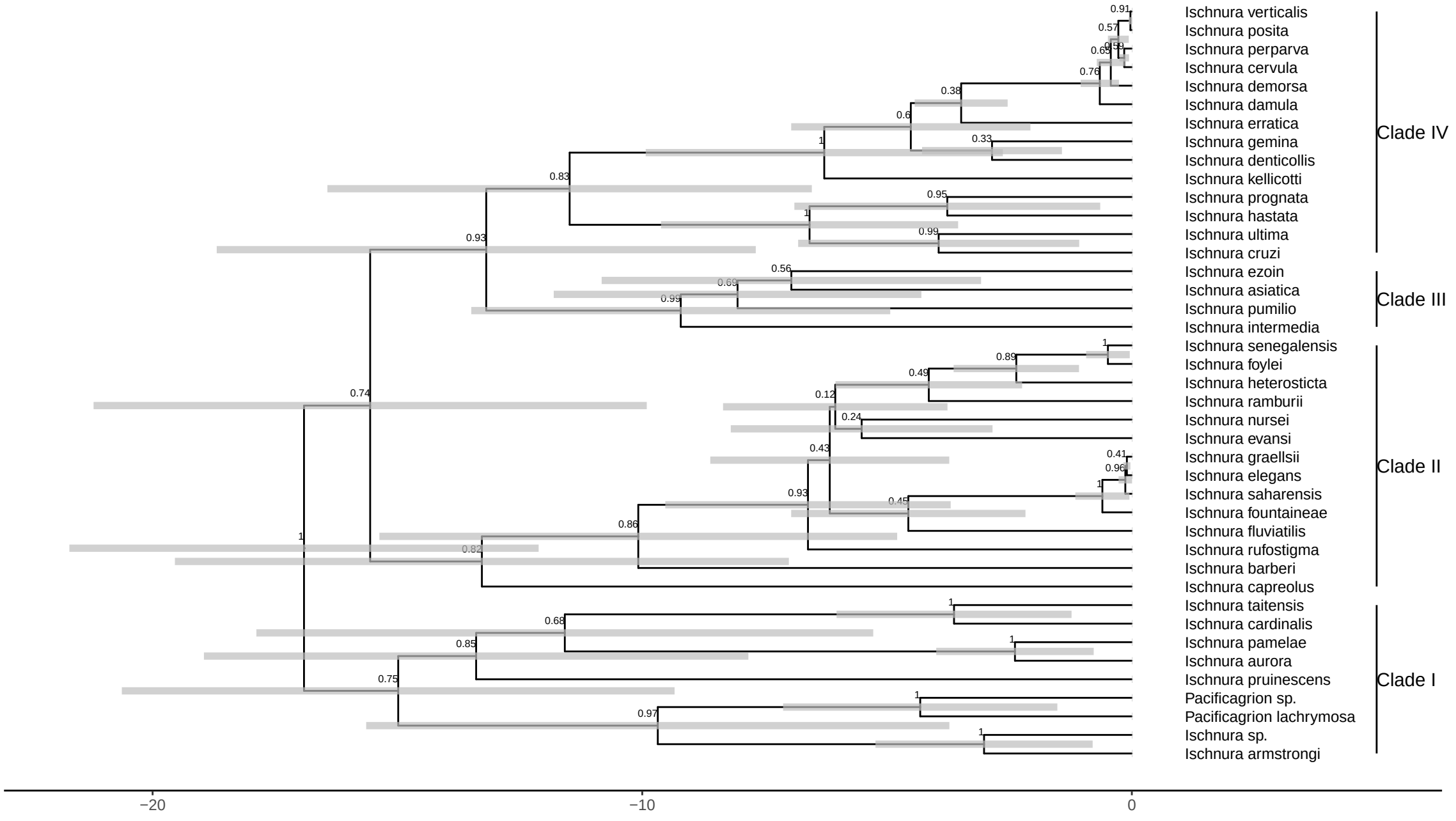
1018

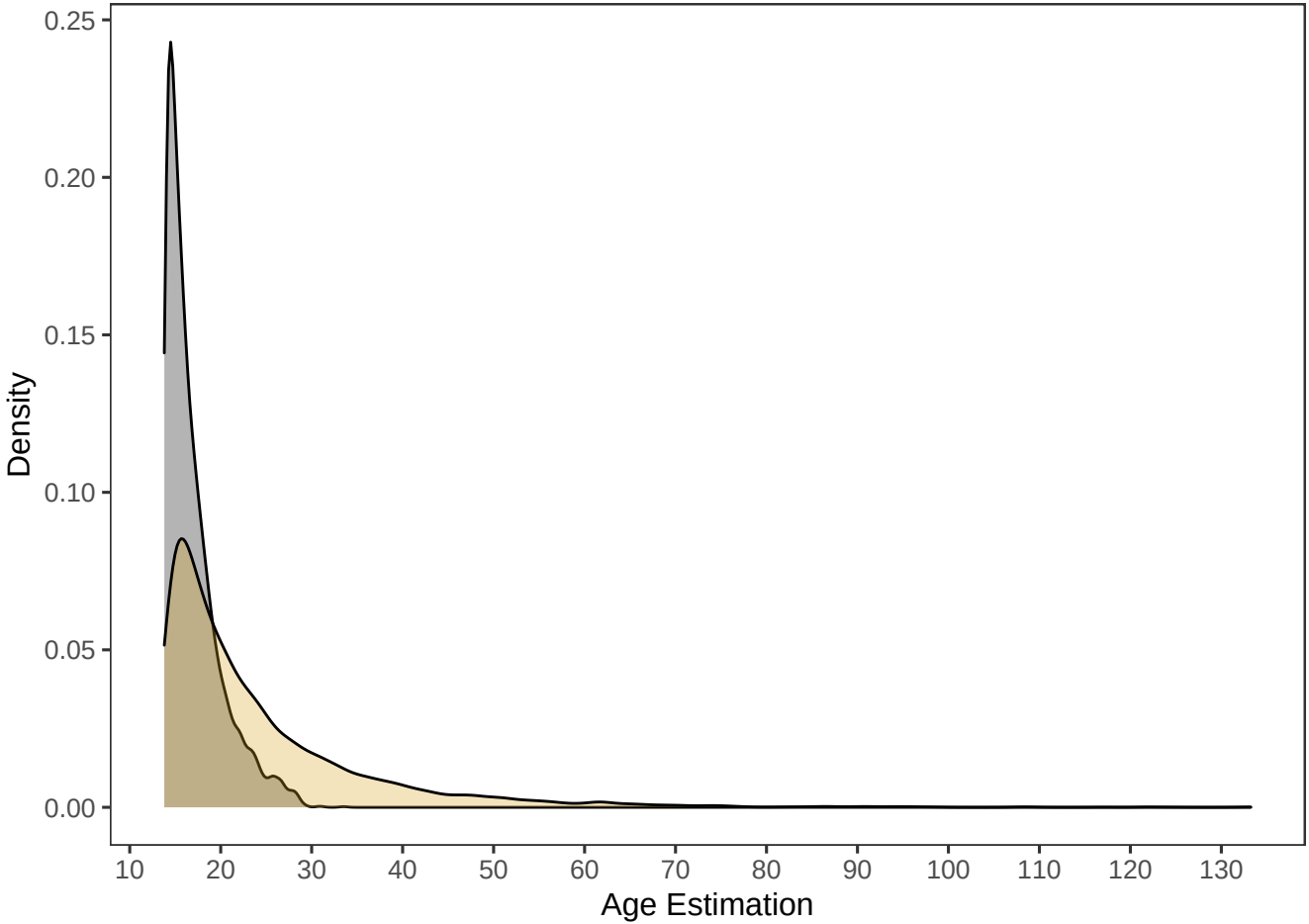
1019 **Figure 4.** Ancestral state reconstruction across the *Ischnura* phylogeny. Extant morph states
1020 are represented by circles at tips and reconstructed ancestral states are represented by piecharts
1021 at nodes (female-heterochromatic only, green; female-andromorphic only, blue; female-
1022 dimorphic, red; female-trimorphic, pink). Piecharts show the mean posterior probability for
1023 each morph state and are plotted on the MCC tree.

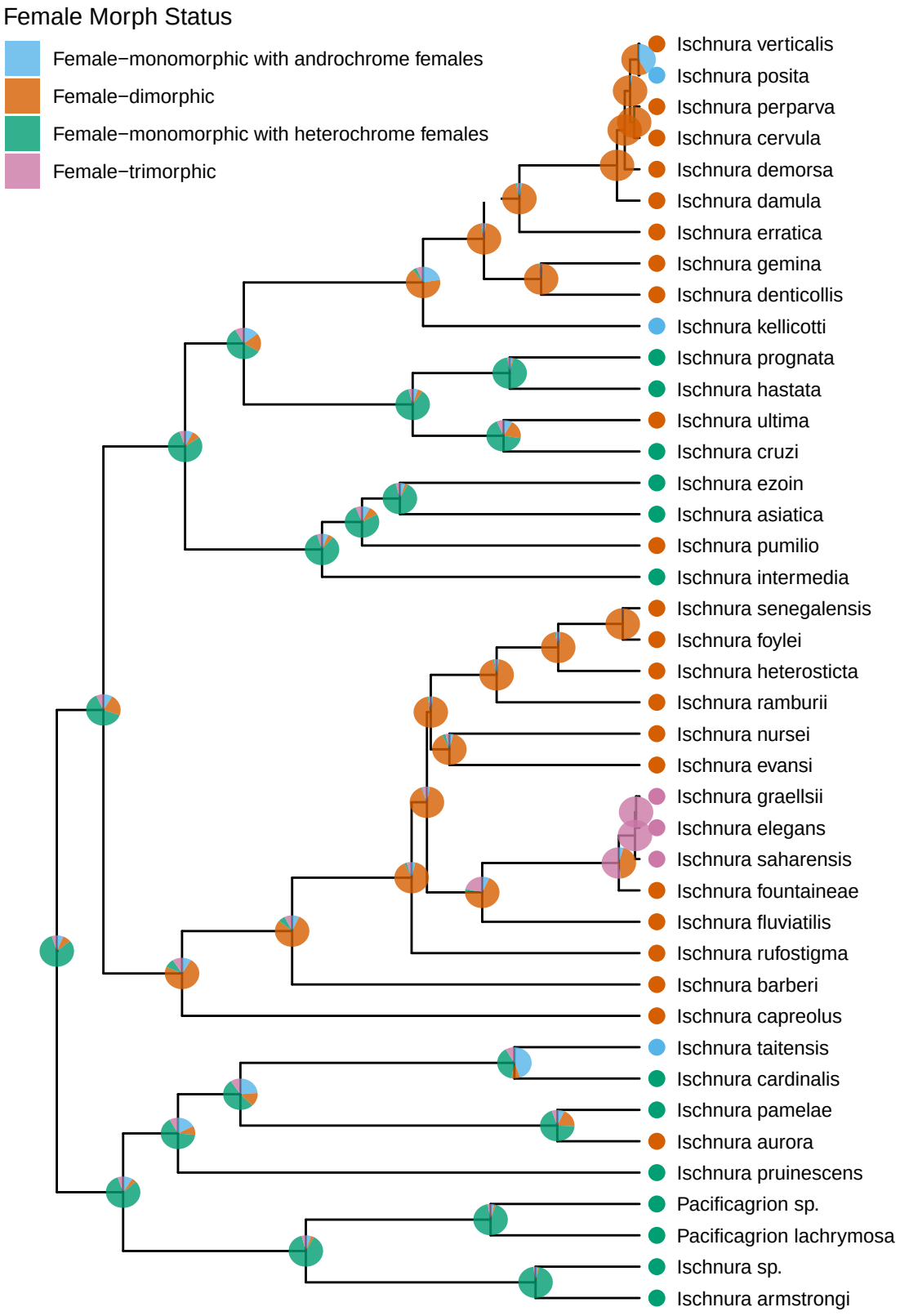
1024

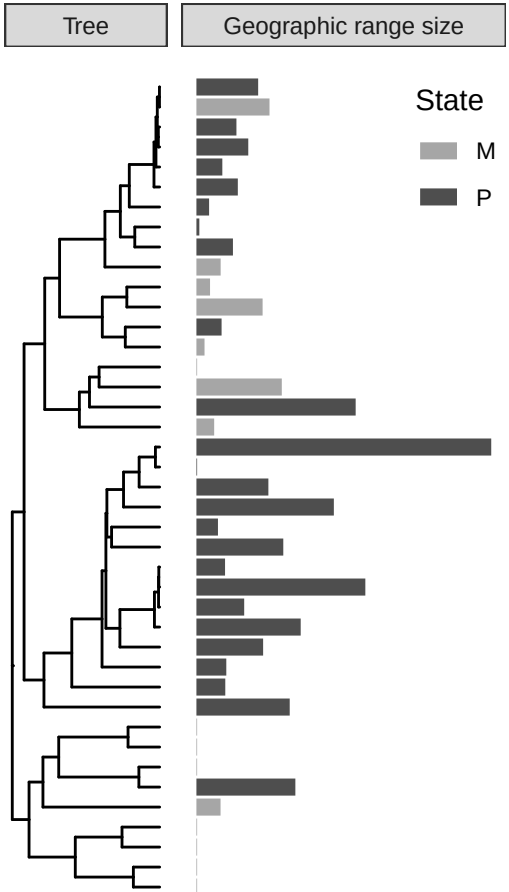
1025 **Figure 5.** Probability of species having female polymorphism in relation to the species
1026 geographic range size, estimated in millions of squared kilometres. The left panel shows the
1027 MMC tree for the genus *Ischnura*. Bars on the centre panel are proportional to the geographic
1028 range of each species, except for species with very small ranges ($<10^5$ Km^2) which are all
1029 depicted by a similarly thin line. Light grey bars represent female-monomorphic (M) species
1030 and dark grey bars correspond to female-polymorphic (P) species. On the right panel, each
1031 circle represents an extant species. The black line shows the prediction from a Bayesian
1032 phylogenetic mixed-effect model and the grey area shows the 95% credible interval of the
1033 prediction. Species with larger geographic ranges are more likely to have polymorphic females
1034 (see Results).

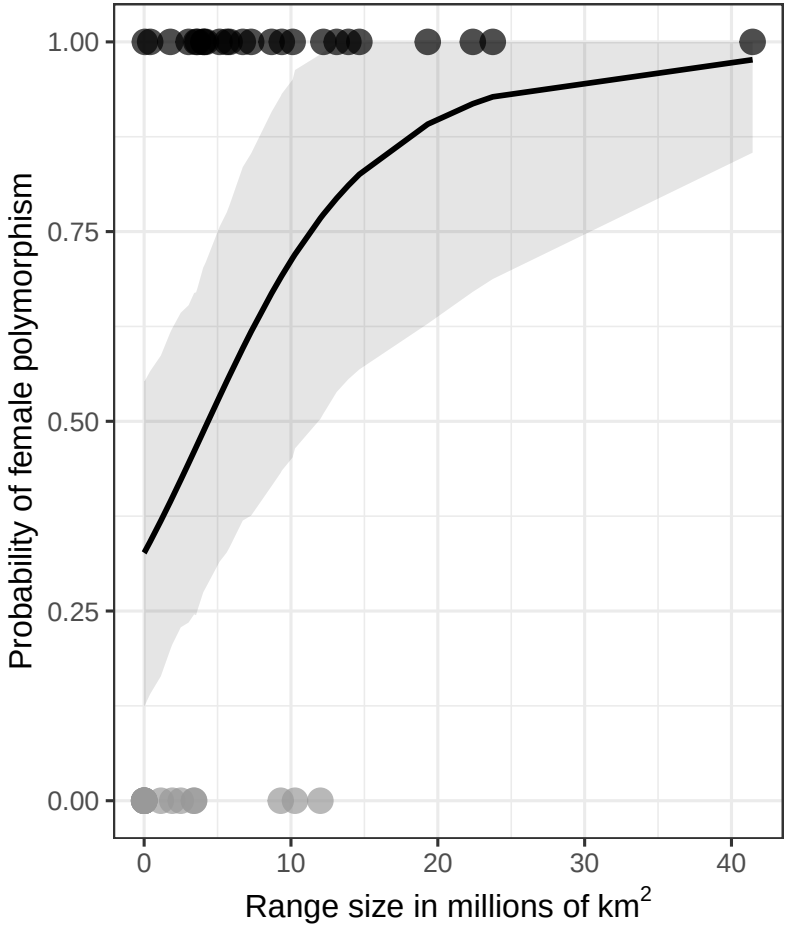

A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.


Ischnura verticalis
Ischnura posita
Ischnura perparva
Ischnura cervula
Ischnura demorsa
Ischnura damula
Ischnura erratica
Ischnura gemina
Ischnura denticollis
Ischnura kellicotti
Ischnura prognata
Ischnura hastata
Ischnura ultima
Ischnura cruzi
Ischnura ezoin
Ischnura asiatica
Ischnura pumilio
Ischnura intermedia
Ischnura senegalensis
Ischnura foylei
Ischnura heterosticta
Ischnura ramburii
Ischnura nursei
Ischnura evansi
Ischnura graellsii
Ischnura elegans
Ischnura saharensis
Ischnura fountaineae
Ischnura fluviatilis
Ischnura rufostigma
Ischnura barberi
Ischnura capreolus
Ischnura taitensis
Ischnura cardinalis
Ischnura pamelae
Ischnura aurora
Ischnura pruinescens
Pacificagrion sp.
Pacificagrion lachrymosa
Ischnura sp.
Ischnura armstrongi
Clade IV
Clade III
Clade II
Clade I
−20
−10
0


0.25
0.20
0.15
0.10
0.05
0.00
Density
10
20
30
40
50
60
70
80
90
100
110
120
130
Age Estimation


Female Morph Status
Female−monomorphic with androchrome females
Female−dimorphic
Female−monomorphic with heterochrome females
Female−trimorphic
Ischnura verticalis
Ischnura posita
Ischnura perparva
Ischnura cervula
Ischnura demorsa
Ischnura damula
Ischnura erratica
Ischnura gemina
Ischnura denticollis
Ischnura kellicotti
Ischnura prognata
Ischnura hastata
Ischnura ultima
Ischnura cruzi
Ischnura ezoin
Ischnura asiatica
Ischnura pumilio
Ischnura intermedia
Ischnura senegalensis
Ischnura foylei
Ischnura heterosticta
Ischnura ramburii
Ischnura nursei
Ischnura evansi
Ischnura graellsii
Ischnura elegans
Ischnura saharensis
Ischnura fountaineae
Ischnura fluviatilis
Ischnura rufostigma
Ischnura barberi
Ischnura capreolus
Ischnura taitensis
Ischnura cardinalis
Ischnura pamelae
Ischnura aurora
Ischnura pruinescens
Pacificagrion sp.
Pacificagrion lachrymosa
Ischnura sp.
Ischnura armstrongi


Tree
Geographic range size
State
M
P


Probability of female polymorphism
1.00
0.75
0.50
0.25
0.00
0
10
20
30
40
Range size in millions of km^2