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

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Abstract

Colour polymorphisms are popular study systems among biologists interested in evolutionary dynamics, genomics, sexual selection and sexual conflict. In many damselfly groups, such as in the globally distributed genus *Ischnura* (forktails), sex-limited female colour polymorphisms occur in multiple species. Female-polymorphic species contain two or three female morphs, one of which phenotypically matches the male (androchrome or male mimic) and the other(s) which are phenotypically distinct from the male (heterochrome). These female colour polymorphisms are thought 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) and incorporating both molecular and fossil data for 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 female polymorphisms, evidence of trans-species female polymorphisms and a significant positive relationship between female 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

Colour polymorphisms are widespread among many animal taxa and provide unique opportunities to study the evolutionary processes shaping genetic diversity within- and between-populations. The genetic basis of such colour polymorphisms is generally due to multiple segregating alleles at a single locus, with different degrees of dominance in relation to each other (Svensson, 2017; Svensson et al., 2009; Wellenreuther et al., 2014). Population-level studies aim to disentangle the evolutionary mechanisms responsible for the coexistence of alternative morphs in sympatry (Le Rouzic et al., 2015; Svensson et al., 2005). Yet, the turnover of alternative phenotypes and their underlying alleles on a macroevolutionary scale is more poorly understood (Corl et al., 2010; Jamie and Meier, 2020). Phylogenetic comparative methods can be used to explore how discrete phenotypic polymorphisms arise and are maintained or lost across macroevolutionary time scales (Corl et al., 2010; Hugall and Stuart-Fox, 2012; Jamie and Meier, 2020) . However, these methods are reliant on robust phylogenetic inferences (Rangel et al., 2015), and are affected by the incidence of evolutionary transitions between character states (Uyeda et al., 2018). An important step to elucidate how microevolutionary processes influence the macroevolutionary fate of discrete and alternative phenotypes is therefore to reconstruct, expand and improve phylogenies of clades with a dynamic history of phenotypic polymorphisms.

Discrete and heritable polymorphisms such as colour morphs are often sex-limited in their expression (Neff and Svensson, 2013; Svensson et al., 2009). Whereas male colour polymorphisms are typically associated with different strategies used in competition over access to females (Neff and Svensson, 2013), female-limited colour polymorphisms have been linked either to predator-avoidance through interspecific mimicry (Kunte, 2009), avoidance of

male mating harassment (Gosden and Svensson, 2009; Lee et al., 2019; Neff and Svensson, 2013) or a combination of these two factors (Cook et al., 1994). In the insect order Odonata (dragonflies and damselflies), female-limited colour polymorphisms occur in several genera and many species (Fincke et al., 2005). Female colour polymorphisms are particularly common in the globally distributed genus of pond damselflies *Ischnura* (family Coenagrionidae; Fig. 1) (Gering, 2017; Gosden and Svensson, 2009; Sirot and Brockmann, 2001; Takahashi and Watanabe, 2010). Within *Ischnura,* there are both female-monomorphic (FM) and female-polymorphic (FP) species, but males are always monomorphic (Fincke et al., 2005; Sanchez-Guillen et al., 2011; Willink et al., 2019)(Fig. 1B). Of the approximately 75 species in the genus *Ischnura*, c.a. 40% are FM, 40% are FP, and the female-colour status of the remaining 20% is currently unknown (Willink et al., 2019).

In FP species, females are either similar in colouration (Fig. 1B) and sometimes also in body shape to males, here referred to as androchrome females or male mimics, or they are phenotypically different from males (referred to here as heterochrome females) (Gosden and Svensson, 2009). Amongst these FP taxa, some species are female-dimorphic (FP(D)) with one androchrome and one heterochrome female morph, whereas others are female-trimorphic (FP(T)), with one androchrome and two distinct heterochrome female morphs (Fig. 1B). In FM species of *Ischnura*, both males and females are therefore monomorphic (Willink et al., 2019)(Fig. 1B). A species can therefore be either sexually monomorphic with androchrome females (FM(A)) or sexually dimorphic with heterochrome females (FM(H))(Willink et al., 2019)(Fig. 1B). Such interspecific variation in female colour states is particularly useful for phylogenetic comparative studies on the macroevolutionary dynamics of discrete and heritable polymorphisms (Corl et al., 2010; Jamie and Meier, 2020).

87 The classical explanation for the evolutionary origin and adaptive function of female colour
88 polymorphisms in *Ischnura,* and other damselflies is the "male-mimicry hypothesis" (Cordero,
89 1989; Robertson, 1985). Male-mimics are thought to have evolved as a response to selection
90 from sexual conflict over mating, which favoured visual features that made such male-like
91 females less recognizable as potential mates to males (Cordero et al., 1998; Gosden and
92 Svensson, 2009; Willink et al., 2019; Xu et al., 2014). This mimicry hypothesis would
93 phylogenetically predict that the common ancestor of FP damselflies was an FM(H) species, in
94 which a novel male-mimicking female phenotype emerged and subsequently increased in
95 frequency due to a frequency-dependent fitness advantage of male mimicry. An alternative,
96 though not mutually exclusive hypothesis for the evolution and maintenance of such female
97 polymorphisms are flexible search images in males (Fincke, 2004; Miller and Fincke, 1999).
98 Under this alternative hypothesis, males are expected to switch mating preferences between
99 female morphs, depending on their frequency, resulting in increased male preferences for
100 currently common morphs (Van Gossum et al., 2001). The most common female morph is then
101 expected to be disproportionally harassed, resulting in negative frequency-dependent selection
102 and the stable maintenance of female polymorphism across many generations (Gosden and
103 Svensson, 2009; Iserbyt et al., 2013, 2011; Le Rouzic et al., 2015; Svensson et al., 2005;
104 Takahashi et al., 2014, 2010). Unlike a pure version of the male-mimicry hypothesis (described
105 above), female colour polymorphisms in *Ischnura* could under this latter scenario have evolved
106 from either an FM(H) or FM(A) ancestral state (Fig. 1B).
107

108 FM species of *Ischnura* have received less attention compared to FP taxa, and consequently we
109 know very little about why there is such a striking variation in the presence or absence of female
110 polymorphisms in different subclades within this genus. One explanation could be that FM
111 lineages reflect an ancestral state with low intensity of sexual conflict. If so, female

112 polymorphisms may have subsequently evolved in lineages experiencing novel selective
113 regimes of more intense sexual conflict. This could occur, for instance, due to a rapid increase
114 in population density or transition to a new mating system. Alternatively, FM lineages could
115 reflect transitions from an ancestral state of female polymorphism (FP), via founder effects or
116 genetic drift, or through the selective fixation of one of the alternative previously sympatric
117 morphs (Corl et al., 2010; West-Eberhard, 1986). Such speciation by morph loss has been
118 termed "morphic speciation" (Corl et al., 2010; West-Eberhard, 1986). The morphic speciation
119 hypothesis explicitly predicts that monomorphic taxa should primarily be located at the terminal
120 tips on a phylogeny, with polymorphism being the ancestral state (Corl et al., 2010). Regardless
121 of the generality and validity of the morphic speciation hypothesis, our knowledge about the
122 relationship between polymorphism and speciation is limited and there are only a few
123 phylogenetic comparative studies in this area (Hugall and Stuart-Fox, 2012; McLean and
124 Stuart-Fox, 2014; Schwander and Leimar, 2011). Recently, it has also been emphasized that
125 some colour polymorphisms can be maintained across speciation events resulting in trans-
126 species polymorphisms (Jamie and Meier, 2020).
127
128 There have been a few previous comparative studies in the genus *Ischnura,* aiming to
129 understand the ecological correlates of female colour polymorphisms and their evolutionary
130 origin. These previous studies suggested that female colour polymorphism was the ancestral
131 state in *Ischnura* (Mattern and Van Gossum, 2008; Sánchez-Guillén et al., 2020). They further
132 argued female polymorphisms are more common in species with female polyandry, than in
133 species with (putative) female monandry, consistent with female polymorphism being
134 promoted by sexual conflict (Mattern and Van Gossum, 2008; Robinson and Allgeyer, 1996;
135 Sánchez-Guillén et al., 2020). However, these previous comparative studies suffer from several
136 limitations, such as ignoring the shared evolutionary history of closely related species

(Robinson and Allgeyer, 1996), using highly sensitive methods of ancestral state reconstruction like maximum parsimony(Mattern and Van Gossum, 2008), and ignoring phylogenetic and model uncertainty in ancestral state estimation (Sánchez-Guillén et al., 2020). Ignoring both phylogenetic uncertainty, model uncertainty and using highly sensitive methods like maximum parsimony is problematic, particularly for rapidly evolving characters (Cunningham et al., 1998; Schluter et al., 1997), which is likely the case for these colour polymorphisms and other traits that are involved in sexual conflict (Rice and Holland, 1997). Here, we revisit these previous hypotheses for the evolution of female colour polymorphism in *Ischnura*, with a broadened taxon sampling in our phylogenetic inference, and using contemporary methods to simulate trait evolution under a Bayesian framework. This approach allows for model-based inference of ancestral states, while taking into account model and phylogenetic uncertainty (Pagel et al., 2004; Pagel and Meade, 2006).

Our novel phylogenetic and comparative data and analyses in this study aimed to shed light on the origin of female colour polymorphisms. We also estimate the age and study the evolutionary history of the genus *Ischnura*. We use a multispecies coalescent model to simultaneously estimate a posterior distribution of species tree topologies and divergence times for 55% of species in the genus *Ischnura.* We then investigate the macroevolutionary history of female colour polymorphisms by reconstructing the ancestral state of this trait. Finally, we investigated whether the presence of female polymorphisms are associated with large geographic range sizes, a factor that is likely to reflect population size and the potential for morphic speciation by neutral processes. A positive association between the presence of female colour polymorphisms and geographic range would be expected in both of the following scenarios: 1) if morphs are lost by neutral or selective processes after a reduction of population (and range)

161 size or 2) if the effects of female colour polymorphisms on population fitness allow FP species
162 to sustain larger populations over a greater geographic range.

163

164 Our study contributes to increasing our understanding of the macroevolutionary history of this
165 genus, the origin of female colour polymorphism and the demographic factors promoting the
166 maintenance of these female polymorphisms. In presenting these results, we hope to stimulate
167 future empirical investigations on these and other damselfly clades, and we discuss some
168 promising hypotheses for follow-up studies. We note that many damselfly groups, although
169 popular in much ecological and evolutionary research (Cordoba-Aguilar, 2008) are still
170 relatively poorly investigated with respect to phylogenetic relationships and evolutionary
171 history, although some recent progress has been made, particularly within the family
172 Coenagrionidae (Beatty et al., 2017; Callahan and McPeek, 2016; Dijkstra et al., 2014;
173 Swaegers et al., 2014; Torres-Cambas et al., 2019; Vega-Sánchez et al., 2019).

174

175 **1. Materials and Methods**

176

177 *2.1 Data collection*

178 We have gathered DNA-sequence data, phenotypic data and geographic range size data for
179 41 species of *Ischnura*. Two of the taxa in this dataset are nominally in the genus *Pacificagrion*,
180 however recent molecular data shows they are nested within the *Ischnura* clade (Willink et al.
181 2019). Likewise, two other taxa, including a yet undescribed species, were classified in the
182 genus *Amorphostigma* (Marinov et al., 2015). However, Dijkstra et al. (2014) subsumed
183 *Amorphostigma* into *Ischnura*, and recent molecular data support this change (Willink et al.
184 2019). Here, we use the generic names *Ischnura* and *Pacificagrion*, according to the "World
185 Odonata List" (https://www.pugetsound.edu/academics/academic-resources/slater-

186 museum/biodiversity-resources/dragonflies/world-odonata-list2/), which maintains a
187 taxonomic classification of Odonata with currently valid names.
188
189 We obtained sequences for three nuclear and two mitochondrial markers. The nuclear markers
190 include conserved and hypervariable regions of the 28S ribosomal DNA (D7; alignment length:
191 693 bp), coding and intronic regions of the arginine methyltransferase gene (PMRT; alignment
192 length: 544 bp), and a coding region of the histone 3 (H3; fragment length: 328 bp). The
193 mitochondrial markers sampled were the third domain of the mitochondrial 16S ribosomal
194 DNA (16S; alignment length: 397 bp) and a fragment of the cytochrome oxidase subunit I (COI;
195 fragment length: 481 bp). Sequence data was collected for 147 specimens, of which 64 were
196 sequenced for this study (Table S1). Genomic DNA was extracted from 1-2 legs using a Qiagen
197 DNeasy tissue kit and following the manufacturer's instructions, except that we increased the
198 amount of proteinase K and incubation time for museum samples collected before 2005, in
199 order to obtain a higher DNA yield. Polymerase chain reaction (PCR) primers and amplification
200 programs used for the molecular markers were the same as in Willink et al. (2019).
201 Amplification products were purified using the Affymetrix ExoSAP-IT reagent (Cat. No.
202 78200) and sequenced on an ABI 3100 capillary sequencer at Lund University. These novel
203 sequence data were complemented with already-published sequences (Table S1), downloaded
204 from the National Centre for Biotechnology Information
205 (https://www.ncbi.nlm.nih.gov/genbank/). There were no indels in the sequences of two
206 molecular markers (COI and H3), which were aligned using MUSCLE v. 3.8.31 under default
207 settings (Edgar, 2004). To reduce gap overmatching in ribosomal sequences (16S and 28S) and
208 intronic regions (present in PMRT), we used the phylogenetically informed algorithm PRANK
209 v.150803 (Löytynoja and Goldman, 2008, 2005) to align these data. PRANK was iterated 20
210 times, inferring a new guide tree after each alignment and the highest scoring alignment was

selected by randomly breaking score ties at each iteration. The remaining settings were left in their default values, except for the alignment of 28S rDNA, for which a branch length multiplier of two was used to produce more mismatches (fewer gaps) in the hypervariable region.

Data on female colour morph status (i.e. FM, or FP) has been recently compiled for *Ischnura* damselflies, based on published literature, field observations, examination of museum specimens and online resources (Willink et al., 2019). We complemented these data with information on whether FP species had two or three morphs (i.e. FP(D) or FP(T)) and whether FM species had androchrome or heterochrome females (i.e. FM(A) or FM(H)) (Table S2). Here, species with a single female morph were classified as FM(A), if they had only one female morph with colouration and patterning similar to that of conspecific males, or as FM(H), if they had only one female morph with colouration and patterning markedly different from the conspecific males. If a species contained two distinct female morphs (always one androchrome and one heterochrome), we classified it as FP(D). Finally, species that had three morphs (which were always one androchrome and two heterochrome female morphs), were classified as FP(T).

We complemented these colour-state data with coarse-grained estimates of geographic range size (Table S2). Because accurate geographic distribution data is lacking for many species, we estimated geographic range using occurrence data from administrative regions, mainly countries, and where available, states or provinces (Table S2). Presence data was translated to geographic range size (km^2) by adding the areas of all the countries or other administrative regions where a species is known to occur (Table S2), as has been done previously in comparative studies where geographic range is taken as a proxy for population size (Ross et al., 2013). The areas of administrative regions were extracted from the GADM database of global administrative areas v. 3.6 (Global Administrative Areas, 2018).

236

237 1.2 *Time-calibrated species tree*

238 We analysed sequence data under a multispecies coalescent (MSC) model using StarBEAST2
239 (Ogilvie et al., 2017). The MSC models the evolution of gene trees independent from each other
240 but conditional on a shared species tree (Degnan and Rosenberg, 2009). When implemented in
241 a Bayesian framework, the MSC provides a means to estimate the joint conditional distribution
242 of gene trees and species trees in the face of gene tree discordance due to incomplete lineage
243 sorting (Rannala et al., 2020). Incomplete lineage sorting occurs whenever two alleles in the
244 same population fail to coalesce, going backwards in time, before at least one of them coalesces
245 with an allele from a more distantly related population. Such incomplete lineage sorting is
246 commonplace, particularly during rapid radiations, such as the one described in the closely
247 related damselfly genus *Enallagma* (Callahan and McPeek, 2016). By using the MSC model,
248 we allowed genealogical histories to vary among nuclear loci and between nuclear and
249 mitochondrial genes. As there is no recombination amongst mitochondrial genes, we linked the
250 gene tree models of mitochondrial sequences (COI and 16S).

251

252 We placed a birth-death prior, conditional on the root, on the species tree to allow for extinction,
253 set the gene ploidy to 0.5 for mitochondrial sequences and 2.0 for nuclear sequences and
254 allowed population-size to be analytically integrated to avoid over-parameterization. We used
255 a relaxed uncorrelated log normal (UCLN) clock per locus to allow for variation in evolutionary
256 rates between lineages. Substitution models for each locus were inferred during the analysis
257 using the package "bModelTest" (Bouckaert and Drummond, 2017) to allow for site model
258 uncertainty. We placed a lognormal prior with a mean of 0.0115 and standard deviation of 1.0
259 on each relaxed mitochondrial clock and a broader lognormal prior with a mean of 0.0115 and
260 standard deviation of 2.0 on each relaxed nuclear clock to centre clock rates on the suggested

261 insect mitochondrial divergence rate of 2.3% per million years (Brower, 1994) whilst allowing
262 for clock rate uncertainty, particularly in the nuclear loci.

263

264 Data for *Ischnura* fossils were downloaded from the Paleobiology database (www.pbdb.org)
265 on 14th May 2018. There were three fossils classified as either in or closely related to the genus
266 *Ischnura*. Two of these fossils were relatively young (7.2 - 5.3 mya), each one consisting of a
267 single wing with missing fragments (Gentilini and Bagli, 2004). The third fossil was preserved
268 in Dominican amber (20.44 – 13.82 mya) and was identified and described as *Ischnura velteni*
269 based on a combination of wing venation and morphological traits in other body parts (Bechly,
270 2000). The combination of these characters suggests that *I. velteni* belongs to the extant genus
271 *Ischnura* (Bechly, 2000), and we therefore considered it the most appropriate crown fossil with
272 which to calibrate the root age of the *Ischnura* tree. We placed an exponentially distributed
273 prior on the root age of the species tree with an offset of 13.8 to ensure the most recent common
274 ancestor (MRCA) of *Ischnura* could not have appeared after the minimum age of this known
275 member of the genus. The mean of the exponential was drawn from a gamma hyperprior with
276 a shape parameter $\alpha = 5.0$ and an inverse scale parameter $\beta = 2.0$. This hyperprior specification
277 centred the root age prior distribution around 10 mya prior to the offset, allowing a moderately
278 high level of uncertainty in the age difference between the fossil and the MRCA of *Ischnura.*

279

280 We ran two separate Markov Chain Monte Carlo (MCMC) algorithms for 200 million iterations
281 with a sampling rate of 20000. We confirmed that independent chains were stationary and had
282 converged for these and all subsequent analyses in R v.3.6.1 (R Core Team 2019) using the
283 "coda" package (Plummer et al., 2006). We combined posterior trees using LogCombiner
284 (available as part of BEAST v2.4.7)(Bouckaert et al., 2014), simultaneously discarding 20% of
285 the posterior as burnin, and summarized them into a maximum clade credibility (MCC) tree

286 using TreeAnnotator (available as part of BEAST v2.4.7). The MCC tree and all further
287 phylogenetic plots were annotated in R v.3.6.1 (R Development Core Team, 2019) using the
288 packages "ape" (Paradis and Schliep, 2019), "ggtree" (Yu et al., 2017) and "ggplot2"
289 (Wickham, 2016).
290

291 1.3 *Reconstruction of ancestral character states of female colour polymorphism*

292 We used the Multistate-method in BayesTraits v.3.0 (Pagel et al., 2004) to infer female-colour
293 character states at ancestral nodes, given this new *Ischnura* phylogeny and the female-colour
294 character states of extant species. To account for phylogenetic uncertainty, we ran the model
295 using a sample of 1000 trees from the combined species-tree posterior of the two independent
296 runs. Additionally, we ran the model using reversible jump (RJ) MCMC to account for
297 uncertainty in the number of model parameters. In this model, the number of different transition
298 rate parameters and their values are sampled in proportion to their posterior probability.
299 Consequently, in each posterior sample a number of transition rates are set to zero, and the non-
300 zero rates belong to one or more categories. We placed an exponential prior on all rate
301 parameters, with the mean seeded from a uniform hyperprior bounded between 1 and 100. We
302 ran two separate chains for 10 million iterations, sampling every 1000 with 20% posterior
303 burnin. Trees were scaled to an average branch length of 0.01 to prevent various rates from
304 becoming too small. Although our main focus is on ancestral state reconstructions, we present
305 the posterior distributions of transition rate parameters in the Supporting Material (Fig. S1).
306 A recent study estimated ancestral female-colour states, using a single MCC tree and stochastic
307 character mapping (Sánchez-Guillén et al., 2020). We investigated if the conflicting results
308 between the present study and Sánchez-Guillén et al. (2020) could be explained by differences
309 in taxon sampling. Specifically, we examined if inclusion of taxa sampled by Sánchez-Guillén
310 et al. (2020), but not included in the present study would qualitatively change our conclusions.

311 To do so, we used the R package "pastis" (Thomas et al., 2013) to include the missing taxa in
312 our MCC species-tree, by randomly assigning their phylogenetic placement and branch lengths
313 within their inferred clade, according to Sánchez-Guillen et al. (2020) (See Supporting
314 Methods). We then repeated ancestral state inferences as explained above on the sample of
315 expanded species trees.
316

317 1.4 *Linking female colour polymorphism to geographic range size*

318 We investigated if the occurrence of female colour polymorphisms could be influenced by
319 demographic changes, by using geographic range size of extant species as a proxy for
320 population size. Geographic range size is also a biologically interesting variable in the light of
321 recent work showing that female colour polymorphisms can increase population fitness
322 (Takahashi et al., 2014). We used a Bayesian phylogenetic mixed-effect model (BPMM)
323 (Hadfield and Nakagawa, 2010) implemented in the package MCMCglmm v. 2.29 (Hadfield,
324 2010) to test for such phylogenetic correlation. Female-morph status was treated as a binary
325 response variable, with species being classified as either FM or FP. Since the occurrences of
326 FP(T) species and FP(A) were low, we classified these species-states as being FP and FM,
327 respectively. In this analysis, we thus investigated if the probability of a species being FP was
328 significantly and positively related to geographic range size (a continuous predictor variable).
329

330 We accounted for phylogenetic uncertainty by using a random sample of 2100 species-trees
331 generated in StarBEAST2. To estimate the phylogenetic random effect, each tree was sampled
332 for 10000 iterations, of which only the last iteration was saved to the posterior, and the first 100
333 trees were also discarded as burn in (O'Connor et al., 2018). We used a Knonecker prior (mu=0,
334 V= σ2units + $\pi^{2/3}$) for the fixed effects as it is relatively flat when a logit link function is used
335 (Hadfield, 2015). We used a parameter-expanded χ^2 distribution (V=1 nu=1000, alpha.mu=0,

336 alpha.V = 1) for the prior on the phylogenetic variance components (Villemereuil et al., 2013).
337 The residual variance was fixed to 1, as it cannot be identified for binary response models. As
338 a statistical test of whether the probability of female colour polymorphism increases with range
339 size, we report the fraction of the posterior distribution in which increasing range size does not
340 result in an increasing probability of female colour polymorphism. The results of this analysis
341 were visualized by plotting the expected probability of female colour polymorphism against
342 estimated geographic range size.
343
344 **2. Results**
345
346 3.1 *Divergence time estimation*
347 Our time-calibrated phylogeny suggests that the age of the MRCA of the genus *Ischnura* lies
348 in a 95% highest posterior density (HPD) interval between 13.8 and 23.4 mya, i.e. during the
349 early Miocene (Fig. 2). A comparison of the posterior root age in our model to a model ran
350 without molecular data suggests the sequence data were sufficiently informative to narrow this
351 posterior distribution (Fig. 3), although age estimates had quite broad error margins (Fig. 2).
352 Nonetheless, divergence dating revealed several interesting features, such as the very recent
353 divergence of two of the most intensely studied FP species in Europe (*I. elegans* and *I. graellsii*)
354 (Fig. 2). Our phylogenetic analysis also showed that the species that feature in most
355 evolutionary and ecological studies (*I. elegans*, *I. graellsii*, *I. ramburii* and *I. senegalensis*) are
356 part of a well-supported lineage (*I. senegalensis* to *I. rufostigma;* Fig. 2), with a common
357 ancestor less than 10 mya (Fig. 2). We found that all species from Australia and the South
358 Pacific islands, except *I. heterosticta,* form a single clade *(I. taitensis* to *I. armstrongi*; Fig. 2).
359 Similarly, we recovered a strongly supported Palearctic clade *(I. ezoin* to *I. intermedia;* Fig. 2)

360 and strongly supported lineages distributed primarily in the Neotropical (*I. prognata* to *I. cruzi*)
361 and Nearctic (*I. verticalis* to *I. kellicotti*) regions (Fig. 2).
362
363 2.2 *Ancestral state reconstruction of female colour polymorphism*
364 Out of the 41 species included in this study, three were FM(A), 13 were FM(H), 22 were FP(D),
365 and three were FP(T) (cf. Fig 1B with Fig. 4). The MRCA of *Ischnura* was reconstructed as
366 being FM(H), i.e. the ancestor was sexually dimorphic, with a mean posterior probability (PP)
367 of 81% (Fig. 4). In contrast, the probability that the ancestor was sexually monomorphic (i.e.
368 androchrome females only, being similar to males) was only 7 %, strongly suggesting that male
369 mimicry and FP are derived traits and not ancestral. The clade containing most taxa from the
370 Pacific islands (Fig. 2) was also inferred as being ancestrally FM(H) (PP = 81%; Fig. 4). All
371 FP(T) species shared an FP(T) common ancestor (PP = 100%; Fig. 4), most likely derived from
372 an FP(D) state (Fig. 4). The results presented here contrast strikingly with the conclusions of a
373 recent study, in which the MRCA of *Ischnura* was reconstructed as female-polymorphic with
374 63% probability (Sánchez-Guillén et al., 2020). Repeating the analysis above but with the
375 inclusion of nine additional species from Sánchez-Guillén et al. (2021) that were not included
376 in our original taxa did not qualitatively alter our conclusion that the ancestor of the genus
377 *Ischnura* was instead FM (H) (Fig. S2), although the support for a FM (H) ancestral state in
378 *Ischnura* decreased to 66 % with the additional taxa (Supporting Results).
379
380 Two evolutionary transitions occurred with high posterior probability during the diversification
381 of *Ischnura*, according to the RJ MCMC analysis. The transition rate from FP(D) to FM(A) (i.e.
382 the evolutionary loss of the heterochrome female morph) was above zero in 99.7% of the
383 posterior (Fig. S1). Similarly, the transition from FM(H) to FP(D) (i.e. the evolutionary gain of
384 an androchrome female morph) occurred with non-zero rate in 94.2% of the posterior (Fig. S1).

385 In contrast, the least probable transition, from FM(H) to FP(T) occurred in only 6.7% of the
386 posterior (Fig. S1). The data on female colour states of extant *Ischnura* species and the posterior
387 distribution of *Ischnura* time trees are therefore consistent with multiple evolutionary histories
388 and different combinations of other character-state transitions being present or absent.
389

390 3.3 *Geographic range size and female-colour polymorphism*

391 Current geographic range sizes varied from 4.3 x 10^7 km^2 in the largely subtropical species *I.*
392 *senegalensis*, which is distributed from sub-Saharan Africa to Japan, to 222 km^2 to species
393 endemic to American Samoa (Table S2). We found that all island-endemic species are FM,
394 except for *I. foylei*, which is FP(D) and only known from the Jambi province in Sumatra,
395 Indonesia (Table S2). In contrast, the three species distributed across the longitudinal range of
396 the Old World, *I. senegalensis*, *I. pumilio* and *I. elegans,* are either FP(D) or FP(T). The
397 probability of a species being FP increased significantly with its geographic range size
398 (PMCMC = 0.003; Fig. 5). The probability of female colour polymorphism increased by ~ 5%
399 (posterior mean = 0.051, 95% HPD interval = 0.008 – 0.990) with an increase of 1 x10^6 km^2 in
400 the geographic range size.
401

402 **4. Discussion**

403 Here, we have presented the first molecular time-calibrated phylogeny for the damselfly genus
404 *Ischnura*. Although both dragonflies (suborder Anisoptera) and damselflies (suborder
405 Zygoptera) have long been popular study organisms in ecology, evolution and conservation
406 biology (Cordoba-Aguilar, 2008), time-calibrated molecular phylogenies that could facilitate
407 phylogenetic comparative studies in these two insect groups are still relatively few (Callahan
408 and McPeek, 2016; Damm et al., 2010; Letsch et al., 2016; Toussaint et al., 2019; Waller and
409 Svensson, 2017). Several species in the genus *Ischnura* in particular have been subject to much

410 research on sexual selection, sexual conflict and female colour polymorphism (see
411 Introduction). The general branching patterns in this new phylogeny are largely and
412 qualitatively in agreement with previous molecular phylogenies covering a subset of the taxa
413 included here (Chippindale et al., 1999; Willink, 2018; Willink et al., 2019).

414

415 Using a UCLN molecular clock and fossil calibration, we inferred the MRCA of *Ischnura* as
416 living between 13.8 and 23.4 mya, that is, the early Miocene. This is earlier than the MRCA of
417 the closely related damselfly genus *Enallagma*, which has an estimated age of about 10 my
418 (mid- to late Miocene), and where many speciation events seem to have been driven by glacial
419 cycles during the Pleistocene (Callahan and McPeek, 2016). An early Miocene origin of
420 *Ischnura* is also slightly earlier than the inferred origin of the closely related Palearctic
421 damselfly genus *Coenagrion,* during the latter part of Miocene 15 mya (Swaegers et al., 2014),
422 and the two genera *Melanosobasis* and *Nesobasis* in Fiji originating during late-middle
423 Miocence (8-12 mya)(Beatty et al., 2017). We emphasize that our age estimate for the MRCA
424 of *Ischnura* rests on the assumption that *I. velteni* is a crown fossil (Bechly, 2000). Future
425 studies based on alternative dating strategies will be important to validate this inferred early
426 Miocene origin of *Ischnura*.

427

428 According to our phylogeny and ancestral state reconstruction, the four most well-studied
429 species in *Ischnura* (*I. senegalensis*, *I. graellsii, I. elegans* and *I. ramburii*)(Gering, 2017;
430 Robertson, 1985; Sirot et al., 2003; Sirot and Brockmann, 2001) are part of a single clade, likely
431 descended from an FP(D) ancestor (Fig. 4), that lived less than 10 mya (Figs. 2,4). While the
432 genus *Ischnura* has increasingly attracted attention in ecological and microevolutionary studies,
433 there is much untapped potential in understanding its macroevolutionary history and repeated

434 transitions between female colour states. Our ancestral state reconstruction suggests that the
435 ancestor of the genus *Ischnura* was FM(H), although we caution that the support for this
436 inference was 81% (Fig. 4). Such a sexually dimorphic ancestor would imply multiple
437 evolutionary transitions to obtain female-dimorphism, through the evolutionary emergence of
438 a male-mimic. This evolutionary transition occurred with a non-zero rate in 94.2% of the
439 posterior distribution of transition rate estimates in our RJ MCMC analysis (Fig. S1). This
440 suggests that female colour polymorphism, with heterochrome and androchrome females
441 coexisting within species, has evolved convergently multiple times in this genus, presumably
442 as an evolutionary response to increased intensity of sexual conflict. Specifically, the
443 emergence of a novel male-mimic might have been possible if it gained an initial fitness benefit
444 due to advantages in intersexual mimicry through reduced male mating harassment (Robertson,
445 1985; Svensson et al., 2009; Willink et al., 2019). Such male-mimics might subsequently have
446 increased to high frequencies in several species where they persisted alongside with the
447 ancestral heteromorph as stable trans-species polymorphisms (Jamie and Meier, 2020).
448 Currently, these female-limited polymorphisms are most likely maintained by frequency-
449 and/or density dependent sexual selection caused by male mating harassment (Gosden and
450 Svensson, 2009; Le Rouzic et al., 2015; Svensson et al., 2005; Takahashi et al., 2014, 2010).

451

452 We further note that the only known FP(T) clade, containing the well-studied European species
453 *I. elegans* and *I. graellsii,* most likely evolved from an FP(D) ancestor (Fig. 4). The evolutionary
454 emergence of a third female morph in an already FP system might indicate that sexual conflict
455 through male mating harassment was particularly intense in this clade, which would fit with
456 available empirical evidence from previous field and experimental studies (Cordero, 1992;
457 Cordero et al., 1998; Gosden and Svensson, 2009; Takahashi et al., 2014; Willink et al., 2019).
458 In support of this, *I. graellsii* and *I. elegans* have some of the longest copulation durations of

459 all investigated species so far and mate for several hours (Robinson and Allgeyer, 1996). The
460 long copulation duration in these two FP(T) species contrasts with the much shorter copulations
461 and lower mating rates of some of the North American FM species, some of which have also
462 been suggested to be monandrous (Robinson and Allgeyer, 1996). Thus, high mating rates, high
463 degree of density-dependent male mating harassment (Gosden and Svensson, 2009) and long
464 copulation duration (Robinson and Allgeyer, 1996) in the FP(T) clade containing *I. elegans* and
465 *I. graellsii* might jointly have favoured the evolutionary establishment of a third morph in these
466 species. Theory suggest that one evolutionary outcome of sexual conflict is the emergence of
467 multiple genetic clusters in both males and females (Gavrilets and Waxman, 2002; Hayashi et
468 al., 2007). There is small but growing body of empirical evidence that female phenotypic
469 polymorphisms might have evolved as a response to sexual conflict in several different taxa
470 (Iversen et al., 2019; Karlsson et al., 2013; Lee et al., 2019; Moon and Kamath, 2019; Reinhardt
471 et al., 2007; Svensson et al., 2005).

472

473 The results in the present study run counter to to the conclusions of two previous phylogenetic
474 comparative studies where the MRCA of *Ischnura* was reconstructed as FP (Mattern and Van
475 Gossum, 2008; Sánchez-Guillén et al., 2020). Sánchez-Guillén et al. (2020) used a model of
476 trait evolution, but assumed a single rate of trait evolution and only two possible character states
477 (FM or FP), ignoring variation in sexual dimorphism among FM species and morph number (i.
478 e. two or three morphs) in the FP taxa. In contrast, here we allowed for model uncertainty by
479 using a reversible jump MCMC algorithm that samples alternative trait evolution models in
480 proportion to their posterior probability (Pagel and Meade, 2006). Moreover, unlike Sánchez-
481 Guillén et al. (2020), our ancestral state inference in the present study explicitly incorporated
482 phylogenetic uncertainty, which through the MSC, captured uncertainty in how gene
483 genealogical histories and species genealogical relate to one another.

To assess the possibility of disparate taxonomic sampling causing this discrepancy between studies, we included the unique species in Sánchez-Guillén et al. (2020) in a new posterior sample of species-trees, taking uncertainty in phylogenetic placement into consideration. We obtained qualitatively identical results as in our original analysis (Fig. S2). The newly added taxa from Sánchez-Guillén et al. (2020) were spread across one of the two lineages splitting from the MRCA of *Ischnura* in the present study (Fig. S2). The other early-splitting clade, however, included primarily South Pacific taxa, most of which are island-endemics and FM, and with one exception, are absent from Sánchez-Guillén et al. (2020). Our more extensive taxon sampling of this primarily FM clade likely contributed to inferring a FM ancestral state for *Ischnura* in the present study. It is noteworthy that of the 23 described and remaining species in this genus that were missing in both Sánchez-Guillén et al. (2020) and the present study, ten are endemic to small islands in the Pacific Ocean (Samoan islands, French Polynesian islands, Northern Mariana islands), one is endemic to Mauritius and at least six are highland endemics (Table S3). These relatively isolated taxa with restricted geographic ranges are more likely to be FM (Fig. 5; see below), and are likely to strengthen the hypothesis of ancestral female monomorphism in *Ischnura*, if they would become included in future phylogenetic studies of this genus.

Polymorphisms might promote speciation if different morphs diverge phenotypically in sympatry, followed by selective or random loss in allopatry, resulting in the fixation of alternative morphs in different populations (Jamie and Meier, 2020; West-Eberhard, 1986). This process – termed “morphic speciation” – predicts that monomorphic taxa are commonly found at the terminal tips on a phylogeny (Corl et al., 2010; West-Eberhard, 1986). There is some evidence for such a process among populations of colour polymorphic side-blotched

509 lizards (*Uta stansburiana*)(Corl et al., 2010). Our results do not lend support for widespread
510 morphic speciation, but are consistent with losses of heterochrome females in some FP(D) taxa,
511 as this evolutionary transition occurred in 99.7% of the posterior in the RJ MCMC analysis.
512 The most likely candidate for such potential morphic speciation is the common ancestor
513 between *I. verticalis* and *I. posita* (Fig. 2). These two taxa are part of a North American clade
514 that diversified rapidly during the Pleistocene (*I. damula* to *I. cervula;* Fig. 2). Such rapid
515 speciation and diversification could have resulted from repeated glacial cycles, as in the closely
516 related genus *Enallagma* that contains many sympatric young species in North America
517 (Callahan and McPeek, 2016; McPeek and Gavrilets, 2006). Such glacial cycles and repeated
518 Pleistocene bottlenecks could have led to morph fixation of a male-like female androchrome
519 morph in the ancestor of *I. posita* during a period of allopatry (Fig. 4). *I. posita* is currently a
520 widely distributed species, ranging from Nova Scotia in Canada to Central America (Paulson,
521 2012; Table S2). If morph loss in the ancestor of *I. posita* resulted from genetic drift during a
522 period of allopatry and population contraction, it must then have been followed by rapid and
523 recent range expansion. While this hypothesis remains to be investigated for *I. posita,* closely
524 related and young sympatric taxa in *Enallagma* are thought to be the result of rapid speciation
525 and range expansion after glacial retreat in the Quaternary (McPeek and Gavrilets, 2006;
526 Turgeon et al., 2005).

527

528 Another potential example of morphic speciation leading to fixation of a single female morph
529 is *I. kellicotti*, although it is less clear whether the lineage leading to *I. kellicotti* had an FP
530 ancestor (Fig. 4). Nevertheless, the potential for morphic speciation in *I. kellicotti* is interesting
531 because females display a striking pattern of ontogenetic colour changes. Sexually immature
532 females start with a bright orange colouration that turns blue and male-like in mature females,
533 and develops increasing pruinescence with age thereafter (Paulson, 2012, 2009). If used as a

534 signal of reproductive availability (cf. Willink et al. 2019), the evolution of distinct
535 developmental colour phases may have reduced male mating harassment and facilitated
536 morphic speciation. We note that in several relatively distantly related species of *Ischnura*,
537 immature females express orange or yellow colouration prior to reproductive maturity (e. g. in
538 *I. ramburii, I. pumilio*, *I. senegalensis* and *I. capreolus*; Fig. 1E-G), and such striking immature
539 colouration could signal reproductive unsuitability (cf. Willink et al. 2019). Alternatively, an
540 evolutionary transition in habitat and mating system could have led to morph loss and morphic
541 speciation in the ancestor of *I. kellicotti*. Individuals of *I. kelicotti* ("Lilypad forktail") are
542 typically found sitting on lilypad leaves in ponds (Paulson, 2012, 2009), consistent with a
543 switch from non-territorial male-male scrambling competition over females with high mating
544 harassment, to a new mating system of partial male territoriality with a lower degree of mating
545 harassment. Such relaxed pressure from sexual conflict may have led to the loss of female
546 polymorphism. A combination of "phylogenetic natural history" (Uyeda et al., 2018) and more
547 detailed knowledge about the molecular and genetic basis of female colour development (
548 Takahashi et al., 2018; Willink et al., 2020) could shed light on this issue and increase our
549 general understanding of this and other macroevolutionary events in *Ischnura*.

550

551 The presence of female colour polymorphism was positively correlated with geographic range
552 size (Fig. 5), which could come about in at least three different, but non-mutually exclusive,
553 ways. First, species with large ranges are also likely to have large effective population sizes and
554 this will decrease the effects of genetic drift and morph fixation. It is interesting to note here
555 that several of the FM taxa are endemic to small islands in the Pacific Ocean (Table S2). Second,
556 species with larger ranges and associated larger population sizes might experience stronger
557 sexual conflict due to higher population densities, consistent with more intense male mating
558 harassment under such conditions (Gosden and Svensson, 2009). Finally, FP species might also

559 be able to exploit a broader range of niches and thereby also achieve higher population mean
560 fitness compared to FM species, with positive effects on both population size and geographic
561 range size (Forsman, 2016; Svensson, 2017; Takahashi et al., 2014; Takahashi et al., 2018;
562 Takahashi and Noriyuki, 2019; West-Eberhard, 1986). Although the data in this study does not
563 allow us to firmly distinguish between these non-mutually exclusive explanations for the
564 positive relationship between the incidence of colour polymorphism and geographic range size
565 (Fig. 5), these different scenarios deserve attention in future studies. A positive relationship
566 between geographic range size and presence of colour polymorphism in *Ischnura* and other
567 related damselflies was reported in a recent study, although without considering phylogenetic
568 relatedness among species, as no molecular phylogeny was available at that time for *Ischnura*
569 (Takahashi and Noriyuki, 2019).

570

571 Interestingly, the least supported nodes of our MCC tree are in the lineage from *I. senegalensis*
572 to *I. rufostigma* (Fig. 2) and in the North American clade (*I. kellicotti* to *I. verticalis*; Fig. 2),
573 which are both dominated by FP taxa (Fig. 4). These subsets of taxa were not particularly
574 underrepresented in our dataset (Table S1). There are at least two potential biological
575 explanations for this pattern. First, interspecific reproductive interference may be boosted by
576 sexual conflict over mating rates (McPeek and Gavrilets, 2006; Svensson et al., 2009) which is
577 likely to be more intense in FP species. This could result in more frequent hybridization and
578 introgression in FP clades (Okude et al., 2020; Sanchez-Guillen et al., 2005; Sánchez-Guillén
579 et al., 2011). Second, faster speciation when population sizes are large can result in deep
580 coalescence among lineages and thus increase gene tree discordance (Maddison, 1997). If FP
581 species have larger population sizes as our results suggest (Fig. 5), and colour polymorphisms
582 accelerate speciation (Hugall and Stuart-Fox, 2012), then the speciation dynamics of FP clades
583 may render them particularly challenging for phylogenetic inference. These alternative

evolutionary scenarios are clearly worthy of further research, and require methods of phylogenetic inference such as the MSC, which accommodates incomplete lineage sorting and can be expanded to incorporate other biological processes such as introgression (Wen and Nakhleh, 2018; Zhang et al., 2018).

Finally, in one, and potentially two, of the FP clades, the female colour polymorphism seems to have arisen early and been subsequently maintained across multiple speciation events (Fig. 4). Therefore, the *Ischnura* female colour polymorphisms are examples of trans-species polymorphisms (Jamie and Meier, 2020), when multiple allelic variants become established in a population prior to speciation and are subsequently passed on to descendant taxa across multiple speciation events (Klein, 1987). Other examples of trans-species polymorphisms are rare, the most well-known being immune system polymorphisms and blood group variation in great apes (Azevedo et al., 2015) and self-incompatibility allelic systems in plants (Goldberg et al., 2010; Richman et al., 1996; Richman and Kohn, 2000). However, such trans-species polymorphisms may be more common than the limited current evidence suggests, particularly in colour polymorphic taxa (Corl et al., 2010; Jamie and Meier, 2020). In the damselfly genus *Ischnura*, some FP species have higher population-level fitness when female morph frequencies are even, compared to when they are biased towards any particular morph (Takahashi et al., 2014). In this study, we also showed that FP species have larger range sizes than FM species, indicating higher "ecological success" of the former (Fig. 5). Although these female colour polymorphisms are maintained by negative frequency-dependent selection at the individual level (Le Rouzic et al., 2015; Svensson et al., 2005; Takahashi et al., 2010) the higher-level population effects mentioned above might also indicate an additional role for species-level selection through biased extinction of FM taxa. Such higher level-effects would be similar to

608 how self-incompatibility alleles are maintained in plant mating systems by species selection
609 (Goldberg et al., 2010).

610

611 **Conclusions**

612 The results in this study suggest that the damselfly genus *Ischnura* originated in the early
613 Miocene, with ancestral females being monomorphic and markedly distinct from males in their
614 colour pattern (heterochrome). The female colour polymorphisms in this genus arose on several
615 occasions through repeated origins of a male-mimic in different species. Our ancestral state
616 reconstruction further suggests that female colour polymorphisms have survived through
617 multiple speciation events in at least two clades, contrary to the expectation of speciation by
618 selective or random morph loss in allopatric populations (morphic speciation). However, we
619 find at least one potential loss of the female colour polymorphism that could have resulted in
620 morphic speciation. The multiple transitions in female-morph states across the phylogeny imply
621 that the selective pressures from sexual conflict differ between taxa, and that the strength of
622 frequency-dependent selection might be related to local population sizes and densities, with
623 consequences for geographic range size and potentially also for extinction risk. This study
624 provides a foundation for future micro- and macroevolutionary studies on how selective and
625 neutral processes have shaped the evolution of discrete female colour morphs in *Ischnura*
626 damselflies, by providing a phylogeny for future comparative studies in this genus.

627

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651

652 **Data Availability**

653 Novel sequence data has been made available on the National Centre for Biotechnology
654 Information (https://www.ncbi.nlm.nih.gov/genbank/). All raw data, command files and
655 scripts are available at GitHub
656 (https://github.com/rachelblow/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.

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990 **Figure Legends**

991

992 **Figure 1.** Phenotypic variation in the damselfly genus *Ischnura.* **A.** Some representative
993 species, from both the Old and the New World, exemplifying the extensive phenotypic and
994 colour variation within and between species in the genus *Ischnura,* as well as age-related
995 variation (note that the orange and yellow females in **ii**, **iv**, **v** and **xi** show sexually immature
996 and age-related colour phases expressed in females only). All species in this panel are included
997 in the molecular phylogeny we present in this paper. **i.** Sexually mature male and **ii.** immature
998 female *I. ramburii* (Texas, USA). **iii.** Sexually mature male and **iv.** immature female *I.*
999 *senegalensis* (Japan). **v.** immature female *I. pumilio* (Sweden). **vi-viii:** Some species of
1000 *Ischnura* also show heritable female colour polymorphism at the adult stage. Here are the three
1001 different female colour morphs in the trimorphic species *I. elegan*s (all photos from Sweden).
1002 **vi.** Male *I. elegans* mating with an androchrome (male mimic) female morph. **vii.** Male *I.*
1003 *elegans* mating with a heterochrome morph (*infuscans-obsoleta*) . **viii.** Male *I. elegans* mating
1004 with the other heterochrome morph (*infuscans*). The sister species of *I. elegans* is *I. graellsii*
1005 (photos from Portugal): **ix.** sexually mature male and **x.** sexually mature heterochrome female
1006 morph (*infuscans*). Finally, **xi** shows an immature female *Ischnura capreolus*, revealing the
1007 yellow colour females of this species exhibit prior to sexual maturation (Brazil). All
1008 photographs taken by E.I. Svensson. **B.** The four possible female colour morph states found in
1009 the damselfly genus *Ischnura*. Here, we illustrate these four colour morph states using scans
1010 taken from *I. elegans.* Female-monomorphic (FM) species do not exhibit any discrete
1011 intrasexual female colour variation. However, in all polymorphic *Ischnura* species, emales are
1012 either phenotypically similar to males (androchrome) (FM(A)) or phenotypically distinct from
1013 males (heterochrome) (FM(H)). Female-polymorphic (FP) species have multiple female colour
1014 morphs. Polymorphic *Ischnura* species are either dimorphic (FP(D): one androchrome and one

1015 heterochrome female morph, two morphs in total) or they are trimorphic (FP(T): one
1016 androchrome and two heterochrome female morphs, three morphs in total).

1017

1018 **Figure 2.** Time calibrated phylogeny of 41 species of damselflies from the genus *Ischnura* as
1019 estimated in StarBEAST2. Time axis represents millions of years from the present and grey
1020 bars at nodes indicate 95% HPD intervals for age estimates. Values at nodes indicate posterior
1021 probability of internal nodes.

1022

1023 **Figure 3.** Posterior divergence time estimation at the root node sampling from the prior only
1024 (gold) and sampling with data (grey). Age estimation followed an exponentially distributed
1025 prior with an offset of 13.8 my to prevent improbable age estimation (see Methods).

1026

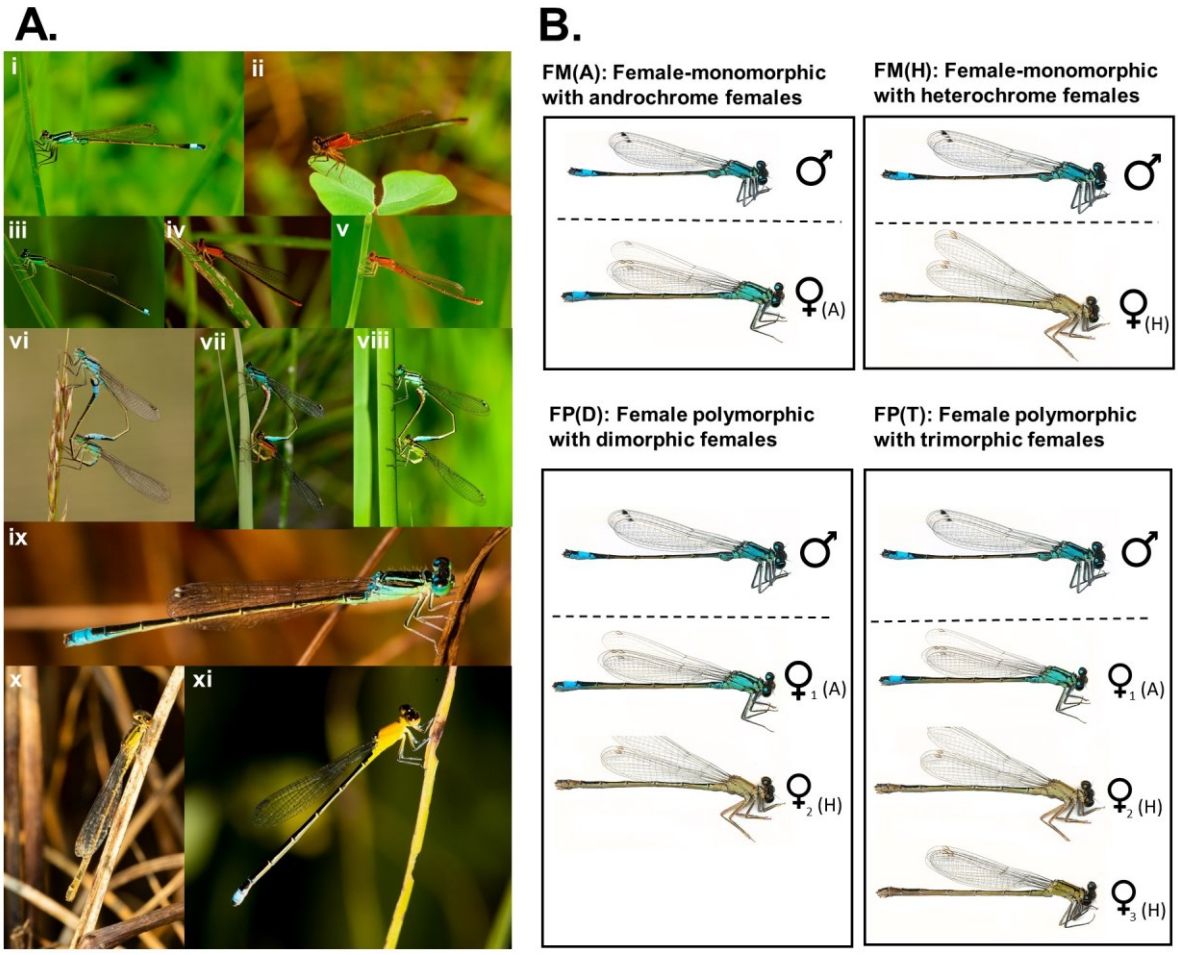
1027 **Figure 4.** Ancestral state reconstruction across the *Ischnura* phylogeny. Extant morph states
1028 are represented by circles at tips and reconstructed ancestral states are represented by piecharts
1029 at nodes (female-heterochromatic only (FM(H)), green; female-andromorphic only (FM(A)),
1030 blue; female-dimorphic (FP(D)), red; female-trimorphic (FP(T)), pink). Piecharts show the
1031 mean posterior probability for each morph state and are plotted on the MCC tree.

1032

1033 **Figure 5.** Probability of species having female polymorphism in relation to the species
1034 geographic range size, estimated in millions of squared kilometres. The left panel shows the
1035 MMC tree for the genus *Ischnura*. Bars on the centre panel are proportional to the geographic
1036 range of each species, except for species with very small ranges ($<10^5$ Km^2) which are all
1037 depicted by a similarly thin line. Light grey bars represent female-monomorphic (M) species
1038 and dark grey bars correspond to female-polymorphic (P) species. On the right panel, each

circle represents an extant species. The black line shows the prediction from a Bayesian phylogenetic mixed-effect model and the grey area shows the 95% credible interval of the prediction. Species with larger geographic ranges are more likely to have polymorphic females (see Results).

1060 **Fig. 1**



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1070 **Fig. 2**

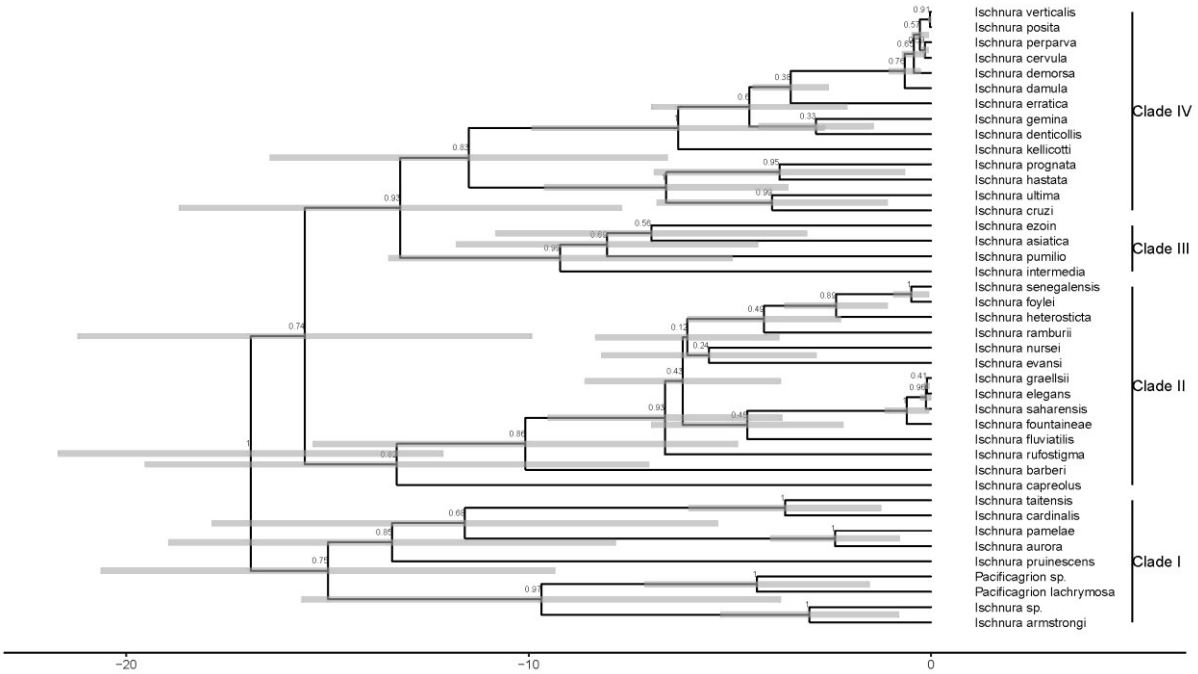

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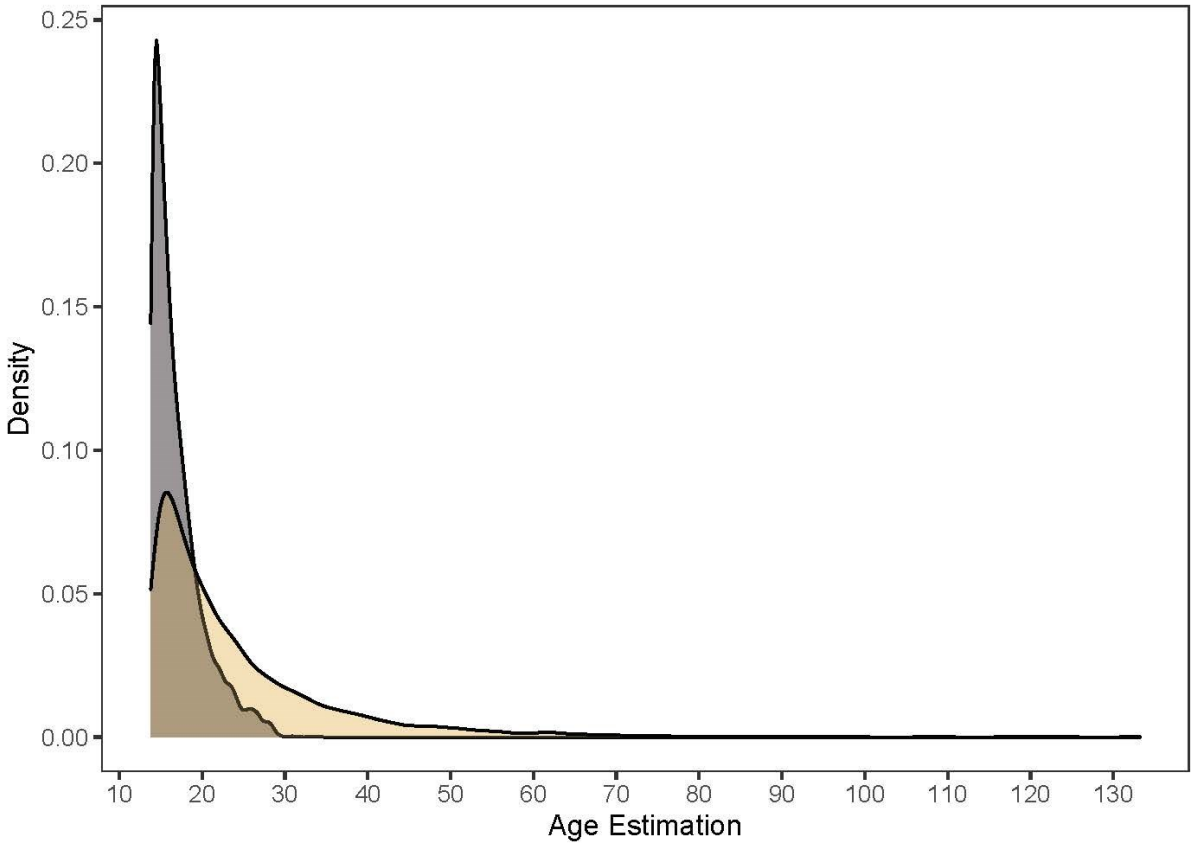
Fig. 3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1095 **Fig. 4**

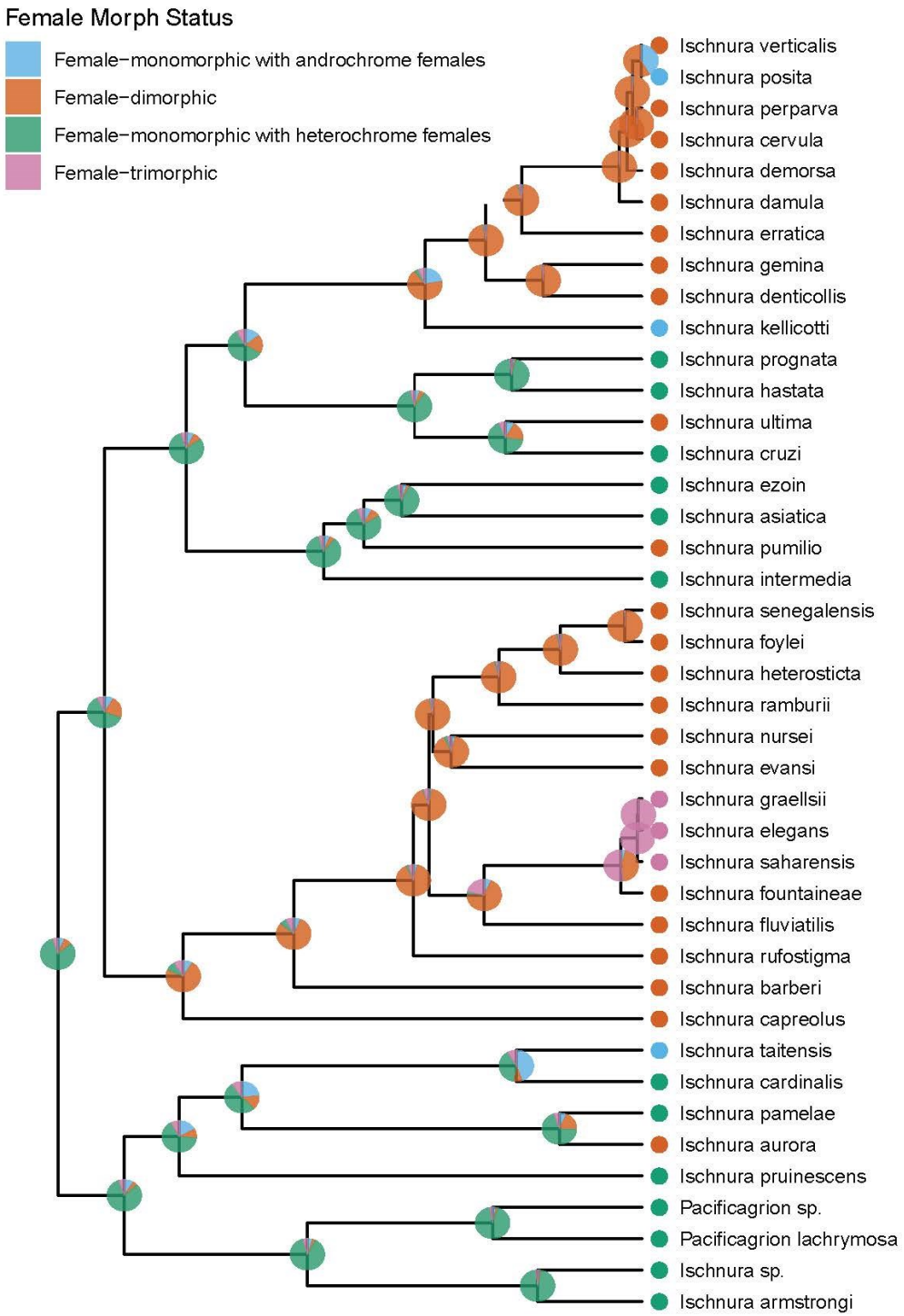

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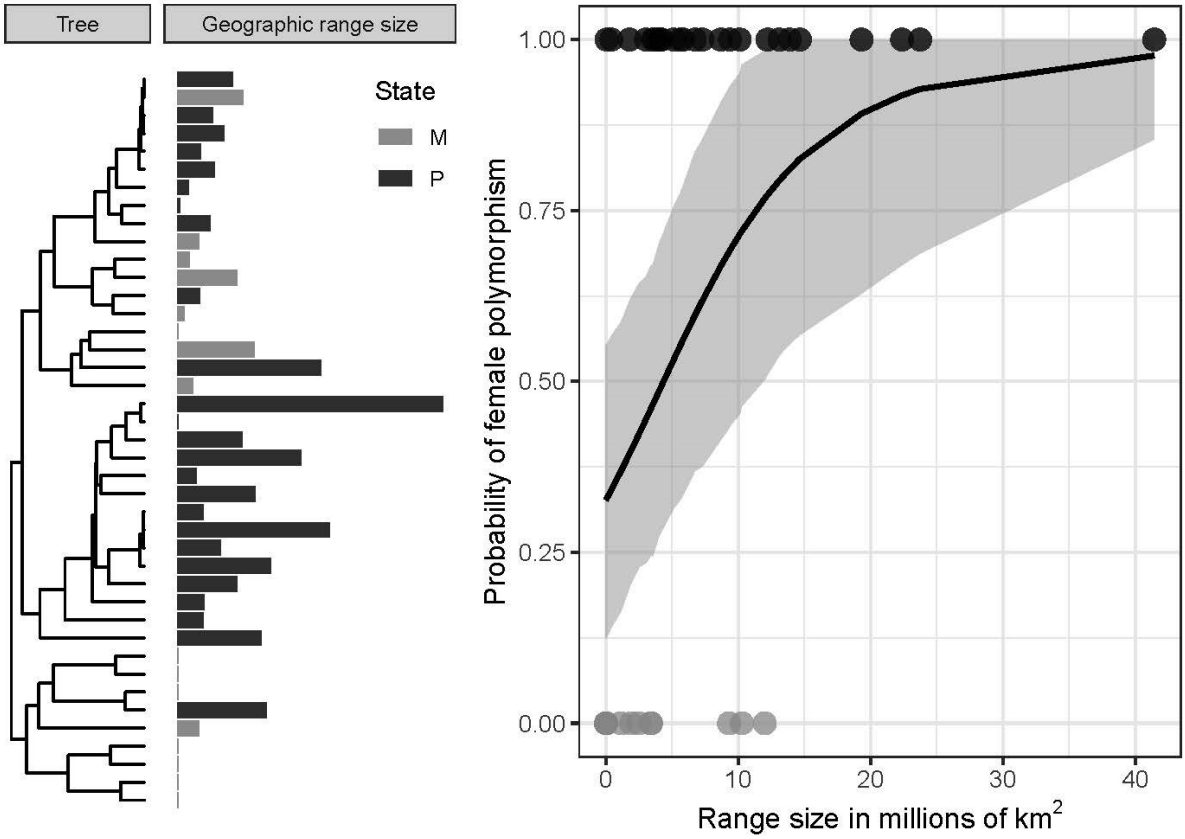
Fig. 5



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