

# The evolutionary history of colour polymorphism in *Ischnura* damselflies (Odonata: Coenagrionidae)

Rosa A. Sánchez-Guillén<sup>1,11</sup>, Sara Fadia-Ceccarelli<sup>2</sup>, Fabricio Villalobos<sup>1</sup>,  
Suman Neupane<sup>3</sup>, Anais Rivas-Torres<sup>4</sup>, Iago Sanmartín-Villar<sup>4</sup>,  
Maren Wellenreuther<sup>5,6</sup>, Seth M. Bybee<sup>7</sup>, María I. Velásquez-Vélez<sup>8</sup>,  
Emilio Realpe<sup>8</sup>, Jesús R. Chávez-Ríos<sup>9</sup>,  
Henri J. Dumont<sup>10</sup> & Adolfo Cordero-Rivera<sup>4</sup>

<sup>1</sup>Instituto de Ecología AC (INECOL), Red de Biología Evolutiva, México

<sup>2</sup>Departamento de Biología de la Conservación, CONACYT-Centro de Investigación Científica y de Educación Superior de Ensenada, México

<sup>3</sup>Department of Biological Sciences, Murray State University, Murray, KY, USA

<sup>4</sup>ECOEXO Lab, E.E. Forestal, Universidade de Vigo, Spain

<sup>5</sup>Plant and Food Research Limited, New Zealand

<sup>6</sup>The University of Auckland, School of Biological Sciences, New Zealand

<sup>7</sup>Department of Biology, Brigham Young University, USA

<sup>8</sup>Laboratorio de Zoología y Ecología Acuática (LAZOE),  
Universidad de los Andes, Colombia

<sup>9</sup>Departamento de Biología Celular y Fisiología, Unidad Periférica Tlaxcala,  
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma  
de México, Tlaxcala, México

<sup>10</sup>Department of Biology, Ghent University, Belgium

<sup>11</sup>Corresponding author: Biología Evolutiva, Instituto de Ecología A.C.  
(INECOL) C.P. 91073 Xalapa, Veracruz, México; <rosa.sanchez@inecol.mx>,  
<rguillenuvigo@hotmail.com>

*Received 22<sup>nd</sup> June 2020; revised and accepted 7<sup>th</sup> October 2020*

**Abstract.** A major challenge in evolutionary biology concerns how genetic and phenotypic variation is created and maintained. In this study, we investigated the origin(s) and evolutionary patterns of the female-limited colour polymorphism in *Ischnura*. This involves the presence of one to three colour morphs: one androchrome morph with coloration that resembles that of the male, and two gynochrome morphs (infuscans and aurantiaca) with a female-specific coloration. We documented the colour of 44 and mating system of 36 of the 76 species within *Ischnura* to investigate the ancestral state of both traits and the correlated

evolution and to infer directionality of trait-state transitions. The ancestral state reconstructions suggest that the most recent common ancestor of the ischnuran damselflies was most likely polymorphic and polyandrous. Our results give some support to the evolutionary correlation between female-limited colour polymorphism and mating system in *Ischnura*. That correlation is consistent with the idea that sexual selection through sexual conflict over the frequency of matings has selected for polymorphic females to reduce the overall intensity of male mating harassment, and our finding that the same phenotypic morphs have evolved multiple times (convergent evolution) suggests that several species in this genus might be experiencing similar selective pressures.

**Further key words.** Dragonfly, Zygoptera, ancestral state, correlated evolution, comparative method

## Introduction

Understanding how genetic variation arises and is maintained is one of the main goals of evolutionary biology. Genetic polymorphisms, such as heritable colour polymorphisms, constitute ideal systems to study the processes that maintain genetic variation over time. Female-limited colour polymorphisms are ubiquitous in nature (ROULIN 2004), from insects, *e.g.*, dragonflies and damselflies (FINCKE et al. 2005) and butterflies (KUNTE 2009), to crustaceans (FAVA 1988), reptiles (FORSMAN & SHINE 1995; VERCKEN et al. 2008) and birds (GALEOTTI et al. 2003).

In odonates, female-limited colour polymorphisms are surprisingly common, occurring in more than 100 Holarctic species (FINCKE et al. 2005), although the degree of intra-family polymorphism varies markedly (CORDERO & ANDRÉS 1996). Some coenagrionid damselfly genera like *Argia*, *Coenagrion*, *Enallagma* and *Ischnura* show a strikingly high prevalence of colour polymorphism (FINCKE et al. 2005); for example, *Ischnura* includes at least 33 polymorphic species (Table 1). Female-limited colour polymorphism in *Ischnura* consists of two or three mature colour morphs, where one (the androchrome morph) resembles the male in coloration, whereas the other morphs (gynochrome morphs: aurantiaca and/or infuscans) have a more cryptic coloration. Colour variation in *Ischnura* is pronounced, and some of this variation is related also to ontogeny and pruinescence, *i.e.*, pale bluish coloration caused by epicuticular wax crystals (HENZE et al. 2019). Genetic studies have shown that female-limited colour polymorphism in *Ischnura* is heritable, and explained by a single Mendelian locus segregating with two alleles in the dimorphic species *I. damula* (JOHNSON 1964),

*I. demorsa* (JOHNSON 1966) and *I. senegalensis* (TAKAHASHI et al. 2014) and three alleles in the trimorphic species *I. elegans* (SÁNCHEZ-GUILLÉN et al. 2005), *I. genei* (SANMARTÍN-VILLAR & CORDERO-RIVERA 2016) and *I. graellsii* (CORDERO 1990a). Several studies have proposed that female morphs use colour in avoiding male harassment (JOHNSON 1975; ROBERTSON 1985; HINNEKINT 1987; UTZERI 1988; CORDERO 1992; SHERRATT & FORBES 2001; SIROT & BROCKMANN 2001; VAN GOSSUM et al. 2001).

In *Ischnura* the mating system is highly diverse in terms of mating frequency, mating duration and oviposition type. ‘Monandrous’ species mate more than once only if their sperm loads are low (FINCKE 1987), and they appear to mate prior to the development of pruinescence (JOHNSON 1975). On the other hand, polyandrous females routinely mate more than once, with highly promiscuous females mating several times (5.83 times in average the gynochrome females of *I. elegans*; SÁNCHEZ-GUILLÉN et al. 2013a). Mating duration is highly variable, with monandrous females mating for briefer periods than polyandrous females (ROBINSON & ALLGEYER 1996). For instance, polyandrous species as *I. elegans* and *I. graellsii* can mate for several hours (more than five) while monandrous species, such as *I. hastata* or *I. kellicotti*, complete mating within 20 minutes (ROBINSON & ALLGEYER 1996). Oviposition can take place alone, both in species with long copulations, which function as a mechanism of ‘in-copula’ guarding (CORDERO 1990b), and also in monandrous species (because females do not re-mate), or with the male guarding the female in tandem (ROBINSON & ALLGEYER 1996).

ROBINSON & ALLGEYER (1996) were pioneers in addressing the correlation between mating system (polyandry/monandry) and intraspecific colour variation (polymorphism/monomorphism) in several *Ischnura* species. The association between mating rates and colour diversity implies a causative link between high mating rates, *i.e.*, strong male mating harassment due to polyandry, and diversity in coloration, *i.e.*, species with polymorphic females. Empirical evidence has linked female colour forms to several life history traits in *Ischnura* such as: (i) mating system (ROBINSON & ALLGEYER 1996); (ii) fecundity (BANHAM 1990; TAKAHASHI & WATANABE 2010; SÁNCHEZ-GUILLÉN et al. 2017); (iii) parasite resistance (SÁNCHEZ-GUILLÉN et al. 2013b); and (iv) behaviour and male harassment avoidance (VAN GOSSUM et al. 2001; SIROT et al. 2003; GALICIA-MENDOZA et al. 2017; SÁNCHEZ-GUILLÉN et al. 2017).

In ischnuran damselflies female-limited colour polymorphism indicates the presence of alternative reproductive strategies (SÁNCHEZ-GUILLÉN et al. 2017), as in polymorphic butterflies (ELLERS & BOGGS 2002), fish (CRAIG & FOOTE 2001) and reptiles (GALEOTTI et al. 2013). Andromorphs are often the minority morph (FINCKE et al. 2005; but see SÁNCHEZ-GUILLÉN et al. 2011), and commonly experience lower levels of harassment and have lower short-term fecundity (SÁNCHEZ-GUILLÉN et al. 2017). On the other hand, gynomorphs tend to have higher fecundity and can thus persist as the majority morph in the population (SÁNCHEZ-GUILLÉN et al. 2017), but have low fecundity when male harassment is high (GALICIA-MENDOZA et al. 2017). The occurrence of female alternative strategies over time and space allows a rapid response of the population to the prevalent ecological conditions (SÁNCHEZ-GUILLÉN et al. 2017). For this reason, colour polymorphisms are frequently under multiple selection pressures, which modulate the covariance between phenotypes and fitness, and can affect speciation rates, either positively or negatively (HUGALL & STUART-FOX 2012). The ‘morph speciation hypothesis’ (WEST-EBERHARD 1986) predicts that the loss of one morph can lead to character release. Thus, this hypothesis predicts that polymorphism is the ancestral state with the monomorphic species at terminal phylogenetic positions (WEST-EBERHARD 1986).

In this study, we reconstructed a phylogeny of *ca* 60% of all species in the genus *Ischnura* (44 of the 76 described species). Using this phylogeny, we investigated the ancestral state of colour polymorphism (monomorphic/polymorphic), ancestral colour (androchrome/gynochrome/ andro-gynochrome), and mating system (monandrous/polyandrous). Moreover, we estimated character correlation using phylogenetic comparative approaches to determine if mating system is associated with colour polymorphism in this genus.

## Material and methods

### Data collection on distribution, colour, and mating system descriptions

We included 44 taxa (42 species and 2 subspecies) for which molecular sequences or DNA were available from five biogeographical regions (Nearctic, Neotropical, Palaearctic, Afrotropical, Australasian/Indomalayan).

We compiled information about: i) species distributions; ii) female mating frequency (polyandrous/monandrous); iii) number of mature colour morphs; iv) type of mature colour morphs (androchrome, aurantiaca or infuscans); and v) genetic system of colour (Table 1). We obtained information from a variety of sources: publications, field guides, web pages and fellow scientists.

### **Species distribution**

To determine the biogeographical region for each species, we compiled information by using multiple databases providing open access to biodiversity data, viz. the ‘Global Biodiversity Information Facility’ (<https://www.gbif.org/>) and ‘The Dragonfiles system’ (<https://medusa.jcu.edu.au/Dragonflies/home.php>).

### **Female colour morphs**

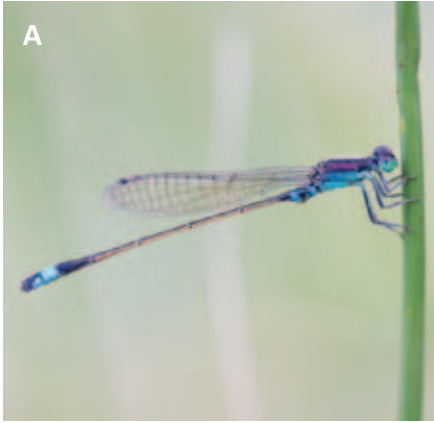
We compiled colour information for 44 species. When colour polymorphism was not previously described for a taxon, we gathered available colour information from multiple sources. We did our data search in Google Scholar using the following terms: i) *Ischnura*; ii) colour/color with the Operators (and, or). Our search was done in both Spanish and English. We also obtained information from publications, field guides, web pages and damselfly experts, who both confirmed the information from the search and provided new information, including personal unpublished observations.

Female-limited colour polymorphism in *Ischnura* is complex, with three genetically determined mature colour morphs and numerous immature colour forms due to colour ontogeny and pruinescence (*cf.* FINCKE et al. 2005). In this study, we use four terms (androchrome, gynochrome, aurantiaca and infuscans) to describe female-limited colour polymorphism. The term androchrome refers to male-like females, *i.e.*, mature females that have the same thoracic coloration and black patterning as males. Figures 1a–c show mature *Ischnura* males, Figure 1d shows a juvenile androchrome female with a mid-dorsal black line on the thorax and two humeral black lines, and Figure 1e shows a mature androchrome female with the same thorax coloration and black patterning as males, including the blue spot at the end of the abdomen (except in the *pumilio*-clade).

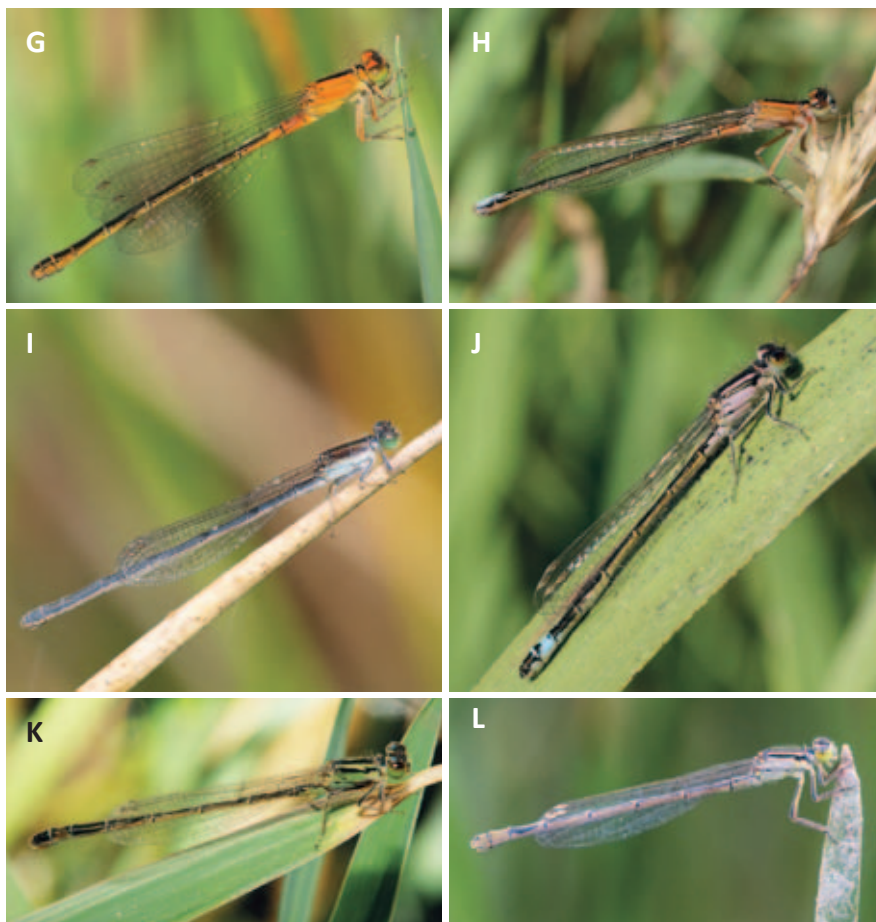
**Table 1.** List of studied *Ischnura* species, with biogeographical regions in which the species occurs, mating system (pa – polyandrous, ma – monandrous), colour (pm – polymorphism, mm – monomorphism), type of colour morph(s) present (A – androchrome, Au – aurantiaca; I – infuscans), genetic system controlling colours and references. Na – data were not available; ? – not included in the analyses. Ref. (References): 1 – CLAUSNITZER & DIJKSTRA (2005); 2 – YANYBAEVA et al. (2006); 3 – HARITONOV (1988); 4 – DUMONT (1996); 5 – SCHMIDT (1954); 6 – SUGIMURA et al. (2001); 7 – TAJIMA & WATANABE (2010); 8 – ROBINSON & ALLGEYER (1996); 9 – MARRÓN et al. (2015); 10 – FINCKE et al. (2005); 11 – VILELA et al. (2017); 12 – DICKERSON et al. (1992); 13 – REALPE (2010); 14 – JOHNSON (1964); 15 – JOHNSON (1966); 16 – SÁNCHEZ-GUILLÉN et al. (2005); 17 – BOUDOT et al. (2009); 18 – ASKEW (1989); 19 – KATBEH-BADER et al. (2004); 20 – SCHMIDT (1954); 21 – MARZOQ (2005); 22 – OZONO et al. (2012); 23 – MCKEE et al. (2005); 24 – BOTA-SIERRA et al. (2019); 25 – KOSTERIN (2015); 26 – DUMONT (1991); 27 – GARRISON & HAFERNIK (1981); 28 – SANMARTÍN-VILLAR & CORDERO-RIVERA (2016); 29 – CORDERO (1989); 30 – HUANG et al. (2012); 32 – DE KNIJF et al. (2016); 33 – JOSHI et al. (2020); 34 – PAULSON (1999); 35 – NOVELO-GUTIÉRREZ & PEÑA-OLMEDO (1989); 36 – VAN GOSSUM & MATTERN (2008); 37 – LAM (2004); 38 – DUNKLE (1990); 39 – CORDERO RIVERA & ANDRÉS ABAD (1999); 40 – ROBERTSON (1985); 41 – ROWE (2010); 42 – SANMARTÍN-VILLAR et al. (2016); 43 – TAKAHASHI & WATANABE (2009); 44 – VON ELLENRIEDER & GARRISON (2007); 45 – FINCKE (1987); 46 – MILENA et al. (2015); 47 – KUNZ (2015); 48 – MONTOYA-Q. et al. (2014); 49 – SCHRÖTER (2010); 50 – PAULSON (2009); 51 – ROBINSON & JORDAN (1996); 52 – DEVICHE (2010); \*1 – M.I. Velásquez-Vélez (unpubl.); \*2 – R.A. Sánchez-Guillén (unpubl.); \*3 – R. & D. Sparrow (pers. comm.); \*4 – I. Sanmartín-Villar (pers. comm.); \*5 – N. von Ellenrieder (pers. comm.); \*6 – C.A. Bota-Sierra (pers. comm.); \*7 – J. Cuéllar-Cardoso (pers. comm.).

Species	Regions	mating system	Colours	Type-colours	Gen. system	Ref.
<i>I. abyssinica</i> Martin, 1908	Afrotropical	Na	pm	A, Au	Na	1
<i>Ischnura</i> sp. "a"	Neotropical	pa	pm	A, Au	Na	*1
<i>I. aralensis</i> Haritonov, 1979	Palaeartic	Na	pm	A, Au	Na	2, 3, 4
<i>I. asiatica</i> (Brauer, 1865)	Palaeartic	pa	mm	Au	Na	5, 6, 7
<i>I. aurora</i> Brauer, 1865	Indomalayan-Australasian	ma	mm	I	Na	5, 6, 8
<i>I. barberi</i> Currie, 1903	Nearctic	pa	pm	A, Au	Na	9, 10, 50, 52
<i>I. capreolus</i> (Hagen, 1861)	Neotropical	pa	pm	A, Au	Na	10, 11, *7
<i>I. cervula</i> Selys, 1876	Nearctic	pa	pm	A, Au	Na	8, 10, 12
<i>I. chingaza</i> Realpe, 2010	Neotropical	ma	pm	A, Au	Na	13, *6
<i>I. cruzi</i> De Marmels, 1987	Neotropical	pa	pm	A, Au	Na	48, *7
<i>I. cyane</i> Realpe, 2010	Neotropical	pa	pm	A, Au	Na	*1, 46

Species	Regions	mating system	Colours	Type-colours	Gen. system	Ref.
<i>I. damula</i> Calvert, 1902	Nearctic	pa	pm	A, Au	Au>A	8, 14
<i>I. demorsa</i> (Hagen, 1861)	Nearctic	ma	pm	A, Au	Au>A	8, 15
<i>I. denticollis</i> (Burmeister, 1839)	Nearctic	pa	pm	A, Au	Na	8, 10
<i>I. elegans</i> (Vander Linden, 1820)	Palaeartic	pa	pm	A, Au, I	A>I>Au	8, 10, 16
<i>I. e. ebneri</i> Schmidt, 1938	Palaeartic	pa	pm	A, Au, I	Na	17, 18, *2
<i>I. erratica</i> Calvert, 1895	Nearctic	pa	pm	A, Au	Na	8, 10
<i>I. evansi</i> Morton, 1919	Palaeartic	pa	pm	A, Au, I	Na	19, 20, 21
<i>I. ezoin</i> (Asahina, 1952)	Palaeartic	Na	mm	Au	Na	22
<i>I. fluviatilis</i> Selys, 1876	Neotropical	pa	pm	A, Au	Na	11, 23, 24
<i>I. foylei</i> Kosterin, 2015	Indomalayan	Na	pm	A, Au	Na	25
<i>I. forcipata</i> Morton, 1907	Palaeartic	pa	mm	Au	Na	5, 49
<i>I. fontaineae</i> Morton, 1905	Palaeartic	pa	pm	A, Au	Na	26, *2
<i>I. gemina</i> (Kennedy, 1917)	Nearctic	pa	pm	A, Au	Na	8, 27
<i>I. genei</i> (Rambur, 1842)	Palaeartic	pa	pm	A, Au, I	A>I>Au	10, 28, *2
<i>I. graellsii</i> (Rambur, 1842)	Palaeartic	pa	pm	A, Au, I	A>I>Au	8, 10, 29
<i>I. hastata</i> (Say, 1839)	Neotropical-Nearctic	ma	mm	Au	Na	8, 10
<i>I. heterosticta</i> (Burmeister, 1839)	Australasia	pa	mm	I	Na	30
<i>I. indivisa</i> (Ris, 1918)	Neotropical	Na	pm	A, Au	Na	24 *1
<i>I. intermedia</i> Dumont, 1974	Palaeartic	pa	mm	Au	Na	32, *3
<i>I. kellicotti</i> Williamson, 1898	Nearctic	ma	mm	A	Na	8, 10
<i>I. nursei</i> (Morton, 1907)	Palaeartic	Na	pm	A, I	Na	33, 47
<i>I. perparva</i> Selys, 1876	Nearctic	ma	pm	A, Au	Na	8, 34, 49
<i>I. p. atezca</i> Novelo & Pena, 1989	Nearctic	ma	mm	I	Na	35, 37
<i>I. posita</i> (Hagen, 1861)	Nearctic	ma	mm	I	Na	8, 37
<i>I. prognata</i> (Hagen, 1861)	Nearctic	Na	mm	Au	Na	36, 37, 38
<i>I. pumilio</i> (Charpentier, 1825)	Palaeartic	pa	pm	A, Au	Na	8, 39
<i>I. ramburii</i> (Selys in Sagra, 1857)	Neotropical-Nearctic	pa	pm	A, Au	Na	8, 40
<i>I. rubilio</i> (Selys, 1876)	Indomalayan-Australasian	pa ?	mm	I	Na	41
<i>I. rufostigma</i> Selys, 1876	Palaeartic	pa	pm	A, Au	Na	42, *4
<i>I. saharensis</i> Aguesse, 1958	Palaeartic	pa	pm	A, Au, I	Na	17, 18, *2
<i>I. senegalensis</i> (Rambur, 1842)	Palaeartic-Afrotropical-Indomalayan	pa	pm	A, Au	Au>A	43, 51
<i>I. ultima</i> Ris, 1908	Neotropical	ma	pm	A, Au	Na	44, *5
<i>I. verticalis</i> (Say, 1839)	Nearctic	ma	pm	A, Au	Na	8, 45







**Figure 1.** Colour diversity in ischnuran damselflies. A – *Ischnura senegalensis* mature male (Namibia). B – *Ischnura chinagza* mature male (Colombia). C – *Ischnura aurora* mature male (Australia). D – *Ischnura elegans* juvenile androchrome (violet thorax coloration) (Spain). E – *Ischnura elegans* mature androchrome (blue thorax coloration) (Spain). F – *Ischnura capreolus* juvenile aurantiaca (citron-fluorescent thorax coloration) (Peru). G – *Ischnura pumilio* juvenile aurantiaca (orange thorax coloration) (Azores, Portugal). H – *Ischnura chingaza* juvenile aurantiaca (orange thorax coloration) (Colombia). I – *Ischnura hastata* mature aurantiaca (body pruinose coloration) (Azores, Portugal). J – *Ischnura graellsii* juvenile infuscans (light violaceous thorax coloration) (Spain). K – *Ischnura genei* mature infuscans (green-brown thorax coloration) (Sardinia, Italy). L – *Ischnura heterosticta* mature infuscans (green-grey thorax coloration) (Australia). Photos by ACR

We used the term gynochrome for all non-male-like females (aurantiaca and infuscans). The term aurantiaca morph was used to denote gynochrome females with an orange thorax coloration (but sometimes yellow or even pink) when juvenile, and in many cases with only one mid-dorsal black line on the thorax, with or without the blue spot on the abdomen (Figures 1f–h). Upon maturation, aurantiaca females become brownish, sometimes with greenish tones, and lose the blue spot on the abdomen (Figure 1i). Also, when more than 7–10 days old, some females may develop two deep brown (rarely black) humeral lines, resembling the black patterning of males. This morph is therefore orange when immature, but not when mature. Exceptionally, in some species (e.g., *I. pumilio*), the juvenile aurantiaca coloration changes to blue in androchromes or brown in gynochrome females.

The term infuscans refers to gynochrome females with a mid-dorsal black line on the thorax and two black humeral lines. They therefore have a black patterning similar to male and androchromes, and usually have a blue spot on the tip of the abdomen when juvenile (Figure 1j). Before maturation, female juvenile thoracic coloration is highly variable and can be white, violet, light blue, or green. Upon maturation, these females become greenish and finally brown and lose the blue tip (Figures 1k–l) and, in many species, are indistinguishable from the mature aurantiaca females. We treated *I. heterosticta* as a monomorphic species because, while it was defined as a dimorphic species consisting of the androchrome and infuscans morph (*cf.* TILLYARD 1905), HUANG & REINHARD (2012) found that the androchrome morph is in fact the immature ontogenetic colour stage of the infuscans morph. Thus, *I. heterosticta* is a genetically monomorphic species when mature.

### **Mating frequency (mating system)**

We compiled information about mating frequency (mating system) for 36 species. When the mating system was not previously described, we determined field mating frequency again via a data search in Google Scholar using the following terms: i) *Ischnura*; ii) mating; iii) copula; and iv) alone, with the Operators (and, or). Our search was done in Spanish and English. We also obtained information from publications, field guides, web pages and damselfly experts to confirm and expand our dataset.

In our study, we used copulation frequency as an indicator of mating system (cf. ROBINSON & ALLGEYER 1996; FINCKE 1987). These authors considered all species to be polyandrous when females were seen to mate more than once, even at a low rate, and monandrous for all species that rarely mate after maturity (only if their sperm load is low). Additionally, ROBINSON & ALLGEYER (1996) defined those species commonly seen in copula as polyandrous, and those species rarely seen in copula as monandrous. ROBINSON & ALLGEYER (1996) identified several life characters in the monandrous vs polyandrous species: i) smaller individuals; ii) greater sexual size dimorphism; iii) shorter copulation duration; and iv) no male biased operational sex ratios at aquatic sites, compared to polyandrous species. Sixteen species were previously categorized by ROBINSON & ALLGEYER (1996) as monandrous (*Ischnura aurora*, *I. demorsa*, *I. hastata*, *I. kellicotti*, *I. perparva* and *I. posita posita*) and polyandrous (*Ischnura cervula*, *I. damula*, *I. denticollis*, *I. elegans*, *I. erratica*, *I. gemina*, *I. graellsii*, *I. pumilio*, *I. ramburii* and *I. senegalensis*). One more species, *I. verticalis*, was classified as monandrous by FINCKE (1987) since mature females mated only if their sperm loads were low. Following that methodology, we comprehensively reviewed information about mating frequency (mating system), operational sex ratio at the aquatic sites, for a further 19 species (see Results).

### DNA extraction and sequencing

Previous phylogenetic studies included 15 Nearctic and Neotropical species (CHIPPINDALE et al. 1999) and 24 old-world species (mainly Palearctic species; DUMONT 2013) and a recent phylogenetic reconstruction added five new species from the Australasian region (WILLINK et al. 2019). We included 44 species in our study and used two mitochondrial genes, cytochrome oxidase II (COII) and cytochrome b (CYTB), and a part of two nuclear genes, the small sub-unit 18S nrDNA (ITS1) and 5.8S (ITS2), and the large sub-unit rDNA 28S nrDNA (18S-ITS). For species lacking published sequences (*Ischnura* sp. “a”, *I. chingaza*, *I. cruzi*, *I. cyane*, *I. foylei*, and *I. indivisa*), DNA was extracted and sequenced. Details are given in supplementary material; for GenBank accession numbers see Table S1 of Supplementary File 1. The inclusion of *Ischnura* sp. “a”, *I. chingaza*, *I. cruzi*, *I. cyane* and *I. indivisa* represents a completely new clade from the Neotropical region

that has not previously been studied, while including *I. foylei* increases the number of Australasian species.

### Phylogeny

Sequence data were aligned using MAFFT v.7 (KATO & STANDLEY 2013) and then uploaded to CIPRES v3.3 (MILLER et al. 2010) for phylogenetic reconstruction. All aligned sequences are given in Supplementary File 2. *Enallagma basidens* and *E. civile* were designated as outgroup species (BYBEE et al. 2008; DUMONT et al. 2010).

Phylogenies were estimated under Bayesian and maximum likelihood frameworks. Maximum likelihood (ML) analysis was carried out using RAxML (STAMATAKIS 2014) under GTR+GAMMA model. Nodal support was estimated with 1 000 bootstrap replicates. Bayesian phylogenetic analysis was performed using BEAST v2.4.8 (BOUCKAERT et al. 2014) assuming relaxed molecular clock model, under GTR+GAMMA model of DNA substitution. The most unconstrained form of GTR family substitution model (implemented in BEAST and RAxML) was chosen because it is preferred over other more restricted models (e.g., HKY or JC) (ABADI et al. 2019). A log-normal distributed secondary root calibration (*Ischnura-Enallagma* split = 44 Ma; TOUSSAINT et al. 2019) was enforced with mean (in real space) of 44 Ma and standard deviation of 0.25. Additionally, a second log-normal distributed secondary calibration prior was assigned for *Ischnura* crown group (mean in real space = 30, standard deviation = 0.25, with monophyly enforced) following the date estimates of *Ischnura* in TOUSSAINT et al. (2019). Multiple runs of 30 000 000 MCMC iterations were performed and checked for convergence using the program Tracer v.1.6.0 (available at <http://beast.community/>). The sampled posterior trees (Supplementary File 3) were used for further downstream analyses (Bayesian posterior probabilities of clades, ancestral state estimation and correlated character evolution).

### Phylogenetic signal, ancestral state estimation and correlated character evolution

To evaluate the presence of a phylogenetic pattern in our traits of interest (female-limited colour polymorphism and mating system), where trait states/values are more prevalent in certain clades but not in others, we estimated

the phylogenetic signal of each trait using the maximum clade credibility (MCC) tree obtained from the BEAST analysis. Since we were interested in the presence or absence of polymorphism (monomorphic/polymorphic) and mating system (polyandry/monandry), we classified species as possessing (1) or not (0) possessing a polymorphism and based on the type of mating system observed (1 = polymorphic/polyandry; 0 = monomorphic/monandry). We measured the phylogenetic signal of both traits separately by calculating the D statistic, specifically designed for binary traits (FRITZ & PURVIS 2010). Phylogenetic signal is considered when D values are around 0 or negative, *i.e.*, clumped or extremely clumped, respectively, according to Brownian motion (BM) evolution. Conversely, D values around 1 or higher (random or overdispersed pattern, respectively, according to BM evolution) imply a lack of phylogenetic signal in the binary trait. We used 1 000 permutations to calculate the probability of the observed D value under simulated BM and under a null model of no phylogenetic structure as implemented in the R package ‘caper’ (ORME *et al.* 2013).

To estimate the ancestral states of female-limited colour polymorphism and mating system types for the *Ischnura* crown, a Bayesian Stochastic Mapping (HUELSENBECK *et al.* 2003) was used as implemented in the R package ‘phytools’ (REVELL 2012). For female-limited colour polymorphism, the morph types were grouped into (i) two categories: monomorphic *vs* polymorphic; and (ii) three categories: androchrome *vs* gynochrome *vs* androgynochrome. Mating system was categorized into binary traits (monandry, polyandry). One thousand stochastic maps were simulated on the MCC tree from BEAST, and based on weighted Akaike Information Criteria (AKAIKE 1974), the character state transition models applied were the “equal rates” model for the binary state coding (colour polymorphism grouping (i) above and mating system) and the “symmetrical” model for the polymorphic data (colour polymorphism grouping (ii) above).

An evolutionary correlation test between female-limited colour polymorphism and mating system was carried out in ‘phytools’ on the MCC tree using Pagel’s binary character correlation test (PAGEL 1994) with a likelihood ratio test to determine the model fit of either independent or dependent rate transition models (PAGEL & MEADE 2006) and to test whether the evolution of one trait was dependent on the other.

## Results

### Distribution, female-limited colour polymorphism and mating frequency

The 44 *Ischnura* species studied came from five biogeographical regions. Of these, 37 came from one biogeographical region (13 species from the Nearctic, eight from the Neotropical, 15 from the Palaearctic, one from the Afrotropical, one from the Indomalayan and two from the Australasian); four from two biogeographical regions, two of them from the Neotropical/Nearctic and two from Indomalayan/Australasian; and one from three biogeographical regions, Palaearctic/Afrotropical/Indomalayan (Fig. 2 and Table S2 of Supplementary File 1). We included 44 out of the currently 76 described species; the species that appear in several biogeographic regions were included in all the regions in which they occur to calculate the percentages of the colour polymorphism by biogeographical region: the majority of the Nearctic (100%), Neotropical [71% (14)], Palaearctic [75% (20)]; half of the Afrotropical [50% (4)]; and a small proportion of the Indomalayan-Australasian [14% (29)] (Fig. 2 and Table S2 of Supplementary File 1).

Out of the 44 species for which colour morphs have already been described, 12 were monomorphic (6 aurantiaca, 5 infuscans and 1 androchrome), distributed in all except the Afrotropical region. Twenty-six were dimorphic (22 with the androchrome and the aurantiaca morphs, and one taxon with the androchrome and infuscans morph), distributed in all biogeographical regions; and six species were polymorphic, presenting the three colour morphs (androchrome, aurantiaca and infuscans) and being distributed exclusively in the Palaearctic region (Table 1 and Fig. 3).

For the mating system, we included information for 36 of the 44 species studied. Out of the 36 species, seventeen species were previously categorized by ROBINSON & ALLGEYER (1996) and FINCKE (1987; Table 1), and 19 species were for the first time categorized in this study, based on published data (7 species); personal communication from expert colleagues on the topic (3 species); or unpublished data from the authors (9 species; details in Table 2). Out of the 36 species, twenty-six were polyandrous, and the remaining ten species were monandrous (Tables 1 and 2).

### Phylogenetic reconstruction

The phylogenetic tree estimated using Bayesian methods (Fig. 3) showed no major topological incongruence for the clades discussed below. The clades supported in the ML analysis (Fig. S1 of Supplementary File 1) (BS  $\geq 60\%$ ) were also supported in the Bayesian analysis (Bayesian posterior probability, BPP  $\geq 0.95$ ), consistent with the two earlier phylogenies published for this genus. CHIPPINDALE et al. (1999) focused on the North American species and included 15 Nearctic and Neotropical species. More recently, DUMONT (2013) focused on the old-world species, including 24 mainly Palaearctic species. Our phylogeny included 10 additional species: six Neotropical (*Ischnura* sp. "a", *I. chingaza*, *I. cruzi*, *I. cyane*, *I. indivisa* and *I. ultima*), one Australasian (*I. foylei*), and three Palaearctic (*I. e. ebneri*, *I. genei* and *I. intermedia*). All our newly added ten species were resolved within the *Ischnura* phylogeny, which is a sister group to *Enallagma*. Our phylogenetic analysis was able to group 41 out of the 44 species into nine clades. Six geographically structured clades (clades 1–6) and remaining three clades (clades 7–9) that included species from different distributions (Fig. 3).

The Neotropical and Nearctic clade 1, named by CHIPPINDALE et al. (1999) as the *hastata*-clade, was strongly supported (BPP = 1; Fig. 3) and included two monomorphic (aurantiaca morph) species and three dimorphic species (androchrome and aurantiaca morphs; Table 1): one Nearctic-Neotropical (*I. hastata*), one Nearctic (*I. prognata*) and three Neotropical species (*I. chingaza*, *I. cruzi* and *I. ultima*). This clade includes at least three monandrous (*I. hastata*, *I. ultima* and *I. chingaza*) and one polyandrous (*I. cruzi*) species (Table 1).

The Nearctic clade 2 (*denticollis*-clade) was strongly supported (BPP = 1; Fig. 3) and comprised 11 species with Nearctic distribution. Our phylogenetic reconstruction reconfirms the close relationship between *I. gemina* and *I. cervula*, and among *I. damula*, *I. verticalis*, *I. perparva*, *I. p. posita*, *I. denticollis*, *I. demorsa* and *I. p. atezca*, consistent with CHIPPINDALE's et al. (1999) phylogenetic reconstruction. We were also able to corroborate the finding that *I. p. atezca* is more closely related to *I. demorsa* than to *I. p. posita* (CHIPPINDALE et al. 1999) supporting the species status of *I. p. atezca*. The *denticollis*-clade includes three monomorphic species; one of them with the androchrome morph (*I. kellicotti*), and two with the infuscans morph (*I. p.*

*posita* and *I. p. atezca*); and eight dimorphic species, with the androchrome and the aurantiaca gynochrome morph (Table 1). In this clade, the three monomorphic species are monandrous, while only three out of the eight polymorphic species are monandrous (*I. demorsa*, *I. perparva* and *I. verticalis*; Table 1).

The Palaearctic clade 3 (*pumilio*-clade) was strongly supported (BPP = 0.99; Fig. 3) and included five Palaearctic species: *I. asiatica*, *I. pumilio*, *I. ezoin*, *I. forcipata* and *I. intermedia*. In agreement with a recently published phylogeny (DE KNIJF et al. 2016) the *pumilio*-clade is a well-supported clade. *Ischnura pumilio* is a dimorphic species that consists of the androchrome and aurantiaca morphs. This species, together with *I. kellicotti* have a unique androchrome female morph that shows an indistinguishable immature coloration from the immature aurantiaca morph coloration (Table 1). The remaining four species (*I. asiatica*, *I. ezoin*, *I. forcipata* and *I. intermedia*) are monomorphic for the aurantiaca morph. The four species for which mating system is known are polyandrous (Table 1).

The Indomalayan-Australasian clade 4 (*aurora*-clade) was strongly supported (BPP = 1; Fig. 3) and included two Indomalayan-Australasian species (*I. aurora* and *I. rubilio*), both of them with a similar and unique abdominal coloration. In these species, the first five male abdominal segments are orange, followed by the sixth black and the seventh and eighth blue segments (PAPAZIAN et al. 2007; SCHNEIDER et al. 2015). There are slight colour differences between the two. Females have a typical black abdomen and are monomorphic for the infuscans (gynochrome) morph (Table 1). The mating system in *I. aurora* is unusual: In Australia (A.G. Orr pers. comm.) and New Zealand (ROWE 1978), females mate only once (monandrous) immediately after emergence. In India, females formerly identified as *I. aurora*, only mate when fully mature and are polyandrous (ANDREW 2001). However, these populations were regarded as *I. rubilio* and not as *I. aurora* by PAPAZIAN et al. (2007). Given these taxonomic uncertainties, we have refrained from including *I. rubilio* in our analysis of mating systems, waiting for a critical evaluation of the *aurora*-clade.

The Neotropical clade 5 (*capreolus*-clade) was strongly supported (BPP = 1; Fig. 3) and comprised four recently diverged Neotropical species. *Ischnura cyane* is the most basal species, from which *I. capreolus*, *I. indivisa* and one

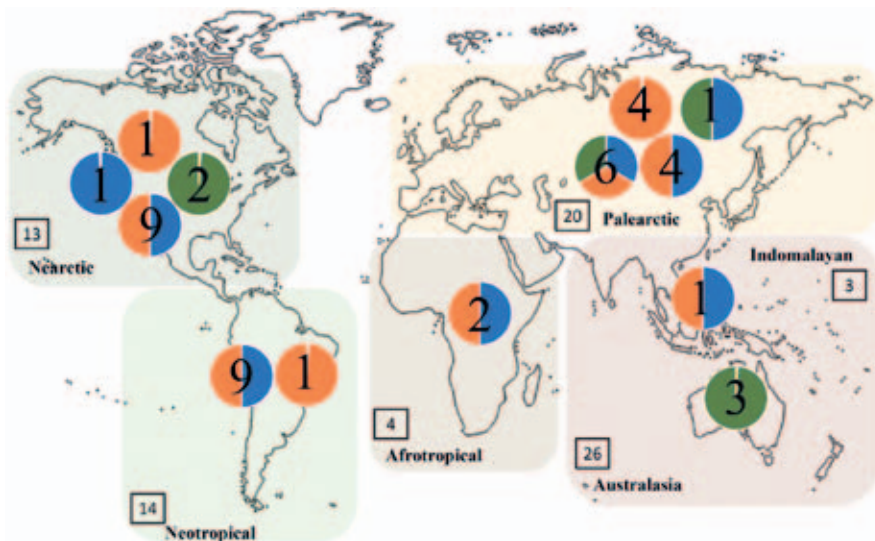


not yet described species (*Ischnura* sp. “a”) have recently evolved and they together form the youngest clade in the genus. All four species are dimorphic (Table 1). Coloration is different from other *Ischnura* clades, in that all immature females have bright coloration, with intense orange in the aurantiaca morph, and intense green in the androchrome morph (REALPE 2010). All species for which reproductive behaviour is described are polyandrous; only the mating system of *I. indivisa* is unknown) (Table 1).

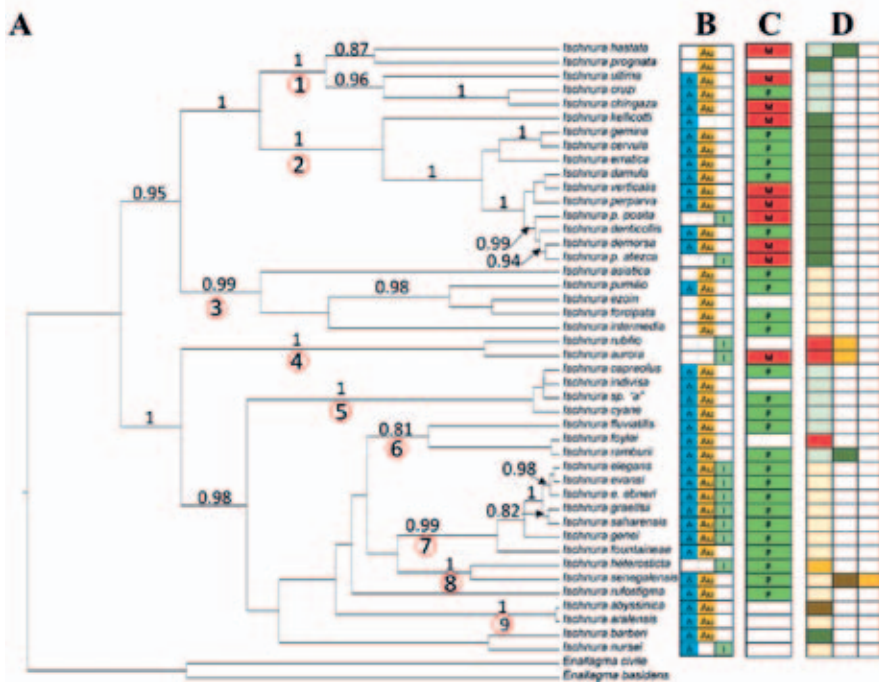
Clade 6 (weakly supported; BPP = 0.81; Fig. 3) forms the *ramburii*-clade and included two Nearctic-Neotropical (*I. ramburii*, *I. fluviatilis*) and one Australasian (*I. foylei*) species. In a previous phylogeny by WILLINK et al. (2019), *I. foylei* was resolved in clade 8; that inconsistency could be due to the weak support of clade 6 in our reconstruction. All species have dimorphic females consisting of the androchrome and gynochrome aurantiaca morphs (Table 1) and the two species for which we have information about the reproductive system, *I. ramburii* and *I. fluviatilis*, are polyandrous.

The Palaearctic clade 7 (*elegans*-clade) was strongly supported (BPP = 0.99; Fig. 3). It included seven Western Palaearctic species (*I. elegans*, *I. e. ebneri*, *I. evansi*, *I. fontaineae*, *I. genei*, *I. graellsii* and *I. saharensis*) and is a recent radiation from the Mediterranean basin. In a previous phylogeny by DUMONT (2013), *I. evansi* was resolved in the *I. elegans* clade but not in the core group, however that relationship had very low support (50 %). Those species are closely related and almost completely reproductively isolated (SÁNCHEZ-GUILLÉN et al. 2013c), but where ranges overlap, hybridization takes place between *I. elegans* and *I. graellsii* in Spain, between *I. elegans* and *I. genei* in Sardinia, and between *I. graellsii* and *I. saharensis* in Morocco (SÁNCHEZ-GUILLÉN et al. 2014a, b). All species except for *I. fontaineae*, which has two colour morphs (androchrome and aurantiaca), have the three colour morphs and are highly polyandrous (mean = 5.83 matings in gynochromes of *I. elegans*; SÁNCHEZ-GUILLÉN et al. 2013b), so that this clade contains all trimorphic species in the genus *Ischnura* (Table 1).

Clade 8 (strongly supported, BPP = 1; Fig. 3), the *senegalensis*-clade, comprises only two species: one widely distributed (Palaearctic, Afrotropical and Indomalayan; *I. senegalensis*); and one Australasian (*I. heterosticta*). Both species are polyandrous but *I. heterosticta* is monomorphic (infuscans morph) while *I. senegalensis* is dimorphic (androchrome and aurantiaca).



**Figure 2.** Distribution by geographic regions of the female colour diversity of the 44 *Ischnura* species sampled. In each geographic region, colour combinations for each species are shown. Blue colour in circles represents the androchrome morph, orange colour the aurantiaca morph, and green colour the infuscans morph. Nearctic region (13 species): one monomorphic species for the androchrome morph, one monomorphic species for the aurantiaca morph, two monomorphic species for the infuscans morph, and nine dimorphic species with the aurantiaca and the androchrome morphs. Neotropical and Neotropical-Nearctic region (10 species): one monomorphic species for the aurantiaca morph and nine dimorphic species with the aurantiaca and the androchrome morphs. Afrotropical and Afrotropical-Palaeartic-Indomalayan region (2 species): two dimorphic species with the aurantiaca and the androchrome morphs. Palaeartic region (15 species): four monomorphic species for the aurantiaca morph, four dimorphic species with the androchrome and the aurantiaca morphs, one dimorphic species with the androchrome and infuscans morphs, and six trimorphic species. Indomalayan region (1 species): one dimorphic species with the infuscans and the androchrome morphs. Australasian-Indomalayan region (3 species): all with monomorphic species for the infuscans morph. Numbers in squares indicate the number of described species for each biogeographical region. Species present in several regions are included in each region.



**Figure 3.** A – Maximum-clade credibility tree of COII, CYTB, and ITS sequences for the 44 *Ischnura* species, and two *Enallagma* species (*E. basidens* and *E. civile*) as outgroups. Values above branches represent the Bayesian posterior probability (only those which have a posterior probability >80 %). Red circles below branches indicate some highlighted *Ischnura* clades. B – Type and number of female colour morphs: Blue rectangle – androchrome; orange rectangle – aurantiaca; green rectangle – infuscans. Females with a rectangle with single colour are monomorphic, females with a rectangle with two colours are dimorphic, and females with a rectangle with three colours are polymorphic. C – Mating system: Green – polyandrous; red – monandrous. D – Geographical affinities of species indicated by colour. Dark green – Nearctic region; pale green – Neotropical region; cream – Palaearctic region; brown – Afrotropical region; red – Indomalayan region; orange – Australasian region.

**Table 2.** List of *Ischnura* species for which reproductive behaviour has been inferred based on mating frequencies and sex ratio. We have done our data search in Google Scholar using the following terms: i) *Ischnura*; ii) mating and iv) copula, with the Operators (and, or). \* – Mature females seen in tandem or mating, but pairs rarely seen considering the abundance of the females. Ref. (References): 1 – ROBINSON & ALLGEYER (1996); 2 – ROWE (1978); 3 – PAULSON (2009); 4 – VILELA et al. (2017); 5 – DICKERSON et al. (1992); 6 – GALINDO (2018); 7 – JOHNSON (1964); 8 – JOHNSON (1975); 9 – CÓRDOBA-AGUILAR (1992); 10 – PARR & PALMER (1971); 11 – CANNINGS & DOERKSEN (1979); 12 – PAULSON & CANNINGS (1980); 13 – KATBEH-BADER et al. (2004); 14 – MCKEE et al. (2005); 15 – SCHRÖTER (2010); 16 – HAFERNIK & GARRISON (1986); 17 – CORDERO (1989); 18 – HUANG et al. (2012); 19 – DE KNIJF et al. (2016); 20 – BICK & BICK (1957); 21 – ROBINSON (1983); 22 – CORDERO RIVERA & ANDRÉS ABAD (1999); 23 – ROBERTSON (1985); 24 – FURTADO (1972); 25 – FINCKE (1987). \*1 – J. Cuéllar Cardozo (pers. comm.); \*2 – F. Palacino-Rodríguez (pers. comm.); \*3 – N. Galindo (pers. comm.); \*4 – N. von Ellenrieder (pers. comm.).

Species	♀ Alone	Tandems	Matings	Mating-Freq.	Sex ratio	Locality/Country	Ref.
<i>I. asiatica</i>	191		78	Common	(260/191) Equal	China	This study
<i>I. aurora</i>	Many	1	0	Rare	–	New Zealand	1, 2
	7	0	0	Rare	(9/7) Equal	China	This study
<i>Ischnura</i> sp. "a"	>100	–	30	Common	–	Colombia	This study
<i>I. barberi</i>	–		Many	Common		West, USA	3
<i>I. capreolus</i>	Minority		Majority (30)	Common	–	Colombia	*1
<i>I. capreolus</i>	316		33	Common	–	Brazil	4
<i>I. cervula</i>	96		34	Common	(592/96) Male	California, U.S.A.	1, 5
<i>I. chingaza</i>	1118		10	Rare	Female	Colombia	*2
<i>I. cruzi</i>	Minority		Majority (46)	Common	–	Colombia	*3
<i>I. cyane</i>	>100		18	Common	–	Colombia	6
<i>I. damula</i>	>500	220	–	Common	–	New Mexico, USA	1, 7, 8
<i>I. demorsa</i>	>500	140	–	Rare*	–	New Mexico, USA	1, 7, 8
<i>I. denticollis</i>	>100	–	36	Common	Male	Veracruz, Mexico	1, 9
<i>I. elegans</i>	2073		158	Common	Male	Wales, UK	1, 10

Species	♀ Alone	Tandems	Matings	Mating-Freq.	Sex ratio	Locality/Country	Ref.
<i>I. erratica</i>	Common						1, 11, 12
<i>I. evansi</i>		Common		Common			13
<i>I. fluviatilis</i>	107		921	Common	(2078/921) Male	Uruguay	14
<i>I. forcipata</i>			Common	Common	–	Kyrgyzstan	15
<i>I. fountaineae</i>	>20	–	>5	Common		Morocco	This study
<i>I. gemina</i>	51	–	228	Common	Male	San Francisco, USA	1, 16
<i>I. genei</i>	555	–	294	Common	(879/555) Male	Sicily, Sardinia	This study
<i>I. graellsii</i>	1727	–	732	Common	Male	Galicia, Spain	1, 17
<i>I. hastata</i>	>500	0	0	Rare	Female	Arlington, USA	1
<i>I. heterosticta</i>	382		41	Common	387/382 Equal	Australia	18
<i>I. intermedia</i>	78	7	24	Common	(1478/78) Males	Cyprus	19
<i>I. kellicotti</i>	>500	0	0	Rare	Female	Arlington, USA	1
<i>I. perparva</i>	–			Rare *		West, USA	1, 3
<i>I. posita</i>	248	0	0	Rare	(184/248) Female	Louisiana, USA	1, 20
	1080	0	0	Rare	(1135/1080) Equal	Arlington, USA	21
<i>I. pumilio</i>	100	–	143	Common	(±400/142) Male	Galicia, Spain	1, 22
<i>I. ramburii</i>	90	–	164	Common	Male	Florida, USA	1, 23
<i>I. rufostigma</i>	192	–	19	Common	(272/192) Male	China	This study
<i>I. saharensis</i>	217	–	131	Common	(217/251) Equal	Morocco	This study
<i>I. senegalensis</i>	300	–	12	Common	–	Malaysia	1, 24
	65	–	5	Common	145/111 (Equal)	China	This study
<i>I. ultima</i>	Common	2	0	Rare	–	Argentina	*4
<i>I. verticalis</i>				Rare	Female	Michigan, USA	25

Clade 9 (strongly supported, BPP = 1; Fig. 3), also included two species, *I. aralensis* with Palearctic distribution and *I. abyssinica* with Afrotropical distribution. Both species are dimorphic for the androchrome and the auro-antia (gynochrome) coloration. No information is available about mating system in both species.

### Phylogenetic signal, ancestral state reconstruction and correlated character evolution

Both female-limited colour polymorphism and mating system of *Ischnura* species showed significant phylogenetic signal. D values for both traits were around 0 (-0.03 and 0.06, respectively) and significantly different from those expected under a random distribution of traits in the phylogeny ( $p = 0.003$ , for both traits).

The results of the ancestral state estimations based on a combination of 1 000 Bayesian stochastic maps for female-limited colour polymorphism and mating system are shown in Figures 4 and 5. Ancestral state estimation of colour morphs was polymorphic with a probability of 0.63 and on average there were 14.33 changes between the two states, divided into 5.57 changes from mono- to polymorphic and 8.76 *vice versa*. Over the evolutionary history of the genus, the mean proportional time spent in the polymorphic state was 0.63 and 0.37 in the monomorphic state. For the mating system, the ancestral state was estimated as polyandrous with a probability of 0.71 and on average, there were 13.11 changes between the two states, divided into 7.46 changes from monandrous to polyandrous and 5.65 *vice versa*. The mean proportional time spent in the polyandrous state was 0.69 and 0.31 in the monandrous state.

Based on the evolutionary correlation tests between female-limited colour morph and mating system as was previously proposed by ROBINSON & ALLGEYER (1996), the likelihood ratio of the independent *vs* dependent model fit was 5.76 ( $p = 0.056$ ) in favour of the dependent model (dependent model log-marginal likelihood: -38.86 and independent model log-marginal likelihood: -41.74) (Fig. S2 of Supplementary File 1). For the models testing the dependence of one trait on the other, female colour morph was found to be dependent on the mating system (LR: 4.26,  $p = 0.039$ ; Fig. S3 of Supplementary File 1), but not *vice versa* (LR: 1.71,  $p = 0.19$ ; Fig. S2 of Supplementary File 1).

## Discussion

Heritable colour polymorphisms, frequently associated with other life history traits, are ideal systems to study the processes that maintain genetic variation over time (ROULIN 2004; GRAY & MCKINNON 2007; WELLENREUTHER et al. 2014). Ancestral state reconstructions of *Ischnura* suggest that their most recent common ancestor was most likely polymorphic and polyandrous, and there were probably several independent evolutionary transitions to colour monomorphism and monogamy. Our results also confirmed the evolutionary correlation between female-limited colour polymorphism and mating system in *Ischnura*, as was previously proposed by ROBINSON & ALLGEYER (1996), and further showed that colour depends on mating system but not *vice versa*.

### Phylogenetic reconstruction

Our phylogenetic trees based on both Bayesian and ML methods showed no major topological incongruences with previous published reconstructions focused on the North American species (CHIPPINDALE et al. 1999), the Palaearctic species (DUMONT 2013), and the phylogeny of WILLINK et al. (2019), which added five new species from the Australasian region. Our phylogenetic trees have added, to previous phylogenetic reconstructions, six Neotropical, three Palaearctic and one Australasian species. However, more effort to include species from the Indomalayan-Australasian region (currently only 14% of the species) is needed to provide a better balance with the remaining biogeographical regions.

### Phylogenetic signal and ancestral state of colour and mating system traits

Closely related species tend to exhibit similarities in their traits (phylogenetic signal), including life-history among others such as morphological, behavioural and ecological characteristics (HARVEY & PAGEL 1991). However, it is recommended that such phylogenetic signal should not be assumed *a priori* but instead explicitly tested using trait values and a phylogeny (BLOMBERG et al. 2003; REVELL et al. 2008). In our study we found that both binary traits, female-limited colour polymorphism and mating system, showed a significant phylogenetic signal with values of the D statistic indicating a clumped structure (instead of random) following Brown-

ian motion evolution. As such, closely related species do tend to exhibit a similar female-limited colour polymorphism and mating system. In fact, six clades included only polymorphic species and one clade only monomorphic species, while only two clades clustered both polymorphic and monomorphic species. Similarly, monandry was more prevalent in the Nearctic and Neotropical species (with 3/5 species in the *hastata*-clade and 6/11 species in the *denticollis*-clade) than in the Palearctic, Afrotropical, Australasian and Indomalayan species with one monandrous species in the six clades (28 species).

A robust inference of an ancestral trait state not only requires an appropriate choice of optimality criterion but also a complete species-level sampling (LITSIOS & SALAMIN 2012). Although we have included a high number of species for female colour polymorphism (44 species, 59 % of the genus) and for mating system (36 species, 48 % of the genus), including species from the five biogeographical regions where the genus occurs, our phylogenetic reconstruction remains incomplete. However, it still covers more species than previous studies seeking to infer the ancestral state of female colour polymorphism and mating system (FINCKE et al. 2005; VAN GOSSUM & MATTERN 2008). Our ancestral state reconstruction revealed that the most recent common ancestor of *Ischnura* was most likely polymorphic (polymorphic: 63 %, monomorphic: 36 %). ROBINSON & ALLGEYER (1996) assumed that polymorphism is the ancestral condition arguing that in *Ischnura*, as in many other damselflies, the ancestral mating system included mate guarding by postcopulatory tandem where the male remains in tandem during oviposition (Group 1), then evolved to male mate guarding by extending time in copula, without postcopulatory tandem (Group 2), and then to monandrous species which mate for short periods and whose monomorphic females also oviposit unguarded (Group 3). This assumption, partly supported by our findings, is consistent with the 'morph speciation hypothesis' (WEST-EBERHARD 1986) that predicts that polymorphism is the ancestral state with the monomorphic species at terminal phylogenetic positions. WEST-EBERHARD (1986) proposed that alternative mating strategies (alternative adaptations) are likely to be involved in speciation and that they can even speed up the process itself. Her postulated morph speciation hypothesis predicts that the loss of one morph can lead to a character release.

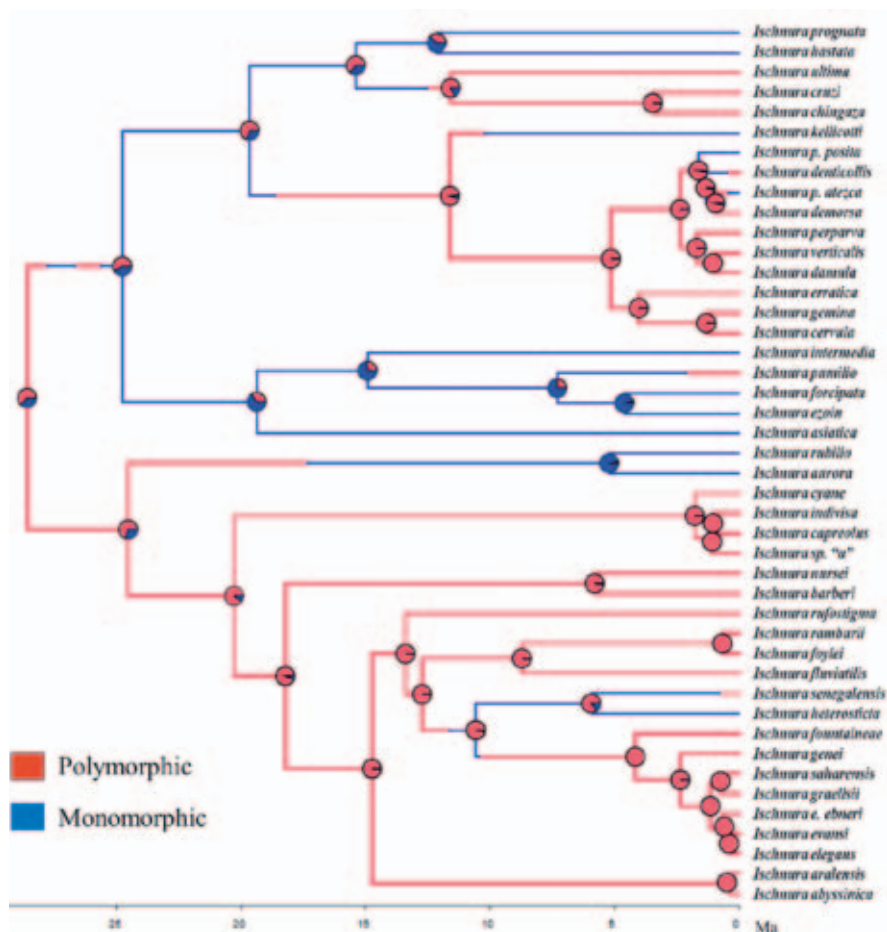


This is because the loss of a mating morph strategy breaks an evolutionary constraint (on the genome of a polymorphic species) imposed by the ancestral state comprising multiple phenotypes. Once the constraint is broken, WEST-EBERHARD (1986) proposed that new alleles can spread in the population and lead to a phenotypic specialization of the remaining morphs. Finally, this can exert a direct effect on traits for mate recognition via sexual selection and can thus ultimately lead to a generation of genetic divergence and reproductive isolation (WEST-EBERHARD 1983; RIESEBERG et al. 1986).

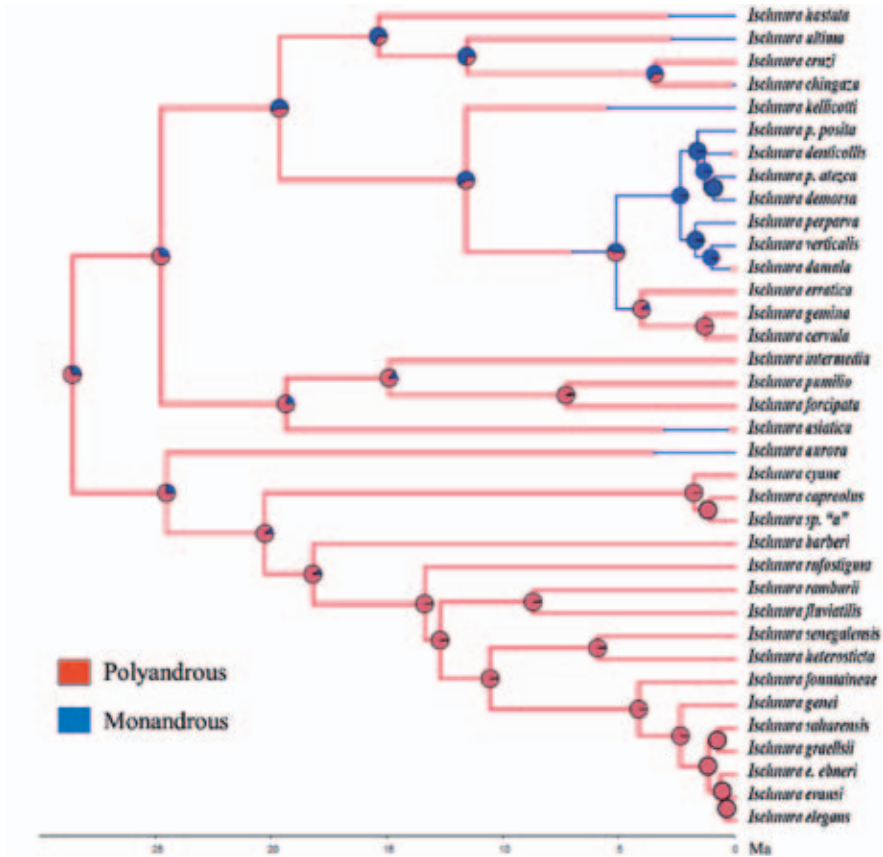
Results from sampling 1 000-character histories detected 14.33 changes between the two states and suggest that colour polymorphism most likely evolved 5.57 times and was subsequently lost 8.76 times throughout the evolutionary history of *Ischnura*. Similarly, the most recent common ancestor of *Ischnura* was most likely polyandrous (71 %) rather than monandrous (29 %), with 13.11 changes between the two states suggesting that polyandry most likely evolved 7.46 times and was subsequently lost 5.65 times throughout their evolutionary history. In *Ischnura*, female-limited polymorphism is controlled by a single autosomal Mendelian locus segregating with two or three alleles, with sex-limited expression probably due to differential gene expression: In *I. elegans* melanin, pteridine and ommochrome colour pathways have already been identified, and two of them, the ommochrome and melanin pathways, showed evidence for differential expression between the sexes (CHAUHAN et al. 2016). Accordingly, few changes are required to express or suppress a trait in both sexes and thus many losses and gains are expected, as our data have demonstrated.

### **Evolutionary history of the colour polymorphism**

A large body of work has related colour polymorphism to several other traits, ranging from life history traits to behaviour (MCKINNON & PIEROTTI 2010). Detailed eco-evolutionary work has revealed several ways by which the occurrence of two or more alternative colour morphs can influence the ecological success and evolutionary dynamics of a species (FORSMAN et al. 2008). For example, individuals from different morphs have been shown to occupy different subsets of available resource dimensions, such that when summed across alternative morphs the species as a whole occupies a wider niche (VAN VALEN 1965; GALEOTTI & RUBOLINI 2004; SVANBÄCK et



**Figure 4.** Maximum-clade credibility tree of *Ischnura* species with a random Bayesian stochastic map of female-limited colour morphs along the branches and ancestral character states shown at the nodes. Pies at the nodes represent the relative probabilities of each character state (polymorphic vs monomorphic) obtained by averaging over 1 000 Bayesian stochastic maps.



**Figure 5.** Maximum-clade credibility tree of *Ischnura* species with a random Bayesian stochastic map of mating strategy along the branches and ancestral character states shown at the nodes. Pies at the nodes represent the relative probabilities of each character state (polyandrous vs monandrous) obtained by averaging over 1 000 Bayesian stochastic maps.

al. 2008; KARPESTAM & FORSMAN 2011). The wide utilisation of resources is thought to be due to colour associated effects on performance and fitness, because individuals that differ in coloration utilize different microhabitats that best match their needs, such as temperature, humidity and background matching (FORSMAN et al. 2008). At a species level, the degree of experienced niche variation of monomorphic *versus* polymorphic species is therefore thought to differ, with polymorphic species occupying a wider fundamental niche compared to monomorphic species (FORSMAN et al. 2008; FORSMAN & WENNERSTEN 2016). Accordingly, the presence of polymorphism has been suggested to increase speciation rates (GRAY & MCKINNON 2007; GAVRILETS et al. 2000). Together, previous studies suggest that colour polymorphism in *Ischnura* may confer some advantages in terms of long-term survival and resilience. This agrees with our finding that the polymorphic state is more common than the monomorphic state in *Ischnura*. Other damselfly genera with colour polymorphism, like *Argia*, *Ceriagrion*, *Coenagrion*, *Enallagma* or *Mesamphiagrion*, are geographically more restricted than *Ischnura*, to one or two biogeographical regions, and apparently show less variation in reproductive strategies. In fact, we are unaware of species described as ‘monandrous’ in these genera. We hypothesize that the high prevalence of polymorphism in *Ischnura*, and its association with male harassment, has been an engine for behavioural diversification in this genus.

Our correlation test suggests (marginally significant) the view that coloration diversity is associated with the type of mating system (colour depends on mating system but not *vice versa*). A significant association between female-limited colour polymorphism and mating system supports that the phenotypic evolution of additional colour morphs, and thus the diversification at the morph level of the clade could be linked to the intense sexual selection and conflict in this group of damselflies. Indeed, female-limited colour polymorphism has frequently been proposed to be an evolutionary response to sexual conflict over the mode and frequency of matings, and male mating harassment may hence have selected for polymorphic females to reduce harassment levels (CORDERO-RIVERA & EGIDO PÉREZ 1998; FINCKE et al. 2005; VAN GOSSUM & SHERRATT 2008; SÁNCHEZ-GUILLÉN et al. 2017). Morph-specific female behaviour and fecundity combined with context-de-

pendent male behaviour can maintain long-term polymorphic states, even in populations that differ in morph frequencies (SÁNCHEZ-GUILLÉN et al. 2017; GALICIA-MENDOZA et al. 2017). In *I. elegans*, regional variation in stochastic (genetic drift) and deterministic factors (divergent/balancing selection) is responsible for the highly differentiated female morph frequencies, from 3.3 to 74.6% for the androchrome morph (SÁNCHEZ-GUILLÉN et al. 2011), whereas the stable and similar frequencies over the whole distribution of *I. graellsii* (ANDRÉS et al. 2000; SÁNCHEZ-GUILLÉN et al. 2005), *I. damula*, *I. demorsa*, *I. ramburii* and *I. denticollis* (SÁNCHEZ-GUILLÉN et al. 2011) indicate that some sort of uniform selection is acting over a wide distributional range.

### Conclusions

Our results support the evolutionary correlation between female-limited colour and mating system in *Ischnura*, as proposed by ROBINSON & ALLGEYER (1996), and investigated by FINCKE et al. (2005) and VAN GOSSUM & MATTERN (2008) in *Enallagma* and *Ischnura*. Ancestral state reconstructions suggest that the most recent common ancestor of the ischnuran damselflies was most likely polymorphic and polyandrous. The correlation between female-limited polymorphism and mating system is consistent with idea that sexual selection through sexual conflict over the frequency of matings has selected for polymorphic females to reduce the overall extent of male mating harassment.

### Acknowledgements

This work was possible thanks to many people who provided samples: Jean-Pierre Boudot, Alex Córdoba, Andrea Corso, Geert de Knijf, Rassim Khelifa, Oleg Kosterin, Patricia Martín, Jürgen Ott and Phill Watts. We are grateful to Janet Nolasco Soto for technical support. This research was funded by the Spanish Ministry of Science, grants CGL2005-00122, CGL2008-02799, CGL2011-22629, CGL2014-53140-P and PGC2018-096656-B-I00 to ACR, which included FEDER funds, and by CONACYT to RAS-G [Ciencia Básica 282922] and FV [Ciencia Básica A1-S-34563].

## References

- ABADI S., AZOURI D., PUPKO T. & MAYROSE I. 2019. Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* 10 (1): 934
- AKAIKE H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6): 716-723
- ANDRÉS J.A., SÁNCHEZ-GUILLÉN R.A. & CORDERO RIVERA A. 2000. Molecular evidence for selection on female colour polymorphism in the damselfly *Ischnura graellsii*. *Evolution* 54: 2156-2161
- ANDREW R.J. 2001. Evidence of sperm displacement in *Ischnura aurora* (Brauer) (Zygoptera: Coenagrionidae). *Odonatologica* 30: 435-439
- ASKEW R.R. 1989. The dragonflies of Europe. Harley Books, Colchester
- BANHAM W.M.T. 1990. Non-random mating in the polymorphic damselfly *Ischnura elegans*. PhD Thesis, University of Manchester
- BICK G.H. & BICK J.C. 1957. The Odonata of Oklahoma. *The southwestern Naturalist* 2: 1-18
- BLOMBERG S.P., GARLAND T. JR, IVES A.R. & CRESPI B. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717-745
- BOTA-SIERRA C.A., VELÁSQUEZ-VÉLEZ M.I. & REALPE E. 2019. A new species of *Ischnura* from the Colombian Central Andes (Odonata: Coenagrionidae). *Odonatologica* 48: 115-132
- 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 Computational Biology* 10 (4): e1003537
- BOUDOT J.-P., KALKMAN V.J., AZPILICUETA AMORÍN M., BOGDANOVIĆ T., CORDERO RIVERA A., DEGABRIELE G., DOMMANGET J.-L., FERREIRA S., GARRIGÓS B., JOVIĆ M., KOTARAC M., LOPAU W., MARINOV M., MIHOKOVIĆ N., RISERVATO E., SAMRAOUI B. & SCHNEIDER W. 2009. Atlas of the Odonata of the Mediterranean and North Africa. *Libellula Supplement* 9: 1-256
- BYBEE S.M., OGDEN T.H., BRANHAM M.A. & WHITING M.F. 2008. Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. *Cladistics* 24: 477-514
- CANNINGS R.A. & DOERKSEN G.P. 1979. Description of the larva of *Ischnura erratica* (Odonata: Coenagrionidae) with notes on the species in British Columbia. *The Canadian Entomologist* 111: 327-331
- CHAUHAN P., WELLENREUTHER M. & HANSSON B. 2016. Transcriptome profiling in the damselfly *Ischnura elegans* identifies genes with sex-biased expression. *BMC Genetics* 17: 985
- CHIPPINDALE P.T., DAVE V.K., WHITMORE D.H. & ROBINSON J.V. 1999. Phylogenetic relationships of North American damselflies of the genus *Ischnura* (Odonata: Zygoptera: Coenagrionidae) based on sequences of three mitochondrial genes. *Molecular Phylogenetics and Evolution* 11: 110-121
- CLAUSNITZER V. & DIJKSTRA K.-D.B. 2005. The dragonflies (Odonata) of Ethiopia, with notes on the status of endemic taxa and the description of a new species. *Entomologische Zeitschrift* 115: 117-130
- CORDERO[RIVERA] A. 1989. Reproductive behaviour of *Ischnura graellsii* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica* 18: 237-244

- CORDERO[-RIVERA] A. 1990a. The inheritance of female polymorphism in the damselfly *Ischnura graellsii* (Rambur) (Odonata: Coenagrionidae). *Heredity* 64: 341-346
- CORDERO[-RIVERA] A. 1990b. The adaptive significance of the prolonged copulations of the damselfly, *Ischnura graellsii* (Odonata: Coenagrionidae). *Animal Behaviour* 40: 43-48
- CORDERO[-RIVERA] A. 1992. Density-dependent mating success and colour polymorphism in females of the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). *Journal of Animal Ecology* 61: 769-780.
- CORDERO[-RIVERA] A. & ANDRÉS [ABAD] J.A. 1996. Colour polymorphism in odonates: females that mimic males? *Journal of the British Dragonfly Society* 12: 50-60
- CORDERO[-]RIVERA A. & ANDRÉS ABAD J.A. 1999. Lifetime mating success, survivorship and synchronized reproduction in the damselfly *Ischnura pumilio* (Odonata: Coenagrionidae). *International Journal of Odonatology* 2: 105-114
- CORDERO-RIVERA A. & EGIDO PÉREZ F.J. 1998. Mating frequency, population density and female polychromatism in the damselfly *Ischnura graellsii*: an analysis of four natural populations. *Etología* 6: 61-67
- CÓRDOBA-AGUILAR A. 1992. Reproductive behavior and polychromatism in *Ischnura denticollis*. *Bulletin of American Odonatology* 1: 57-64.
- CRAIG J.K. & FOOTE C.J. 2001. Counter gradient variation and secondary sexual colour: phenotypic convergence promotes genetic divergence in carotenoid use between sympatric anadromous and non-anadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution* 55: 380-391
- DE KNIJF G., SPARROW D.J., DIMITRIOU A., KENT R., KENT H., SIEDLE K., LEWIA J. & CROSSLEY L. 2016. Distribution, ecology and status of a threatened species *Ischnura intermedia* (Insecta: Odonata), new for Europe. *International Journal of Odonatology* 19: 257-274
- DEVICHE P. 2010. Heterospecific copulating pair of forktails (*Ischnura*). Online on the internet, URL (08-x-2020): <http://azdragonfly.org/news/heterospecific-copulating-pair-forktails-ischnura>
- DICKERSON J.E., TYLER T. & ROBINSON J.V. 1992. Aspects of the life-history of adult *Ischnura cervula* (Selys) (Zygoptera: Coenagrionidae). *Notulae odonatologicae* 3: 137-156
- DUMONT H.J. 1991. Fauna Palaestina. Insecta V – Odonata of the Levant. The Israel Academy of Sciences and Humanities, Jerusalem
- DUMONT H.J. 1996. On the nature of *Ischnura aralensis* Haritonov, 1979 (Zygoptera: Coenagrionidae). *Odonatologica* 25: 179-182
- DUMONT H.J. 2013. Phylogeny of the genus *Ischnura* with emphasis on the Old World taxa (Zygoptera: Coenagrionidae). *Odonatologica* 42: 301-308
- DUMONT H.J., VIERSTRAETE A. & VANFLETEREN J.R. 2010. A molecular phylogeny of the Odonata (Insecta). *Systematic Entomology* 35: 6-18
- DUNKLE S.W. 1990. Damselflies of Florida, Bermuda and the Bahamas. Scientific Publishers, Gainesville
- ELLERS J. & BOGGS C.L. 2002. The evolution of wing color in *Colias* butterflies: heritability, sex linkage, and population divergence. *Evolution* 56: 836-840

- FAVA G. 1988. Genetic differentiation in experimental populations of the copepod *Tisbe clodiensis* Battaglia & Fava from two Adriatic lagoons. *Journal of experimental Marine Biology and Ecology* 97: 51-61
- FINCKE O.M. 1987. Female monogamy in *Ischnura verticalis* Say (Zygoptera, Coenagrionidae). *Odonatologica* 16: 129-143
- FINCKE O.M., JÖDICKE R., PAULSON D.R. & SCHULTZ T. 2005. The evolution and frequency of female color morphs in Holarctic Odonata: why are male-like females typically the minority? *International Journal of Odonatology* 8: 183-212
- FORSMAN A. & SHINE R. 1995. The adaptive significance of colour pattern polymorphism in the Australian scincid lizard *Lampropholis delicata*. *Biological Journal of the Linnean Society* 55: 273-291
- FORSMAN A. & WENNERSTEN L. 2016. Inter-individual variation promotes ecological success of populations and species: evidence from experimental and comparative studies. *Ecography* 39: 630-648.
- FORSMAN A., AHNESJÖ J., CAESAR S. & KARLSSON M. 2008. A model of ecological and evolutionary consequences of color polymorphism. *Ecology* 89: 34-40
- FRITZ S.A. & PURVIS A. 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology* 24: 1042-1051
- FURTADO J.I. 1972. The reproductive behaviour of *Ischnura senegalensis* (Rambur), *Pseudagrion microcephalum* (Rambur) and *P. perfuscatum* Lieftinck (Odonata, Coenagrionidae). *Malaysian Journal of Science* 1(A): 57-69
- GALEOTTI P. & RUBOLINI D. 2004. The niche variation hypothesis and the evolution of colour polymorphism in birds: A comparative study of owls, nightjars and raptors. *Biological Journal of the Linnean Society* 82: 237-248
- GALEOTTI P., RUBOLINI D., DUNN P.O. & FASOLA M. 2003. Colour polymorphism in birds: causes and functions. *Journal of evolutionary Biology* 16: 635-646
- GALEOTTI P., SACCHI R., PELLITTERI-ROSA D., BELLATI A., COCCA W., GENTILLI A., SCALI S. & FASOLA M. 2013. Colour polymorphism and alternative breeding strategies: effects of parent's colour morph on fitness traits in the common wall lizard. *Evolutionary Biology* 40: 385-394
- GALICIA-MENDOZA I., SANMARTÍN-VILLAR I., ESPINOSA-SOTO C. & CORDERO-RIVERA A. 2017. Male biased sex ratio reduces the fecundity of one of three female morphs in a polymorphic damselfly. *Behavioral Ecology* 28: 1183-1194
- GALINDO N. 2018. Evidencia de hibridación en dos especies de *Ischnura* (Odonata: Coenagrionidae) a partir de su ciclo de vida y análisis ecológico. Master Thesis. Universidad de los Andes
- GARRISON R.W. & HAFERNIK J.E. 1981. The distribution of *Ischnura gemina* (Kennedy) and a description of the andromorph female (Zygoptera: Coenagrionidae). *Odonatologica* 10: 85-91
- GAVRILETS S., ACTON R. & GRAVNER J. 2000. Dynamics of speciation and diversification in a metapopulation. *Evolution* 54: 1493-1501
- GRAY S.M. & MCKINNON J.S. 2007. Linking color polymorphism maintenance and speciation. *Trends in Ecology and Evolution* 22: 71-79
- HAFERNIK J.E. & GARRISON R.W. 1986. Mating success and survival rate in a population



- of damselflies: results at variance with theory? *The American Naturalist* 128: 353-365
- HARITONOV A.YU. 1988. [Dragonflies of the genus *Ischnura* Charp. (Insecta, Odonata) in the fauna of the USSR]. In: Zolotareno G.S. (Ed), *Taksonomiya zhivotnykh Sibiri. Novye i Maloizvestnye Vidy Fauny Sibiri* 20: 32-46. Nauka, Novosibirsk [in Russian]
- HARVEY P.H. & PAGEL M.D. 1991. The comparative method in evolutionary biology (Oxford series in ecology and evolution 1). Oxford University Press
- HENZE M.J., LIND O., WILTS B.D. & KELBER A. 2019. Pterin-pigmented nanospheres create the colours of the polymorphic damselfly *Ischnura elegans*. *Journal of the Royal Society Interface* 16 (153): 20180785
- HINNEKINT B.O.N. 1987. Population dynamics of *Ischnura e. elegans* (Vander Linden) (Insecta: Odonata) with special reference to morphological colour changes, female polymorphism, multiannual cycles and their influence on behaviour. *Hydrobiologia* 146: 3-31
- HUANG S.C. & REINHARD J. 2012. Color change from male-mimic to gynomorphic: a new aspect of signalling sexual status in damselflies (Odonata: Zygoptera). *Behavioural Ecology* 23: 1-7
- HUANG S.C., REINHARD J. & NORVAL G. 2012. The reproductive biology and daily activity patterns of *Ischnura heterostica* (Burmeister) in eastern Australia (Zygoptera: Coenagrionidae). *Odonatologica* 41: 99-107
- HUELSENBECK J.P., NIELSEN R. & BOLLBACK J.P. 2003. Stochastic mapping of morphological characters. *Systematic Biology* 52: 131-138
- HUGALL A.F. & STUART-FOX D. 2012. Accelerated speciation in colour-polymorphic birds. *Nature* 485: 631-634
- JOHNSON C. 1964. The inheritance of female dimorphism in the damselfly, *Ischnura damula*. *Genetics* 49: 513-519
- JOHNSON C. 1966. Genetics of female dimorphism in *Ischnura demorsa*. *Heredity* 21: 453-459
- JOHNSON C. 1975. Polymorphism and natural selection in ischnuran damselflies. *Evolutionary Theory* 1: 81-90
- JOSHI S., DAWN P., ROY P. & KUNTE K. (Eds) 2020. Odonata of India, v. 1.57. Indian Foundation for Butterflies. Online on the internet, URL (08-x-2020): <http://www.indianodonata.org>
- KARPESTAM E. & FORSMAN A. 2011. Dietary differences among colour morphs of pygmy grasshoppers revealed by behavioural experiments and stable isotopes. *Evolutionary Ecology Research* 13: 461-477
- KATBEH-BADER A., AMR Z., BAKER M.A. & MAHASNEH A. 2004. The dragonflies (Insecta: Odonata) of Jordan. *Denisia* 14: 309-317
- KATOH K. & STANDLEY D.M. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30: 772-780
- KOSTERIN O.E. 2015. *Ischnura foylei* sp. nov. (Odonata, Coenagrionidae) from the highlands of Sumatra. *Zootaxa* 4032: 179-189
- KUNTE K. 2009. Female-limited mimetic polymorphism: a review of theories and a critique of sexual selection as balancing selection. *Animal Behaviour* 78: 1029-1036
- KUNZ B. 2015. First record of *Ischnura nursei* Morton, 1907 from Oman (Odonata: Coenagrionidae). *Libellula* 34: 117-124
- LAM E. 2004. Damselflies of the Northeast: a guide to the species for eastern Canada

- and the northeastern United States. Biodiversity Books, Forest Hills, NY
- LITSIOS G. & SALAMIN N. 2012. Effects of Phylogenetic Signal on Ancestral State Reconstruction. *Systematic Biology* 61: 533-538
- MARRÓN G., CARMONA R. & HERNÁNDEZ-ÁLVAREZ A. 2015. Primeros registros de *Erythemis vesiculosa* (Fabricius, 1775; Odonata: Libellulidae) y de *Ischnura barberi* Currie, 1903 (Odonata: Coenagrionidae) para Baja California Sur, México. *Acta zoológica mexicana* 31: 502
- MARZOQ M.K. 2005. Relative growth and morphological variability in *Ischnura evansi* (Morton) (Odonata: Coenagrionidae). *Marina mesopotamica* 20: 27-38
- MCKEE D., HARVEY I.F., THOMPSON D.J. & SHERRATT T.N. 2005. Frequency of female colour morphs in populations of four coenagrionid damselflies (Zygoptera: Coenagrionidae). *Odonatologica* 34: 37-49
- MCKINNON J.S. & PIEROTTI M.E.R. 2010. Colour polymorphism and correlated characters: genetic mechanisms and evolution. *Molecular Ecology* 19: 5101-5125.
- MILENA L., REALPE E. & VELÁSQUEZ VÉLEZ M.I. 2015. Estudio colorimétrico y genético de *Ischnura capreolus* e *Ischnura cyane* y su posible morfotipo intermedio en el departamento de Cundinamarca. Bachelor thesis, Universidad de los Andes, Bogotá
- MILLER M.A., PFEIFFER W. & SCHWARTZ T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *2010 Gateway Computing Environments Workshop, GCE 2010*
- MONTOYA-Q. Á., PÉREZ L., PÉREZ H.A. & MAHECHA-J. O. 2014. Common Odonata from Córdoba, Salitre and La Florida wetlands, Cundinamarca, Bogotá-Colombia. Online on the internet, URL (07-x-2020): <https://fieldguides.fieldmuseum.org/sites/default/files/rapid-color-guides-pdfs/608%20COLOMBIA%20Common%20Odonata%20a1.pdf>
- NOVELO-GUTIÉRREZ R. & PEÑA-OLMEDO J. 1989. The subspecies of *Ischnura posita* (Hagen, 1861), with description of *I. p. atezca* ssp. nov. (Zygoptera: Coenagrionidae). *Odonatologica* 18: 43-49
- ORME D., FRECKLETON R., THOMAS G., PETZOLDT T., FRITZ S. & ISAAC N. 2013. The caper package: comparative analyses of phylogenetics and evolution in R. R package version 0.5.2. Available at: <https://CRAN.R-project.org/package=caper> (accessed on 1 November 2017)
- OZONO A., KAWASHIMA I. & FUTAHASHI R. 2012. Dragonflies of Japan. Bunichi-Sogo Syuppan, Japan
- PAGEL M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London. (B, Biological Sciences)* 255 (1342): 37-45
- PAGEL M. & MEADE A. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible jump Markov chain Monte Carlo. *American Naturalist* 167: 808-825
- PAPAZIAN M., DUMONT H.J. & MARY-SASAL N.J. 2007. The Odonata of the Pacific Ocean Islands of Wallis and Futuna, with special reference to speciation in *Ischnura aurora* (Brauer). *Odonatologica* 36: 53-62
- PARR M.J. & PALMER M. 1971. The sex ratios, mating frequencies and mating expectancies of three coenagriids (Odonata: Zygoptera) in Northern England. *Entomologica scandinavica* 2: 191-204

- PAULSON D.R. 1999. *Ischnura perparva* McLachlan (Zygoptera: Coenagrionidae) has an andromorphic female, and another suggestion to modify the terminology of female color polymorphism in Odonata. *International Journal of Odonatology* 2: 101-103
- PAULSON D.R. 2009. Dragonflies and damselflies of the West. Princeton University Press
- PAULSON D.R. & CANNINGS R.A. 1980. Distribution, natural history and relationships of *Ischnura erratica* Calvert (Zygoptera: Coenagrionidae). *Odonatologica* 9: 147-153
- REALPE E. 2010. Two new Andean species of the genus *Ischnura* Charpentier from Colombia, with a key to the regional species (Zygoptera: Coenagrionidae). *Odonatologica* 39: 121-131
- REVELL L.J. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217-223
- REVELL L.J., HARMON L.J. & COLLAR D.C. 2008. Phylogenetic signal, evolutionary process, and rate. *Systematic Biology* 57: 591-601
- RIESEBERG L.H., SINERVO B., LINDER C.R., UNGERER M.C. & ARIAS D.M. 1996. Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. *Science* 272: 741-745
- ROBERTSON H.M. 1985. Female dimorphisms and mating behaviour in a damselfly, *Ischnura ramburii*: females mimicking males. *Animal Behaviour* 33: 805-809
- ROBINSON J.V. 1983. Effects of water mite parasitism on the demographics of an adult population of *Ischnura posita* (Hagen) (Odonata: Coenagrionidae). *American Midland Naturalist* 109: 169-174
- ROBINSON J.V. & ALLGEYER R. 1996. Covariation in life-history traits, demographics and behaviour in ischnuran damselflies: The evolution of monandry. *Biological Journal of the Linnean Society* 58: 85-98
- ROBINSON J.V. & JORDAN W.H. 1996. Ontogenetic color change in *Ischnura kellicotti* Williamson females (Zygoptera: Coenagrionidae). *Odonatologica* 25: 83-85
- ROULIN A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews* 79: 815-848
- ROWE R.J. 1978. *Ischnura aurora* (Brauer), a dragonfly with unusual mating behaviour (Zygoptera: Coenagrionidae). *Odonatologica* 7: 375-383
- ROWE R.J. 2010. *Ischnura aurora* (Brauer 1865) (Zygoptera: Coenagrionidae), an Australo-Pacific species. *New Zealand Journal of Zoology* 37: 189-192
- SÁNCHEZ-GUILLÉN R.A., VAN GOSSUM H. & CORDERO-RIVERA A. 2005. Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). *Biological Journal of the Linnean Society* 85:471-481
- SÁNCHEZ-GUILLÉN R.A., HANSSON B., WELLENREUTHER M., SVENSSON E.I. & CORDERO-RIVERA A. 2011. The influence of stochastic and selective forces in the population divergence of female colour polymorphism in damselflies of the genus *Ischnura*. *Heredity* 107: 513-522
- SÁNCHEZ-GUILLÉN R.A., HAMMERS M., HANSSON B., VAN GOSSUM H., CORDERO-RIVERA A., GALICIA-MENDOZA D.I. & WELLENREUTHER M. 2013a. Ontogenetic shifts in male mating preference and morph-specific polyandry in a female colour polymorphic insect. *BMC Evolutionary Biology* 13: 116

- SÁNCHEZ-GUILLÉN R.A., MARTÍNEZ-ZAMILPA S.M.J., JIMÉNEZ-CORTÉS J.G., FORBES M.R. & CÓRDOBA-AGUILAR A. 2013b. Maintenance of polymorphic females: do parasites play a role? *Oecologia* 171: 105-113
- SÁNCHEZ-GUILLÉN R.A., MUÑOZ J., RODRÍGUEZ-TAPIA G., ARROYO T.P.F. & CÓRDOBA-AGUILAR A. 2013c. Climate-induced range shifts and possible hybridisation consequences in insects. *PLoS ONE* 8: 1-10
- SÁNCHEZ-GUILLÉN R.A., CÓRDOBA-AGUILAR A., CORDERO-RIVERA A. & WELLENREUTHER M. 2014a. Rapid evolution of prezygotic barriers in polymorphic damselflies. *Biological Journal of the Linnean Society* 113: 485-496
- SÁNCHEZ-GUILLÉN R.A., CÓRDOBA-AGUILAR A., CORDERO-RIVERA A. & WELLENREUTHER M. 2014b. Genetic divergence predicts reproductive isolation in damselflies. *Journal of Evolutionary Biology* 27: 76-87
- SÁNCHEZ-GUILLÉN R.A., WELLENREUTHER M., CHÁVEZ-RÍOS J.R., BEATTY C.D., RIVAS-TORRES A., VELÁSQUEZ-VÉLEZ M. & CORDERO-RIVERA A. 2017. Alternative reproductive strategies and the maintenance of female color polymorphism in damselflies. *Ecology and Evolution* 7 (15): 5592-5602
- SANMARTÍN-VILLAR I. & CORDERO-RIVERA A. 2016. The inheritance of female colour polymorphism in *Ischnura genei* (Zygoptera: Coenagrionidae), with observations on melanism under laboratory conditions. *PeerJ* 4: e2380
- SANMARTÍN-VILLAR I., ZHANG H.-M. & CORDERO-RIVERA A. 2016. Colour polymorphism and ontogenetic colour changes in *Ischnura rufostigma* (Odonata: Coenagrionidae). *Odonatologica* 45: 77-86
- SCHMIDT E. 1954. Ergebnisse der Österreichischen Iran-Expedition 1949/50. Die Libellen Irans. Sitzungsberichte der Akademie der Wissenschaften in Wien, Mathematisch-Naturwissenschaftliche Klasse, Abteilung I, 163: 223-260, 1 pl.
- SCHNEIDER T., IKEMEYER D. & DUMONT H.J. 2015. New records of dragonflies (Odonata) from Belutschistan-e-Sistan province in Iran. *Zoology in the Middle East* 61: 288-290
- SCHRÖTER A. 2010. The Odonata of Kyrgyzstan, part I – Critical national checklist, annotated list of records and collected data of the summer half-years 2008 and 2009. *International Dragonfly Fund-Report* 28: 1-72
- SHERRATT T.N. & FORBES M.R. 2001. Sexual differences in coloration of Coenagrionid damselflies (Odonata): a case of intraspecific aposematism? *Animal Behaviour* 62: 653-660
- SIROT L.K. & BROCKMANN H.J. 2001. Costs of sexual interactions to females in Rambur's forktail damselfly, *Ischnura ramburi* (Zygoptera: Coenagrionidae). *Animal Behaviour* 61: 415-424
- SIROT L.K., BROCKMANN H.J., MARNIS C., MUSCHETT G., MARINIS C. & MUSCHETT G. 2003. Maintenance of a female-limited polymorphism in *Ischnura ramburii* (Zygoptera: Coenagrionidae). *Animal Behaviour* 66: 763-775
- STAMATAKIS A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312-1313
- SUGIMURA M., ISHIDA S., KOJIMA K., ISHIDA K. & AOKI T. 2001. Dragonflies of the Japanese Archipelago in color. Hokkaido University Press, Sapporo
- SVANBÄCK R., EKLÖV P., FRANSSON R. & HOLMGREN K. 2008. Intraspecific competition drives multiple species resource polymorphism in fish communities. *Oikos* 117: 114-124

- TAJIMA Y. & WATANABE M. 2010. Sperm transfer process in the non-territorial *Ischnura asiatica* (Brauer) during copulation (Odonata: Coenagrionidae). *Odonatologica* 39: 253-258
- TAKAHASHI Y. & WATANABE M. 2009. Diurnal changes and frequency dependence in male mating preference for female morphs in the damselfly, *Ischnura senegalensis* (Rambur) (Odonata Coenagrionidae). *Entomological Science* 12: 219-226
- TAKAHASHI Y. & WATANABE M. 2010. Morph-specific fecundity and egg size in the female-dimorphic damselfly *Ischnura senegalensis*. *Zoological Science* 27: 325-329
- TAKAHASHI Y., KAGAWA K., SVENSSON E.I. & KAWATA M. 2014. Evolution of increased phenotypic diversity enhances population performance by reducing sexual harassment in damselflies. *Nature Communications* 5: 4468
- TILLYARD R.J. 1905. On dimorphism in the female of *Ischnura heterosticta* Burm. *Proceedings of the Linnean Society of New South Wales* 30: 302-306
- TOUSSAINT E.F.A., BYBEE S.M., ERICKSON R.J. & CONDAMINE F.L. 2019. Forest giants on different evolutionary branches: Ecomorphological convergence in helicopter damselflies. *Evolution* 73: 1045-1054
- UTZERI C. 1988. Female «refusal display» versus male «threat display» in Zygoptera: is it a case of interspecific imitation? *Odonatologica* 17: 45-54
- VAN GOSSUM H. & MATTERN M.Y. 2008. A phylogenetic perspective on absence and presence of a sex-limited polymorphism. *Animal Biology* 58: 257-273
- VAN GOSSUM H. & SHERRATT T.N. 2008. A dynamical model of sexual harassment in damselflies and its implications for female-limited polymorphism. *Ecological Modelling* 210: 212-220
- VAN GOSSUM H., STOKS R. & DE BRUYN L. 2001. Frequency-dependent male mate harassment and intra-specific variation in its avoidance by females of the damselfly *Ischnura elegans*. *Behavioural Ecology and Sociobiology* 51: 69-75
- VAN VALEN L. 1965. Morphological variation and width of ecological niche. *The American Naturalist* 99: 377-390
- VERCKEN E., SINERVO B. & CLOBERT J. 2008. Colour variation in female common lizards: why we should speak of morphs, a reply to Cote et al. *Journal of evolutionary Biology* 21: 1160-1164
- VILELA D.S., RICIOLI L.S., DEL-CLARO K. & GUILLERMO-FERREIRA R. 2017. Female color polymorphism of *Ischnura capreolus* Hagen, 1861 (Odonata: Coenagrionidae) with notes on behavior and ontogenetic color changes. *International Journal of Odonatology* 20: 191-200
- VON ELLENRIEDER N. & GARRISON R.W. 2007. Libélulas de las Yungas (Odonata). Una guía de campo para las especies de Argentina. Pensoft Publishers, Sofia
- WELLENREUTHER M., SVENSSON E.I. & HANSSON B. 2014. Sexual selection and genetic colour polymorphisms in animals. *Molecular Ecology* 23: 5398-5414
- WEST-EBERHARD M.J. 1983. Sexual selection, social competition, and speciation. *The quarterly Review of Biology* 58: 155-183
- WEST-EBERHARD M.J. 1986. Alternative adaptations, speciation, and phylogeny (a review). *Proceedings of the National Academy of Sciences* 83: 1388-1392

- WILLINK B., DURYEA M.C. & SVENSSON E.I. 2019. Macroevolutionary origin and adaptive function of a polymorphic female signal involved in sexual conflict. *The American Naturalist* 194: 707-724
- YANYBAEVA V.A., DUMONT H.J., HARITONOV A.Y. & POPOVA O.N. 2006. The Odonata of South Ural, Russia, with special reference to *Ischnura aralensis* Haritonov, 1979. *Odonatologica* 35: 167-185

#### Appendix I: Supplementary data

Supplementary data to this article (supplementary files 1–3) can be found online at <https://doi.org/10.5281/zenodo.4066815> and on the Odonatologica homepage at <http://www.odonatologica.com/supplementary-data/xxxxxxx>