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## Resumen

El cambio climático es quizás el reto actual más desafiante para las especies. Dos consecuencias principales de los cambios en áreas de distribución de especies inducidos por el clima son: el aumento de áreas de simpatria y la probabilidad de hibridación en las nuevas zonas de contacto. El potencial de nuevas interacciones interespecíficas aumentará en estas nuevas áreas de simpatria, y cuando estas interacciones ocurren entre especies recientemente evolucionadas que anteriormente se encontraban en alopatría, podrían desencadenarse eventos de hibridación entre especies. *Comprender* qué especies son vulnerables a la hibridación es de gran relevancia en la actualidad. Para una mejor comprensión de este proceso evolutivo y sus consecuencias en este trabajo hemos analizado: 1) cómo el cambio climático puede afectar las distribuciones de especies, 2) si los cambios en las distribuciones de especies generarán nuevas áreas de simpatria, 3) el grado de divergencia genética entre especies, 4) si las áreas superpuestas pronosticadas pueden presentarse en especies estrechamente relacionadas que podrían hibridar según su divergencia genética, eso significaría que los linajes más cercanos preservarían propiedades más cercanas del nicho, 5) caracterizarán las barreras del aislamiento reproductivo. En el primer capítulo, examiné el potencial de la hibridación en función de las distancias genéticas y las áreas de distribución actuales y futuras en diferentes escenarios de cambio climático para especies del género *Argia*. En el segundo capítulo, hago una revisión crítica de un concepto fundamental en mi trabajo que es el conservadurismo de nicho. Examiné el conservadurismo de nicho a la luz de la hibridación. En el tercer capítulo, documenté las barreras de aislamiento entre cuatro especies del género *Argia* en condiciones naturales, a través de experimentos de atracción sexual entre sexos de diferentes especies. Además, en un primer apéndice modelé la distribución potencial de seis especies de libélulas y, de acuerdo con los criterios de la UICN, proponemos a *A. garrisoni* para su inclusión en la categoría de vulnerable. En un segundo apéndice, modelé las áreas de distribución de las especies de odonatos de Argentina. En un tercer apéndice, describí la morfología genital en varias especies de libélulas, incluidas tres del género *Argia*. Esta descripción es fundamental para comprender el aislamiento entre las especies de libélulas, el cual se basa bastante en las interacciones genitales durante el coito.

## Abstract

Climate change is perhaps the most challenging sources of stress for species. Two major consequences of climate-induced range shifts are: the increase of sympatric areas, and the probability of hybridization in new contact zones. The potential for interspecific interactions increases in these new areas of sympatry, and when these interactions occur between recently-evolved species formerly in allopatry, the process could lead to hybridization. Understanding which species are vulnerable to hybridization is of upmost relevance in nowadays, for a better understand of the evolutionary process and consequences. In this work we have analyzed: 1) how climate change may affect species distributions, 2) whether changes in species distributions will generate new areas of sympatry, 3) the degree of genetic divergence between species, 4) whether predicted overlapping areas may render closely related species to hybridize according to their genetic divergence, that means test if closer lineages will preserve closer niche properties, 5) characterized the barriers of reproductive isolation. In the first chapter, I examined the potential of hybridization based on genetic distances and current and future distribution areas in different climate change scenarios for species of *Argia* genus. In the second chapter, I make a critical review of a fundamental concept in my work that is niche conservatism. I examined conservatism in light of the hybridization. In the third chapter, I documented the hybridization potential between four species of the genus *Argia* on natural conditions, through experiments of sexual attraction between sexes of different species, and distribution models. Also, In a first appendix I estimated the distribution of six species of dragonflies and according to the IUCN criteria, we propose *A. garrisoni* for inclusion in the category of vulnerable. In a second appendix we have model the distribution areas of the odonata species of Argentina. In a third appendix, I described the genital morphology in several species of damselflies including three of the genus *Argia*. This description is fundamental to understand the isolation between species of damselflies which is quite based on genital interactions during intercourse.

## INTRODUCCIÓN GENERAL

Representación del patrón abdominal de *Rhionaeschna variegata* (Fabricius, 1775): la libélula más austral del planeta.

## Introducción general

Tres temas centrales en la evolución de las especies son la divergencia genética, la divergencia ecológica y la acumulación de barreras de aislamiento reproductivo durante la especiación. En el desarrollo de esta tesis investigué estos tres grandes tópicos evolutivos a la luz de quizá, el más grande reto que tienen que enfrentar las especies actualmente, el cambio climático. Como respuesta al cambio climático que registra el planeta, muchas especies han modificado recientemente sus patrones de distribución (Parmesan *et al.*, 1999). Una de las consecuencias de este fenómeno es el aumento de áreas de simpatria entre especies o la generación de nuevos contactos entre especies aisladas previamente (Garroway *et al.*, 2010). Esto da pie a nuevas interacciones ecológicas entre especies, incluyendo la hibridación, es decir al apareamiento entre individuos de diferentes especies (Taylor, Larson, & Harrison, 2015). La hibridación es un factor que ha influido en gran medida en los patrones de diversidad de las especies y provee una excelente oportunidad para el estudio de procesos evolutivos (Rhymer & Simberloff, 1996; Dowling, Secor, & L., 1997). Actualmente existe una gran cantidad de estudios que evidencian la hibridación como consecuencia del cambio climático, siendo quizá uno de los ejemplos más emblemáticos aquel entre el oso polar (*Ursus maritimus*) y el oso grizzli (*U. arctos*) (Kelly, Whiteley, & Tallmon, 2010). Los híbridos de estas especies han surgido gracias a la expansión en la distribución del oso grizzli hacia latitudes más nórdicas en el Ártico (Crispo *et al.*, 2011). Ejemplos como este pueden encontrarse en diferentes grupos de organismos como son peces (Yau & Taylor, 2013), mariposas (Mallet, Wynne, & Thomas, 2011) u odonatos (Sánchez-Guillén *et al.*, 2011) por citar algunos.

A pesar de que la hibridación puede ocurrir debido al cambio climático, no sabemos cuáles especies podrían enfrentarla. Un método que puede ser usado efectivamente para predecir cuáles especies incurrirían en esto es aquel que combine: 1) el modelado de nicho ecológico, con el que se pueden proyectar áreas idóneas para una especie y con ello predecir zonas futuras de simpatria, donde el contacto sexual entre especies potencialmente puede tener lugar; 2) el grado de divergencia genética entre especies; y, 3) la caracterización de la fuerza de las barreras de aislamiento reproductivo entre especies.

Con esta información, podemos predecir cuáles especies genéticamente cercanas, podrían hibridar con aquellas especies que invadan su área de distribución en respuesta al cambio climático.

El estudio de la hibridación con herramientas como la divergencia genética tiene una larga tradición desde el siglo pasado. De hecho, hay alrededor de 500 estudios que analizan la distancia genética como un correlato para predecir hibridación en organismos como aves (Price & Bouvier, 2002), anfibios (Sasa, Chippindale, & Johnson, 1998), plantas (Scopece *et al.*, 2007), insectos como mariposas (Presgraves, 2002) y odonatos (Sánchez-Guillén *et al.*, 2014). En cuanto a las herramientas de modelado de nicho ecológico con énfasis en las cuestiones de hibridación, el desarrollo ha sido más reciente (por ejemplo: (Sánchez-Guillén *et al.*, 2013; Bariotakis *et al.*, 2016; Otis *et al.*, 2017).

Usando las herramientas metodológicas descritas arriba, estudiamos: 1) cómo el cambio climático puede afectar las distribuciones de especies, 2) si los cambios en las distribuciones de especies generarán nuevas áreas de simpatría, 3) el grado de divergencia genética entre las especies, 4) si nuevas áreas de simpatría corresponderán con especies estrechamente relacionadas, por ejemplo, con distancias genéticas cercanas a un umbral de hibridación, 4) si existe una relación entre la divergencia genética y la divergencia ecológica entre especies, 5) la fuerza de las barreras de aislamiento reproductivo, y 6) la importancia del grado de la simpatría en la intensidad de aislamiento sexual. Esto nos permitió evaluar riesgo de hibridación como consecuencia del cambio climático en 30 especies de caballitos del diablo del género *Argia*.

En el contexto del cambio climático, se han documentado alteraciones en la distribución de libélulas del Reino Unido, mediterráneas e incluso africanas (Ott, 2010). La tendencia observada es de aumentar su área de distribución: 37 especies de Gran Bretaña han ampliado su área de distribución hacia el norte, por hasta 87 km (Hickling *et al.*, 2005). Como este, existen otros estudios que documentan cómo algunas especies que se distribuían al sur de Europa se han desplazado hacia el centro y norte del continente. Tal es el caso de *Aeshna affinis*, *A. mixta*, *Anax parthenope*, *Erythromma viridulum* (Ott, 2010).

Además, se han hecho proyecciones al futuro para varias especies del género *Ischnura* en Europa, las cuales proyectan cambios en las distribuciones, con nuevas áreas que pueden conllevar la formación o aumento de simpatría entre especies afines.

En el contexto del aislamiento reproductivo en libélulas, se han documentado diferentes grados de aislamiento relacionados positivamente con la diferencia genética entre especies (Sánchez-Guillén *et al.*, 2014). Esta correlación puede utilizarse para predecir la hibridación (Coyne & Orr, 1989; Moyle, Olson, & Tiffin, 2004). De hecho, se ha predicho que las distancias genéticas entre pares de cinco especies de la familia Coenagrionidae (*I. elegans*, *I. fountaineae*, *I. genei*, *I. graellsii* y *I. saharensis*) caen dentro del umbral de hibridación (Sánchez-Guillén *et al.*, 2014), lo cual se ha corroborado en la naturaleza (Sánchez-Guillén, Wellenreuther, & Cordero-Rivera, 2012).

En relación a los sujetos de estudio que usé en mi tesis, *Argia* es un buen grupo modelo para estudiar la hibridación como consecuencia del cambio climático, debido a que es el género más especioso de odonatos en el mundo, con más de 126 especies descritas (Garrison & Von Ellenrieder, 2017). Además, los eventos de especiación en este grupo han sido relativamente rápidos y recientes, lo cual posiblemente ha reducido las posibilidades de aislamiento reproductivo entre especies hermanas (Paulson, 1974; Nava-Bolaños *et al.*, 2016). Adicionalmente, la mayoría de las especies de este género se encuentran en Norte América ocupando áreas relativamente grandes, por lo cual se pueden encontrarse fácilmente en simpatría (Nava-Bolaños *et al.*, 2016). Finalmente, existe abundante información de registros de presencia, así como información molecular disponible para las especies de este género (Caesar & Wenzel, 2009; Torres-Pachón, Novelo-Gutiérrez, & Espinosa de los Monteros, 2017), a partir de las cuales es posible derivar umbrales basados en divergencias genéticas y así predecir la hibridación (Sánchez-Guillén *et al.*, 2014).

Así, en este trabajo, identifiqué las especies del género *Argia* que podrían generar zonas híbridas potenciales bajo escenarios de cambio climático. Considerando la divergencia genética entre estas especies, determiné cuáles especies son más susceptibles de hibridar. Caractericé las barreras de aislamiento reproductivo de estos organismos. Y



estudié la relación entre la divergencia genética, la divergencia ecológica y la fuerza del aislamiento reproductivo.

Mi trabajo de tesis se compone de los siguientes capítulos. En el primero examino el potencial de hibridación basado en las distancias genéticas y las áreas de distribución actuales y futuras en diferentes escenarios de cambio climático. En el segundo capítulo hago una revisión crítica de un concepto fundamental en mi trabajo que es el conservadurismo de nicho. Examino el conservadurismo a la luz del potencial de hibridación. En el tercer capítulo documento el potencial de hibridación entre cuatro especies del género *Argia* en condiciones naturales, mediante experimentos de atracción sexual entre sexos de las diferentes especies, y modelos de distribución. Este tercer capítulo cumple el requisito del doctorado ya que fue publicado en la revista *Biological Journal of the Linnean Society*. También presento tres apéndices con diferentes enfoques relacionados con mi trabajo y usando las libélulas como sujetos de estudio. En el primer apéndice estimo la distribución de seis especies de libélulas y de acuerdo con los criterios de la UICN, propongo a *A. garrisoni* para su inclusión en la categoría de vulnerable. Este apéndice ha sido aceptado para publicar en la *Revista Mexicana de Biodiversidad*. En un segundo apéndice modelamos las áreas de distribución de las especies de odonatos de Argentina, como una antesala a predecir híbridos de una manera similar a lo que intentamos en el tercer capítulo. Este segundo apéndice está actualmente en revisión en la revista *Systematics and Biodiversity*. En un tercer apéndice, describo la morfología genital en varias especies de libélulas incluyendo tres del género *Argia*. Esta descripción es fundamental para entender el aislamiento entre especies de libélulas el cual está bastante fundamentado en interacciones genitales durante la cópula. Este último apéndice fue publicado en la revista *Acta Ethologica* (Nava-Bolaños *et al.*, 2014).

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## Predicting hybridization as a consequence of climate change in *Argia* damselflies

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**Running title:** Hybridization as a consequence of climate change

**Key words:** *Argia* – climate change – damselflies – ecological niche – genetic divergence – sympatric patterns.

## Abstract

Climate change is perhaps the most challenging sources of stress for species. Two major consequences of climate-induced range shifts are: the formation of new areas of geographic overlapping (or sympatry), and the probability of hybridization in new contact zones. In these new areas of sympatry, the potential for interspecific interactions increases, and when these interactions occur between recently-evolved species formerly in allopatry, the process can lead to hybridization. Understanding which species are vulnerable to hybridization is of upmost relevance in nowadays, for a better understand of the evolutionary process and consequences. One method that can be used effectively to test the potential of hybridization as a result of climate change is ecological niche modelling combined with genetic divergence studies. In this paper we have combined this methodology to predict the evolutionary consequences of climate change in *Argia* damselflies. In detail:1) how climate change may affect species distributions, 2) whether changes in species distributions will generate new areas of sympatry, 3) the degree of genetic divergence between species, and 4) whether predicted overlapping areas may render closely related species to hybridize according to their genetic divergence, that means test if closer lineages will preserve closer niche properties. According to our climate change scenarios, most species will increase their distribution boundaries, which will be accompanied by enlarged areas of sympatry. These species shown low ecological niche differentiation between species that have been evolve, maybe in a separate way without reinforcement of reproductive barriers. *Argia* species show highly-predicted hybridization by the degree of overlapped in their potential distribution areas and the genetic divergence. Our results can be useful to forecast how *Argia* species will respond to climatic change and how closely related species would increase their hybrid zones due to climatic change.

## Introduction

Climate change causes significant environmental stress for many species. Organisms can respond to such stress in three ways: (1) through short-term phenotypically plastic (often physiological) adjustments, (2) by adapting their life history through a more long-term evolutionary response and/or by modifying their behavior, and (3) extensive movement of the organisms following their niche preferences, and create novel areas of geographic overlap between formerly allopatric taxa, or the increase in overlapping areas between formerly sympatric taxa (Taylor *et al.*, 2006; Garroway *et al.*, 2010; Sánchez-Guillén *et al.*, 2013, 2016). Changes in temperature can have a significant impact on the fitness and life cycle features, patterns of distribution (Angiletta *et al.*, 2002, Wang *et al.*, 2009). Thus, events such as climate change are one of the greatest challenges for species, particularly for ectothermic organisms such as insects.

In areas of *de novo* sympatry, the potential for interspecific interactions increases, and when these interactions occur between closely related species, this process can lead to hybridization. An increasing number of climate-induced range shifts have now been associated with hybridization (Kelly, Whiteley, & Tallmon, 2010; Crispo *et al.*, 2011; Abbott *et al.*, 2013; Mallet, Wynne, & Thomas, 2011). One well known hybridization example is that of the polar bear *Ursus maritimus* and grizzly bear *U. arctos* (Kelly, Whiteley, & Tallmon, 2010). Observations over the last decade indicate that brown and black bears are shifting northward into the Arctic with hybridization between these lineages in their new sympatric areas (Crispo *et al.*, 2011; Abbott *et al.*, 2013). Hybrid zones as a by-product of increasing temperatures also include invertebrates, such as brown Argus butterflies (*Polyommatus agestis* and *P. Artaxerxes*) (Mallet, Wynne, & Thomas, 2011). These species have been shifting their range northwards, potentially threatening the long-term survival of the northern species (Mallet, Wynne, & Thomas, 2011). Another example comes from damselflies, where hybridization and introgression events have been documented in a number of species. A particularly well documented example is that of the hybridization between *Ischnura elegans* and *I. graellsii*, with introgressive hybridization of *I. graellsii* genes into recently established *I. elegans* populations (Sánchez-Guillén *et al.*, 2011, Wellenreuther *et al.*, 2018). One consequence is a rapid species turnover in sympatric regions in favour of *I. elegans* (Sánchez-Guillén *et al.*, 2011).

The evolutionary consequences of hybridization can range from local adaptation, speciation to extinction (Sánchez-Guillén *et al.* 2011; 2016; Presgraves, 2002; Hoffmann and Sgrò 2011). Thus, predicting which species are vulnerable to undergo hybridization and identifying the geographic areas where this could occur is of utmost relevance nowadays. One way this can be achieved is by using ecological niche models (ENM), in combination

with genetic divergence studies. ENM tools are a more recent approach with emphasis on hybridization issues (Bariotakis, Koutroumpa, Karousou, & Pirintsos, 2016 with *origanum*, Otis, Thornton, Rutledge, & Murray, 2017 with wolf-coyote, and Sánchez-Guillén et al., 2013 with odonate insects). These models are useful analytical tools to project the suitable habitat of a species under different climatic scenarios to evaluate range contractions, expansions and to identify areas of persistence (Peterson *et al.*, 2011). This in combination with knowledge about the genetic divergence between species can be used to predict species combinations that are prone to hybridize when forming a secondary contact zone (Sánchez-Guillén *et al.*, 2014). Genetic divergence predicts reproductive isolation in many organisms including damselflies. That is because to the general relationship between genetic divergence (as a surrogate for time) and reproductive isolation (e.g. Coyne & Orr, 1989, 1997). A positive relationship between the strength of reproductive isolation and genetic distance was first detected by Zouros (1973) and Ayala (1975) when working on closely related species of *Drosophila*. A little over a decade later, Coyne & Orr (1989) argued that if the time since species splitting affects genetic distance, then a general relationship with the degree of reproductive isolation should be expected. Indeed, comprehensive work by Coyne & Orr (1989, 1997) detected a positive correlation between the strength of prezygotic (sexual/behavioural) and post-zygotic isolation (hybrid sterility and inviability) and genetic divergence in a meta-analysis of 174 pairs of *Drosophila* species. Indeed, the studies of hybridization risk in relation to genetic divergence have been conducted since the last century on a wide range of animals groups, including birds, amphibians, plants, and insects (Sasa, Chippindale, & Johnson, 1998 in frogs 46 species, Price & Bouvier, 2002 in birds 368 species, angiosperms Moyle *et al.*, 2004 191 species, Scopece, Musacchio, Widmer, & Cozzolino, 2007 with orchids, Presgraves, 2002 in butterflies 182 species, and also odonata Sánchez-Guillén *et al.*, 2014 30 species pairs from eight damselfly genera, including the Coenagrionidae family). These studies are in line with the hypothesis that reproductive isolation is a by-product of gradual genetic divergence, that is, a phenomenon commonly referred to as the 'speciation clock' (Coyne & Orr, 1989, 1997).

Here we have investigated the evolutionary consequences of climate induced range shifts in *Argia* damselflies (Odonata: dragonflies and damselflies). In particular we determined in this group: 1) the extent of range shifts in response to future climate warming using ENM projections, with a particular emphasis to understand the creation of novel sympatric ranges between formerly allopatric species; and 2) the likelihood of species hybridization in these *de novo* sympatric areas following these range shifts based on their genetic distance. Among odonate insects, the genus *Argia* is a good model group to understand the propensity of hybridization for several reasons. First, it is the most speciose genus of Odonata in the world, with more than 126 described species (Schorr & Paulson,



2016; Garrison & Von Ellenrieder, 2017). Second, given the relatively strong dispersal ability of odonates, the fact that most *Argia* species occur exclusively in North America (approximately 81 spp; González Soriano & Novelo-Gutiérrez, 2012), implies large habitat ranges for most species, reduced ecological niche differentiation and, thus, high sympatric areas (Nava-Bolaños *et al.*, 2016). And third, there is molecular information available for *Argia* species (Caesar & Wenzel, 2009; Torres-Pachón, Novelo-Gutiérrez, & Espinosa de los Monteros, 2017) making it possible to calculate the genetic likelihood of hybridization of species pairs (Sánchez-Guillén *et al.*, 2014).

## **Methodology**

### **Species distributional data**

The presence of 30 *Argia* species was compiled based on records from databases (<http://www.odonatacentral.org>, 66% of data, [www.conabio.gob.mx](http://www.conabio.gob.mx), 8% of data, and [www.gbif.org](http://www.gbif.org), 20%), and the literature (publications and thesis dissertations, 6% of data). All data were checked carefully for geographic accuracy. Inconsistent points were removed, such as data with longitude and latitude inconsistent with the location of the species, duplicate information and references of species that were in the sea. Niche models were built only when more than 10 records per species were available. Thus, the final data set included 7560 unique presence data points of 30 species (see Table 1) of *Argia* species from the years 1975 to 2015. Although these records spanned the last 40 years, most of the data was derived from the last 15 years. The database is available.

### **Ecological niche models**

#### ***Study area and environmental predictors***

Our study area included North and Central America between the latitudes 53.00 to 0.00N, and longitudes -130.00 to -55.00W. We downloaded WorldClim 1.4 ([www.worldclim.org](http://www.worldclim.org)) bioclimatic variables for this area (Hijmans *et al.*, 2005) at a cell size of 0.041666669. To define a set of uncorrelated climatic variables, we intersected the variables with target group points, and with 10,000 points randomly selected in the extension of the study area (M). We removed variables with an exploratory data analysis of contribution of variables using jackknife and Pearson correlation analysis (i.e. any value >0.7 following the criteria of Romo 2012). Thus, the final data set included uncorrelated variables which had more biological importance for *Argia* species, and more contribution. The final variables set included: mean diurnal range (bio 02), temperature seasonality (bio 04), mean temperature of warmest quarter (bio 10), precipitation of wettest month (bio 13), precipitation of driest month (bio 14), and precipitation seasonality (bio 15).

#### ***Background niche selection***

To choose the best niche background, preliminary species distribution models were generated with Maxent 3.3.3k (Phillips, Anderson, & Schapire, 2006) with the following variables: 1) characterizing the background with 10,000 points randomly selected points in the extension of the study area, 2) target group points, that is using the points of localities of sister species to characterize the background and 3) characterizing the background to a special area extent delineating it for each particular species with ecoregions (World Wildlife Fund; [www.worldwildlife.org/](http://www.worldwildlife.org/)), and biogeographical provinces (CONABIO; [www.conabio.gob.mx](http://www.conabio.gob.mx)). Models were run using default settings ('Auto features', convergence = 10<sup>-5</sup>, maximum number of iterations = 500) with the exception of the random seed number (with a 30 test percentage), 10 replicates ran with bootstrap replicated type ran, we removed duplicate records, with no extrapolation and no clamping option when projected to regions of environmental space outside the limits encountered during training. The models were evaluated based on the highest area under the curve value (AUC) while minimizing the number of model parameters, as well as producing 'closed', bell-shaped response curves guaranteeing model calibration (Elith, Michael, & Steven, 2010). Thus, of these three criteria to build the background environment, the best performance was 10,000 points randomly.

### ***Training data sets***

Final models were built with the BIOMOD (Biodiversity Modelling) package in R (R Development Core Team (2008). This package is a platform for predicting species' distributional ranges, including the ability to model the distribution combining many algorithms and test statistics for validation (Thuiller *et al.*, 2009). We trained models using four widely used algorithms: Maximum Entropy (Maxent), Random Forest (RF), Generalized Boosting Methods (GBM) and Multivariate Adaptive Regression Splines (MARS). These models have shown good performance in terms of predictive power (Plischoff & Fuentes-Castillo, 2011; Reiss *et al.*, 2011; Broennimann *et al.*, 2012). From individual models obtained with these different algorithms, we generated a "consensus model". Such model combination is the best logistic compromise to avoid both overfitting and overpredicting (Merow *et al.*, 2014). 70 % of data was used for training, and 30 % for model validation, with 10 replicate runs. The final model validation was performed using True Skills Statistics (TSS), which denotes the average net rate of successful presence and absence predictions (Liu, White, & Newell, 2009), ranging from -1 to 1, where positive values indicate a higher degree of accuracy and discrimination model (Allouche, Tsoar, & Kadmon, 2006) (Table 1). It is noteworthy that the result of these models is not the area that species occupy in absolute terms, because these models do not consider population dynamics, dispersal ability, interactions with other species and human impacts. However, these models can make right predictions of suitability areas where species can be potentially found given their

environmental conditions. This is based on the assumption that the known distribution of species provides enough information to characterize its environmental requirements.

A total of 240 models were generated, and model performance was assessed using AUC and TSS statistics, while minimizing the number of model parameters. 10 percentile training presence threshold was used because we applied a conservative approach accepting that a 10% of our presences could be problematic (for a similar rationale, see (Sánchez-Guillén *et al.*, 2014)). The best models of current climatic conditions of species were used to generate projections.

### **Potential future projections**

The data for the future projections included: the most recent data from Global Climate Models (GCM) (CNRM-CM5, HadGEM2-ES and MPI-ESM-LR) from the WorldClim database (<http://worldclim.org/CMIP5v1>). These climate projections were used in the Fifth Assessment (CMIP5) (<http://cmip-pcmdi.llnl.gov/cmip5/>) report of The Intergovernmental Panel on Climate Change (IPCC) (<http://www.ipcc.ch/>), the RCP (representative concentration pathways which are greenhouse gas concentration trajectories) used were RCP4.5 and RCP8.5 (numbers after are the range of radiative forcing values), to 2050 and 2070 years.

We calculated the percentage loss or gain of geographic areas with respect to current potential distributions for each future projection (2050 and 2070) under RCP 4.5 and 8.5. For each year we only used the consensus area predicted by all models to calculate a species range distribution (Table 1). The potential hybrid zones between each pair of species (for current and future potential distributions) were estimated as the proportion of range overlap between two species. We further estimated the overlap areas in km<sup>2</sup> (see Supplementary Table S1) with the function `stringr` and `raster` packages in R.

### **Correlation between genetic divergence and predicted hybrid zones**

The phylogeny of the North American *Argia* species, including our 30 studied species, has already been published by Caesar & Wenzel (2009) using one mtDNA gene (16S), and by Torres-Pachón *et al.* (2017) using two mtDNA genes: 16S and cytochrome oxidase subunit I (COI). We downloaded the 16S sequences from GenBank ([www.ncbi.nlm.nih.gov/Genbank/](http://www.ncbi.nlm.nih.gov/Genbank/)) while the COI sequences were provided by authors of the two phylogenies (Caesar & Wenzel 2009 and Torres-Pachón *et al.* 2017).

Sequences were aligned using the BioEdit software, and pairwise genetic distances were estimated in MEGA V. 7 (Kumar, Stecher, & Tamura, 2016). The best evolutionary models, for genetic divergence estimations were inferred using JModel test. For 16S (N=54

sequences) the best nucleotide substitution model (the lowest Bayesian Information Criterion Scores is considered to describe the best substitution pattern) was "Tamura 3-parameter" model (Tamura *et al.*, 2011), with a discrete Gamma distribution. Tamura 3-parameters considers differences between transitions and transversions and provides equal substitution rates among sites. For COI (N=24 sequences), the best nucleotide substitution model was used based on Tamura-Nei (Nei & Kumar, 2000).

To identify which pairs of species are prone to hybridize we used the 1.78 hybridization threshold range to identify species pairs that are prone to hybridize calculated for the COII marker, data from COII and 18S-28S showed that species with genetic distances below a threshold of  $0.0067 \pm 0.011$ , (range-0.43-1.78%) for COII and  $0.0033 \pm 0.004$ , (range-0.052-0.713%) for 18S-28S are susceptible to hybridize and produce hybrids (Sánchez-Guillén *et al.*, 2014). This threshold is comparable due to Sánchez-Guillén *et al.*, (2014) found a positive and strong correlation between reproductive isolation and genetic distance using both mitochondrial and nuclear genes for 30 species pairs from eight damselfly genera, including the Coenagrionidae family. They have theoretically predicted that species pairs that have genetic distances similar to/or below species pairs forming hybrids are prone to undergo hybridization themselves. The threshold hybridization range was calculated based on the genetic distances between all species pairs, including that are forming hybrids in the wild. Which suggests that these threshold values are suitable to identify species prone to hybridization and that positive isolation–divergence relationships are taxonomically widespread.

To test the relationship between genetic divergence and the divergence of ecological niche across the geographic distribution of *Argia* species, we applied a correlation coefficient, which was estimated using Pearson's correlation between genetic distance of each pair of *Argia* species and their predicted overlap area in R. Here a positive correlation will be used as a proxy for a case where ecological diversification scales with genetic diversification. Our prediction is that we will not find a positive correlation, because niche diversification in several damselfly genera has been shown to negligible (Wellenreuther & Sánchez-Guillén, 2016).

## RESULTS

### Current potential distribution

Our models predicted a wide variation among species in geographic ranges. Some species were predicted to occupy fairly small areas (i.e. less than 20,000 km<sup>2</sup>, *A. cuprea*, *A. funcki*, *A. garrisoni*, *A. harknessi* and *A. munda*) whereas others were predicted to occupy vast

geographic areas (i.e. over one million km<sup>2</sup>, *A. alberta*, *A. apicalis*, *A. emma*, *A. lacrimans*, *A. lugens*, *A. nahuana*, *A. sedula*, *A. tarascana*, *A. tibialis* and *A. vivida*) (see Table 1).

### **Predicted future distribution changes**

The relative change in the area as predicted by the distribution models projected to RCP 4.5 and 8.5 for 2050 and 2070 respectively, are shown in Table 1. From this data a clear general trend emerges, namely that the most species will experience an increase in their distributional area.

For RCP 8.5 and 2070, only *A. extranea* is predicted to remain in the same geographic area as it inhabits now. However, 9 species are forecasted to decrease the area that they can occupy by 9% to 56% depending on the species. Nevertheless, 20 species are predicted to significantly increase the area that they can occupy by 21% to 151%. The future distribution models for the two periods (2050 and 2070) and two scenarios of climate change (RCP 4.5 and RCP 8.5) were consistent, showing a pattern of progressive increase or progressive decrease of the suitable habitats for *Argia* species.

### **Predicted sympatric overlap based on current distribution models**

According to cotemporary climatic conditions 125 *Argia* species combinations show allopatric distributions, while 310 species combinations show sympatric distribution (see Supplementary Table S1 which includes overlapping areas in km<sup>2</sup>, and relative species overlap estimates; Figure 1).

### **Changes in overlapping distribution ranges under different climate scenarios**

The range of overlap for each species combination is predicted to increase for both climate change scenarios for 2050 and 2070. The relative change in sympatric areas are presented in Table 2 and Supplementary TableS1. We found that for 2050 (with a RCP of 4.5) there will be 13 new *Argia* species combinations and a total increase of 27.89 % (Supplementary TableS1). In general, these new interactions will be maintained until 2070, and also with a RCP of 8.5 (Supplementary TableS1). Figure 1 shows the potential hybrid zones (i.e. overlapping areas) for current and future scenarios for 2070 (RCP of 8.5) for all pairwise *Argia* species combinations.

### **Correlation between genetic divergence and degree of sympatry**

Genetic divergence between all species ranged from 0.00% (i.e. *A. apicallis* and *A. tezpi*) to 15.72% (i.e. *A. anceps* and *A. tezpi*; Supplementary Table S1). We did not find a strong correlation between the degree of sympatry and genetic divergence in *Argia* species ( $r=-$

0.04,  $P= 0.485$  for 16S mtDNA;  $r=-0.213$ ,  $P= 0.287$  for 16s rRNA, and  $r=-0.082$ ,  $P= 0.349$  for COI).

A total of 108 species pairs showed a genetic distance lower than 0.37%, which is lower than hybridization umbral threshold of 1.78% for COII (Table 2) as predicted by Sánchez-Guillén et al., 2014. 97 of these species pairs with low genetic distance also showed overlap in their potential current areas of distribution (Table 2 and examples in Figure 2). Our model showed that under climate change RCP8.5: 48 out of the 97 vulnerable species will increase (with more than 10% of increase) their potential future sympatric area under climate change RCP8.5, while 37 species pairs will decrease their potential future sympatric area (with more than 10% of lost) and only 12 will maintain their potential future sympatric distribution with less of 10% of change. In addition, this tendency of change, was remained as a pattern in the other projections of climate change (see Table 2 and examples in Figure 3).

## DISCUSSION

Our climate change scenarios forecasts that 20 of the 30 *Argia* species will increase their distribution and that this will be accompanied by enlarged areas of currently sympatric species. This expansion will be accompanied by de novo sympatric distribution areas, with 97 species pairs vulnerable to hybridization coming into increasing contact. Our projections indicate that increases in distributional ranges will occur predominantly in northern latitudes, as has been reported in other odonate species in central and northern Europe (Hickling *et al.*, 2005; Sánchez-Guillén, Van Gossum, & Cordero Rivera, 2005; Sánchez-Guillén *et al.*, 2011, 2013). Interestingly, contemporary *Argia* distribution overlap to a great extent, indicating that these species largely share ecological niche characteristics. This suggests that these species show minor interspecific niche differences. In odonates, similar minor niche differences have been implicated to lead to frequent interspecific sexual interactions at a local scale (Wellenreuther, Larson, & Svensson, 2012). Reduced niche differences in odonates have been explained by sexual selection rather than ecological niche selection being the main driver of interspecific divergence in this group (Nava-Bolaños *et al.*, 2016 and (Wellenreuther & Sánchez-Guillén, 2016). In fact, this pattern may seem common in odonates which usually show large differences in secondary sexual traits, but often have smaller interspecific niche differences (Wellenreuther *et al.*, 2012).

Hybridization can be facilitated by changes in potential areas of distribution, particularly when species are closely related (show low interspecific genetic difference). Here we detected 108 species pairs with low genetic distance with respect to the threshold suggested by Sánchez-Guillén et al. (2013) of 1.78% of genetic difference for the COI marker. For example, *A. funcki* and *A. lugens* which have 0.08% of genetic divergence, and currently

share 63.39% of their distribution for *A. funcki*'s suitable area and 7.9% for *A. lugens*' suitable area. Our prediction is that this species pair along those listed in Table 2 are facing a high risk to undergo future hybridization. Therefore, the potential risk of hybridization of *Argia* species is high for many of that studied species. It could be explained because this genus has presented a broadly recent diversification in North America, accompanied by recent genetic diversification. Thus many of these *Argia* species showed low genetic differences and high risk of hybridization. Also, these *Argia* species share many ecological niche characteristics and environmental preferences. Whereby, *Argia* species are present in large sympatric zones with some other *Argia* species in most of the potential distribution area. The risk of these high pattern of hybridization may lead to the breakdown of species groups because of climate change. Also, another evolutionary long-term consequences are changes in the genetic architecture of *Argia* species by introgression between hybrids and parental species increasing diversity, or with possible changes in the stability of parental species and hybrids; ecological competition for the niche preferences; high intensity of sexual selection pressures; and, ultimately, speciation and decreasing diversity with extinction rates.

Spatial overlap can promote the formation of hybrid zones and such hybrid zones have been predicted to increase due to climate change on a global level (Sanchez Guillen et al., 2016). Furthermore, climate change may make some of the current hybrid zones unstable, as seen in some bird species (Engler et al., 2013) in which, new climatic conditions might induce opposite demographic trends in sympatric populations of each species. Recent studies have shown that odonate hybrids of the genus *Ischnura* can be more successful compared to their parental species (Sánchez-Guillén et al., 2016). In the ischnuran example, hybrids have displaced parental species from their original habitats (Sánchez-Guillén et al., 2016). Some outcomes of hybridization can even include speciation, as seen in the successful hybrid speciation process documented in *Argus* butterflies (Ording et al., 2010; Mallet et al., 2011) and fruit flies (Schwarz et al., 2005). In the ischnuran example, hybrids have displaced parental species from their original habitats (Sánchez-Guillén et al., 2016). Of course, results are predictive but some experiment in laboratory conditions can be done in the lab to see how hybrids will perform against their parental species.

Finally, our results can help to direct conservation efforts to understand and protect odonate biodiversity. One example of this is that the role of protected natural areas which were created to embrace biodiversity hot-spots, may not have such a role in the future. Analyses of how future odonate species will move into, not being able to occupy or produce hybrids in these areas will be enlightening.

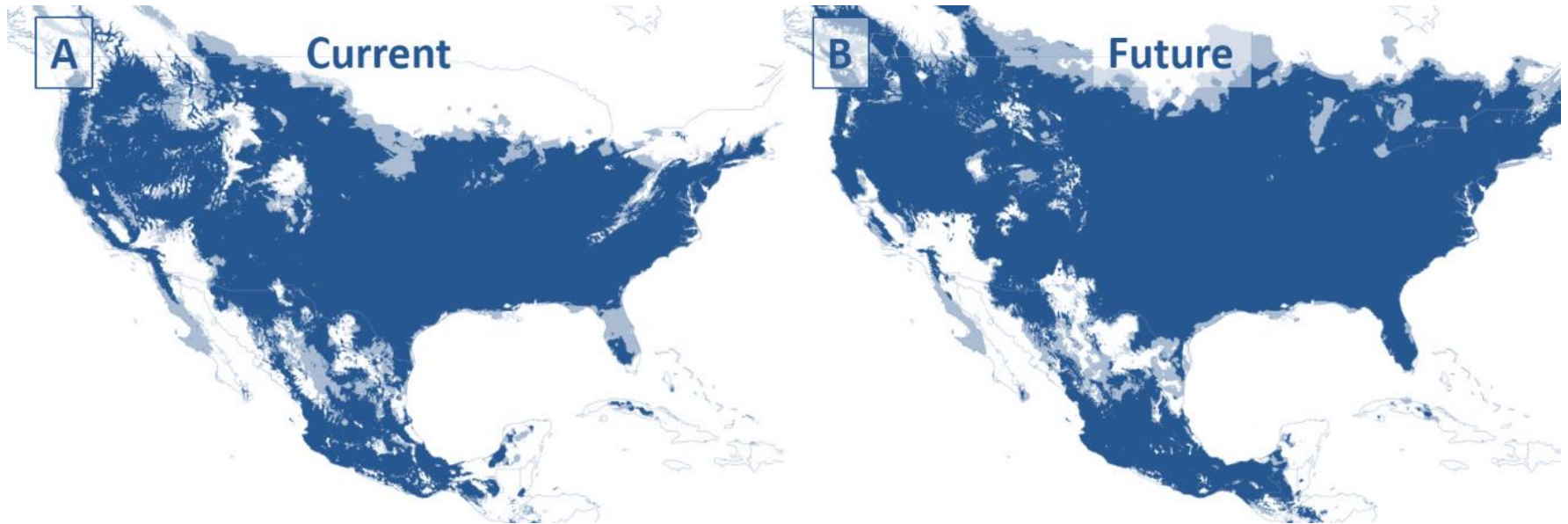
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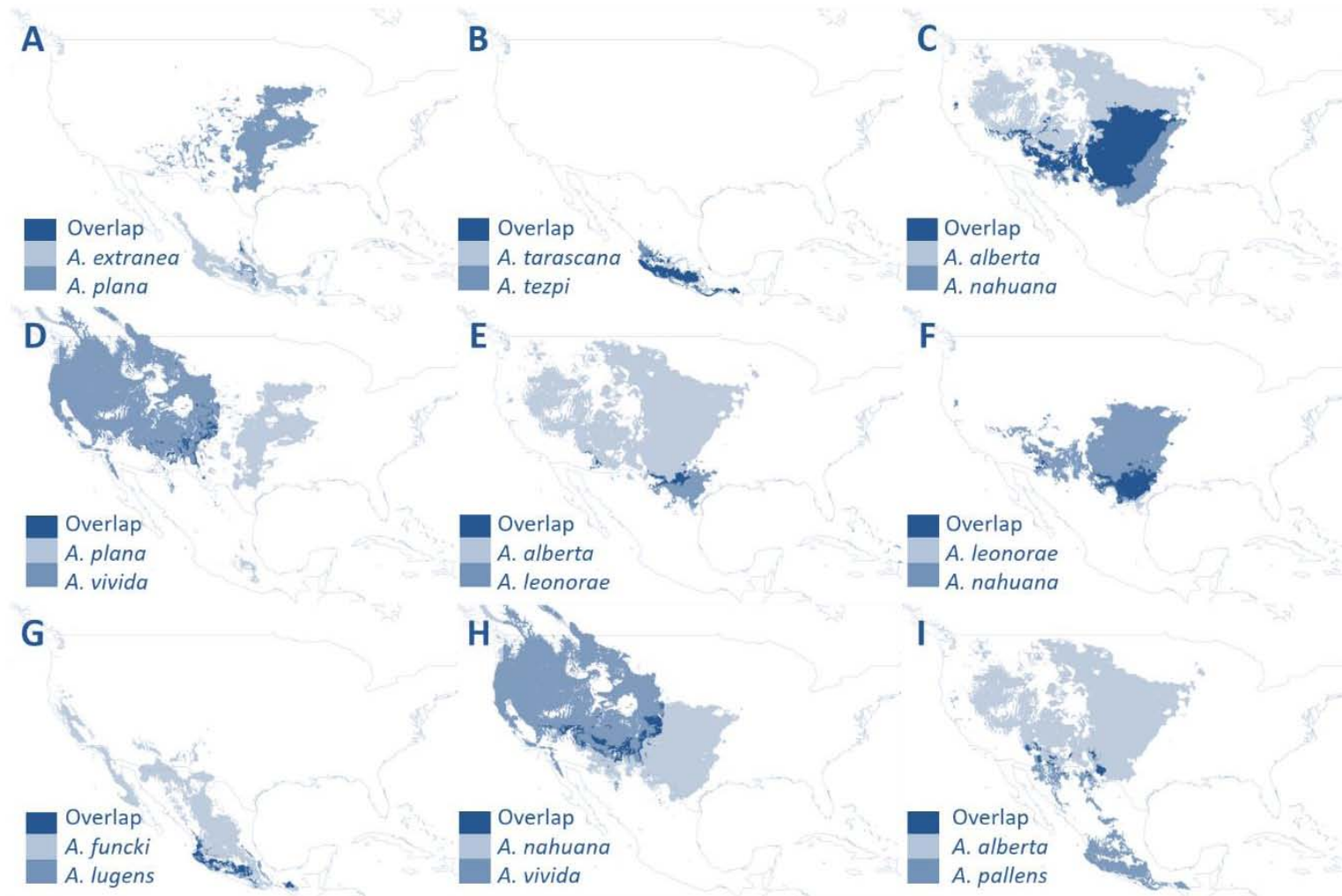


## Figure legends

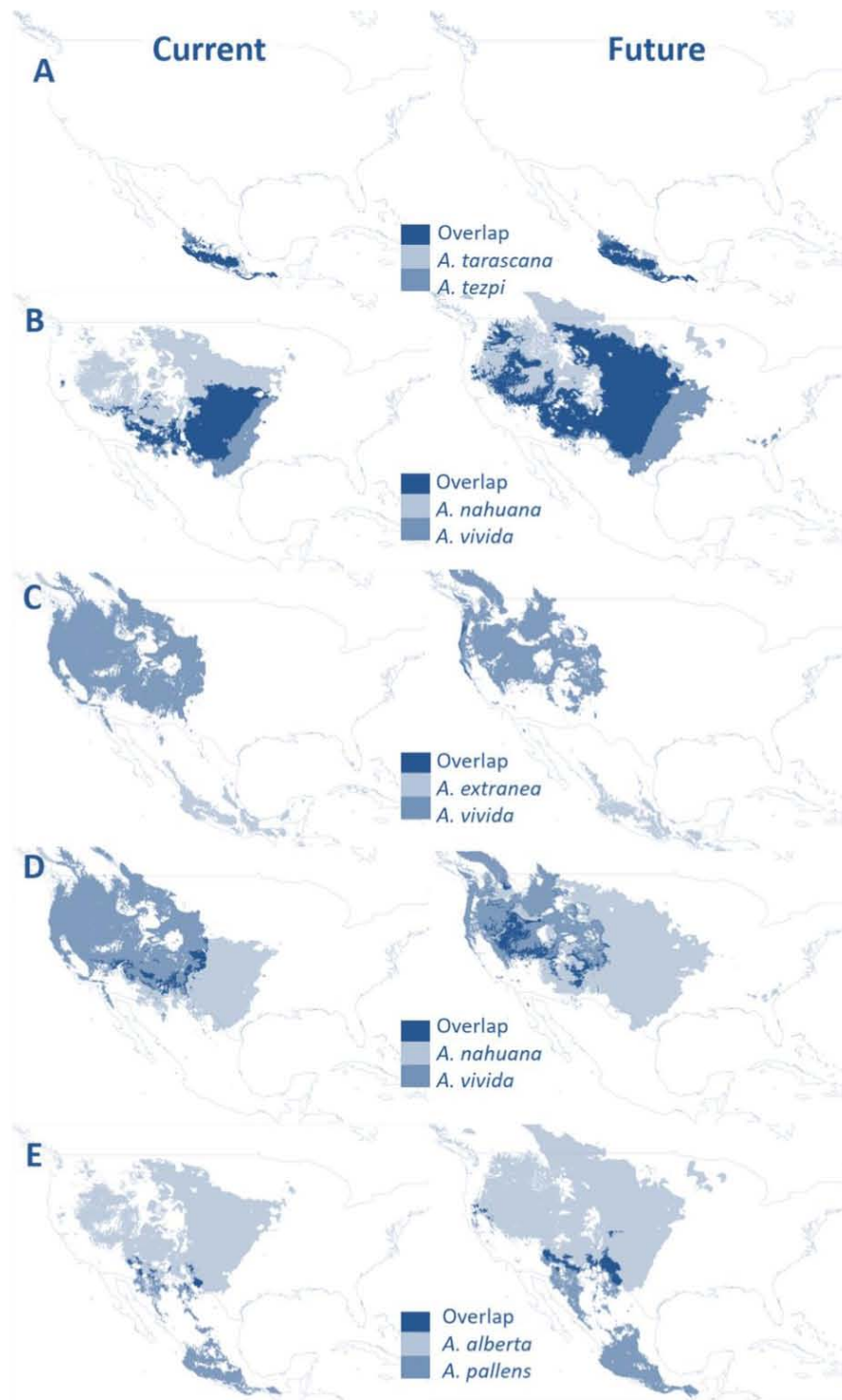
**Figure 1.** A) Current potential sympatric (dark blue) and allopatric (light blue) areas among *Argia* species. B) Future potential sympatric (dark blue) and allopatric (light blue) areas among *Argia* species.



**Figure 2.** Examples of current potential hybrid (i.e. sympatric in darkest blue) and allopatric (light blue) zones appear in different *Argia* species pairs.



**Figure 3.** Examples of future potential hybrid (i.e. sympatric in darkest blue) and allopatric (light blue) zones appear in different *Argia* species pairs. Future was projected for 2070 with RCP8.5 for changes in area of full projections see Table 2.



## Tables

**Table 1.** Modeled *Argia* species, their record number, TSS values of validation, current potential distribution in km<sup>2</sup>, and percentage of change in distribution with respect to the current area using RCP 4.5 and 8.5, to 2050 and 2070 years.

Species	Records	TSS	Current area (km <sup>2</sup> )	Change in future area (%)			
				2050 RCP4.5	2050 RCP8.5	2070 RCP4.5	2070 RCP8.5
<i>A. alberta</i>	1812	0.75	2486397	21	26	23	32
<i>A. anceps</i>	1208	0.89	358411	13	19	21	29
<i>A. apicalis</i>	1080	0.74	2784061	36	45	41	65
<i>A. cuprea</i>	759	0.90	90342	-41	-49	-48	-48
<i>A. emma</i>	437	0.77	2063013	-15	-25	-22	-41
<i>A. extranea</i>	268	0.88	388995	-3	-5	0	0
<i>A. funcki</i>	261	0.85	130143	-9	-10	-7	-20
<i>A. garrisoni</i>	255	0.85	9991	30	103	111	151
<i>A. harknessi</i>	185	0.89	73428	17	48	39	60
<i>A. hinei</i>	167	0.84	915571	-2	-4	-7	-12
<i>A. immunda</i>	119	0.85	893551	69	84	70	92
<i>A. lacrimans</i>	114	0.84	1262049	-7	-7	-7	-20
<i>A. leonora</i>	114	0.88	241601	-2	-32	-29	-56
<i>A. lugens</i>	112	0.76	1040572	-3	-7	-7	-14
<i>A. munda</i>	86	0.89	161815	55	92	79	139
<i>A. nahuana</i>	78	0.83	1342356	51	67	65	105
<i>A. oculata</i>	71	0.88	276273	16	23	27	36
<i>A. oenea</i>	58	0.88	488947	19	33	34	62
<i>A. pallens</i>	57	0.87	533878	43	59	57	78
<i>A. plana</i>	46	0.86	859630	88	104	92	145
<i>A. pulla</i>	42	0.88	310682	29	42	31	61
<i>A. rhoadsi</i>	39	0.89	194479	56	51	47	70
<i>A. sedula</i>	38	0.76	2499501	23	22	23	33
<i>A. tarascana</i>	31	0.87	220928	12	20	17	21
<i>A. tezpi</i>	29	0.88	198298	21	33	30	40
<i>A. tibialis</i>	25	0.80	1936853	23	31	23	40
<i>A. tonto</i>	23	0.87	179823	1	2	5	-9
<i>A. translata</i>	19	0.80	1801334	33	27	29	27
<i>A. ulmeca</i>	16	0.89	173649	26	32	34	60
<i>A. vivida</i>	11	0.77	2224638	-9	-18	-15	-34

**Table 2.** *Argia* species pairs likely to hybridize based on their genetic distance and potential hybrid zones. Current potential hybrid zones are shown in terms of overlapped area (in km<sup>2</sup>) between potential distribution. Percentage of current overlapping is calculated as a proportion of overlapped area with respect to both full areas (species 1 and species 2), percentage of change in future area (using RCP 4.5 and 8.5, to 2050 and 2070 years) with respect to the current overlapping, and genetic distance between species. Information is presented for species with low degree of genetic distance (for the remaining 422 *Argia* species combinations see Supplementary Table S1).

Specie 1	Specie 2	Overlapped area (km <sup>2</sup> )	Percentage of overlapping		Change in future area ( %)					
			From Specie 1	From Specie 2	2050 RCP4.5	2050 RCP8.5	2070 RCP4.5	2070 RCP8.5	16S mtDNA	COI
<i>A. extranea</i>	<i>A. plana</i>	9966	2.56	1.16	7	46	46	-3	0.24	0.06
<i>A. funcki</i>	<i>A. lugens</i>	82503	63.39	7.93	1	-5	-1	-25	1.78	0.08
<i>A. pulla</i>	<i>A. rhoadsi</i>	11326	3.65	5.82	225	268	232	615	2.94	0.1
<i>A. anceps</i>	<i>A. tonto</i>	101	0.03	0.06	-81	-1	-100	-40	5.68	0.1
<i>A. munda</i>	<i>A. tonto</i>	72067	44.54	40.08	27	34	35	28	6.18	0.1
<i>A. hinei</i>	<i>A. rhoadsi</i>	4683	0.51	2.41	421	265	280	180	7.51	0.11
<i>A. hinei</i>	<i>A. tarascana</i>	208	0.02	0.09	-100	-100	-100	-100	6.05	0.14
<i>A. plana</i>	<i>A. rhoadsi</i>	5028	0.58	2.59	-52	-5	5	-8	7.16	0.14
<i>A. extranea</i>	<i>A. rhoadsi</i>	27338	7.03	14.06	22	23	29	39	7.12	0.15
<i>A. cuprea</i>	<i>A. rhoadsi</i>	19886	22.01	10.23	15	29	27	127	9.22	0.15
<i>A. hinei</i>	<i>A. plana</i>	95084	10.39	11.06	2	17	26	7	4.86	0.15
<i>A. plana</i>	<i>A. tarascana</i>	20588	2.39	9.32	-16	1	3	-47	6.02	0.16
<i>A. cuprea</i>	<i>A. hinei</i>	10747	11.9	1.17	-99	-100	-100	-100	8.09	0.17
<i>A. cuprea</i>	<i>A. pulla</i>	45254	50.09	14.57	4	-1	10	23	9.59	0.17
<i>A. plana</i>	<i>A. pulla</i>	2251	0.26	0.72	-53	35	55	-13	8.33	0.17
<i>A. hinei</i>	<i>A. lugens</i>	348105	38.02	33.45	-30	-38	-38	-54	7.39	0.18

<i>A. plana</i>	<i>A. sedula</i>	482773	56.16	19.31	35	44	28	71	9.1	0.19
<i>A. lugens</i>	<i>A. rhoadsi</i>	389	0.04	0.2	161	-100	-45	-95	10.97	0.19
<i>A. rhoadsi</i>	<i>A. translata</i>	61401	31.57	3.41	114	139	112	184	6.06	0.19
<i>A. rhoadsi</i>	<i>A. ulmeca</i>	29826	15.34	17.18	102	140	134	267	7.67	0.2
<i>A. cuprea</i>	<i>A. plana</i>	17590	19.47	2.05	-90	-77	-76	-78	8.46	0.2
<i>A. cuprea</i>	<i>A. oenea</i>	56976	63.07	11.65	-2	-5	1	16	4.25	0.21
<i>A. hinei</i>	<i>A. translata</i>	51400	5.61	2.85	34	5	4	-2	7.16	0.21
<i>A. extranea</i>	<i>A. tarascana</i>	153388	39.43	69.43	11	16	16	10	5.69	0.21
<i>A. cuprea</i>	<i>A. tarascana</i>	383	0.42	0.17	-47	-84	-37	-100	8.47	0.21
<i>A. extranea</i>	<i>A. pulla</i>	217422	55.89	69.98	-1	-1	1	-4	8.28	0.21
<i>A. oculata</i>	<i>A. ulmeca</i>	148755	53.84	85.66	18	22	30	36	5.59	0.22
<i>A. cuprea</i>	<i>A. tezpi</i>	2240	2.48	1.13	-86	-89	-75	-100	8.46	0.22
<i>A. cuprea</i>	<i>A. oculata</i>	57241	63.36	20.72	-1	-5	1	14	7.13	0.22
<i>A. cuprea</i>	<i>A. translata</i>	48921	54.15	2.72	-21	-23	-18	3	6.72	0.22
<i>A. extranea</i>	<i>A. tezpi</i>	138525	35.61	69.86	9	15	15	5	5.69	0.22
<i>A. oculata</i>	<i>A. oenea</i>	262499	95.01	53.69	18	27	30	42	4.5	0.22
<i>A. extranea</i>	<i>A. sedula</i>	12452	3.2	0.5	-20	-10	-8	-22	9	0.22
<i>A. extranea</i>	<i>A. translata</i>	42555	10.94	2.36	-5	-17	0	-18	6.86	0.22
<i>A. funcki</i>	<i>A. pulla</i>	101100	77.68	32.54	-5	-9	-5	-28	11.69	0.22
<i>A. sedula</i>	<i>A. ulmeca</i>	14912	0.6	8.59	6	25	27	32	8.74	0.23
<i>A. plana</i>	<i>A. tezpi</i>	1703	0.2	0.86	-69	-47	-58	-99	6.02	0.23
<i>A. tarascana</i>	<i>A. ulmeca</i>	13448	6.09	7.74	17	12	16	-15	6.38	0.23
<i>A. cuprea</i>	<i>A. lugens</i>	563	0.62	0.05	-64	-89	-93	-100	6.84	0.23
<i>A. oculata</i>	<i>A. tarascana</i>	97675	35.35	44.21	19	23	21	24	7.11	0.23
<i>A. tezpi</i>	<i>A. translata</i>	731	0.37	0.04	-100	-100	-100	-100	8.35	0.23
<i>A. plana</i>	<i>A. translata</i>	391092	45.5	21.71	15	14	5	35	6.92	0.23
<i>A. tarascana</i>	<i>A. tezpi</i>	139119	62.97	70.16	27	42	35	47	0.49	0.23
<i>A. funcki</i>	<i>A. rhoadsi</i>	4006	3.08	2.06	-20	-18	-17	-35	11.46	0.23
<i>A. oenea</i>	<i>A. tarascana</i>	171589	35.09	77.67	22	32	29	41	6.47	0.23

<i>A. lugens</i>	<i>A. plana</i>	54179	5.21	6.3	-18	1	-7	-24	8.91	0.23
<i>A. oenea</i>	<i>A. rhoadsi</i>	27321	5.59	14.05	115	155	156	311	7.16	0.24
<i>A. oculata</i>	<i>A. translata</i>	45917	16.62	2.55	77	95	94	147	6.07	0.24
<i>A. rhoadsi</i>	<i>A. sedula</i>	102288	52.6	4.09	83	68	54	52	6.13	0.24
<i>A. pulla</i>	<i>A. ulmeca</i>	103002	33.15	59.32	32	40	30	58	9.66	0.24
<i>A. pulla</i>	<i>A. translata</i>	34033	10.95	1.89	120	132	106	186	9.15	0.24
<i>A. oenea</i>	<i>A. ulmeca</i>	150280	30.74	86.54	23	31	33	55	4.91	0.24
<i>A. hinei</i>	<i>A. sedula</i>	73268	8	2.93	31	23	27	29	8.53	0.24
<i>A. oculata</i>	<i>A. rhoadsi</i>	28486	10.31	14.65	94	122	120	256	8.77	0.24
<i>A. lugens</i>	<i>A. pulla</i>	145585	13.99	46.86	27	34	29	44	10.72	0.24
<i>A. cuprea</i>	<i>A. ulmeca</i>	58348	64.59	33.6	1	-1	2	26	5.58	0.24
<i>A. cuprea</i>	<i>A. extranea</i>	51897	57.44	13.34	-12	-23	-12	-20	8.1	0.24
<i>A. lugens</i>	<i>A. tezpi</i>	131097	12.6	66.11	33	44	38	50	7.83	0.25
<i>A. pulla</i>	<i>A. tarascana</i>	144726	46.58	65.51	25	36	32	45	9.8	0.25
<i>A. translata</i>	<i>A. ulmeca</i>	46754	2.6	26.92	94	117	103	158	5.36	0.25
<i>A. pulla</i>	<i>A. sedula</i>	3818	1.23	0.15	-23	-4	24	-6	7.66	0.25
<i>A. hinei</i>	<i>A. oenea</i>	3071	0.34	0.63	56	40	41	66	6.64	0.26
<i>A. extranea</i>	<i>A. oenea</i>	327799	84.27	67.04	-5	-7	-2	-5	5.04	0.26
<i>A. extranea</i>	<i>A. lugens</i>	197424	50.75	18.97	16	14	16	17	8.55	0.26
<i>A. lugens</i>	<i>A. tarascana</i>	180047	17.3	81.5	17	25	24	26	7.83	0.26
<i>A. oenea</i>	<i>A. translata</i>	46548	9.52	2.58	89	116	99	165	5.37	0.26
<i>A. sedula</i>	<i>A. tezpi</i>	129	0.01	0.07	-100	-100	-100	-100	10.95	0.27
<i>A. sedula</i>	<i>A. translata</i>	1596645	63.88	88.64	38	34	35	34	9.14	0.27
<i>A. hinei</i>	<i>A. tezpi</i>	387	0.04	0.2	-100	-100	-100	-100	6.05	0.27
<i>A. lugens</i>	<i>A. oenea</i>	205562	19.75	42.04	38	45	46	63	5.85	0.27
<i>A. cuprea</i>	<i>A. sedula</i>	29016	32.12	1.16	-79	-75	-75	-75	9.45	0.28
<i>A. oenea</i>	<i>A. tezpi</i>	191481	39.16	96.56	20	31	29	38	6.47	0.28
<i>A. oculata</i>	<i>A. pulla</i>	198025	71.68	63.74	26	35	32	54	9.92	0.28
<i>A. oenea</i>	<i>A. pulla</i>	284450	58.18	91.56	27	40	32	60	7.44	0.28

<i>A. funcki</i>	<i>A. plana</i>	1708	1.31	0.2	-65	-30	-38	-91	9.85	0.28
<i>A. lugens</i>	<i>A. sedula</i>	203	0.02	0.01	175	334	689	1766	10.84	0.28
<i>A. plana</i>	<i>A. ulmeca</i>	3404	0.4	1.96	-32	38	34	34	6.97	0.29
<i>A. funcki</i>	<i>A. translata</i>	4981	3.83	0.28	-10	-10	-11	-29	8.48	0.29
<i>A. funcki</i>	<i>A. oculata</i>	93692	71.99	33.91	-13	-17	-14	-34	7.81	0.29
<i>A. oculata</i>	<i>A. tezpi</i>	130599	47.27	65.86	16	22	21	24	7.11	0.29
<i>A. funcki</i>	<i>A. ulmeca</i>	39538	30.38	22.77	-24	-30	-24	-46	7.45	0.3
<i>A. tezpi</i>	<i>A. ulmeca</i>	33687	16.99	19.4	9	6	12	-5	6.38	0.3
<i>A. lugens</i>	<i>A. ulmeca</i>	31851	3.06	18.34	4	-13	-4	-30	6.12	0.3
<i>A. funcki</i>	<i>A. tarascana</i>	80819	62.1	36.58	-3	-5	-1	-22	8.76	0.31
<i>A. oenea</i>	<i>A. plana</i>	5861	1.2	0.68	-39	21	4	1	5.36	0.31
<i>A. extranea</i>	<i>A. ulmeca</i>	146277	37.6	84.24	-14	-22	-12	-20	6.91	0.32
<i>A. oculata</i>	<i>A. plana</i>	4118	1.49	0.48	-34	28	21	11	5.68	0.32
<i>A. pulla</i>	<i>A. tezpi</i>	179211	57.68	90.37	25	35	33	43	10.69	0.32
<i>A. funcki</i>	<i>A. oenea</i>	111337	85.55	22.77	-8	-13	-8	-31	7.66	0.32
<i>A. funcki</i>	<i>A. sedula</i>	160	0.12	0.01	-100	-100	-100	-100	12.37	0.33
<i>A. funcki</i>	<i>A. tezpi</i>	82471	63.37	41.59	2	0	2	-26	8.76	0.33
<i>A. cuprea</i>	<i>A. funcki</i>	13473	14.91	10.35	-31	-39	-28	-51	8.7	0.33
<i>A. oenea</i>	<i>A. sedula</i>	12503	2.56	0.5	8	44	41	62	8.22	0.34
<i>A. extranea</i>	<i>A. funcki</i>	106436	27.36	81.78	-15	-19	-12	-36	9.47	0.35
<i>A. oculata</i>	<i>A. sedula</i>	13978	5.06	0.56	4	28	33	32	10.53	0.35
<i>A. lugens</i>	<i>A. oculata</i>	102900	9.89	37.25	22	21	20	20	6.84	0.36
<i>A. extranea</i>	<i>A. oculata</i>	220393	56.66	79.77	-6	-10	-4	-17	5.36	0.36



## Supplementary material

### Supplementary Table S1

Comparison of genetic distance and potential hybrid zones among *Argia* species pairs. Current potential hybrid zones are shown in terms of overlapped area (in km<sup>2</sup>). Percentage of current overlapping is calculated as a proportion of overlapped area with respect to both full areas (between species 1 and species 2), percentage of change in future area (using RCP 4.5 and 8.5, to 2050 and 2070 years) with respect to the current overlapping, and genetic distance between species.

Specie 1	Specie 2	Overlapped area (km <sup>2</sup> )	Percentage of overlapping		Change in future area ( %)				Genetic Distance		
			From Specie 1	From Specie 2	2050 RCP4.5	2050 RCP8.5	2070 RCP4.5	2070 RCP8.5	16S mtDNA	16S rRNA	COI
<i>A. alberta</i>	<i>A. anceps</i>	0	0.00	0.00	0	0	0	0	5.17	NA	NA
<i>A. alberta</i>	<i>A. apicalis</i>	391608	15.75	14.07	42	45	53	42	4.36	NA	NA
<i>A. alberta</i>	<i>A. cuprea</i>	1248	0.05	1.38	-100	-100	-100	-100	6.13	NA	NA
<i>A. alberta</i>	<i>A. emma</i>	1343629	54.04	65.13	-13	-25	-22	-42	3.87	NA	NA
<i>A. alberta</i>	<i>A. extranea</i>	0	0.00	0.00	250	0	0	0	2.34	NA	11.30
<i>A. alberta</i>	<i>A. funcki</i>	0	0.00	0.00	0	0	0	0	8.18	NA	NA
<i>A. alberta</i>	<i>A. garrisoni</i>	0	0.00	0.00	0	0	0	0	6.19	NA	NA
<i>A. alberta</i>	<i>A. harknessi</i>	0	0.00	0.00	0	0	0	0	4.73	NA	NA
<i>A. alberta</i>	<i>A. hinei</i>	395786	15.92	43.23	2	5	-2	-1	3.92	NA	NA
<i>A. alberta</i>	<i>A. immunda</i>	311255	12.52	34.83	70	85	74	110	5.85	NA	NA
<i>A. alberta</i>	<i>A. lacrimans</i>	544450	21.90	43.14	4	11	8	5	4.07	NA	NA
<i>A. alberta</i>	<i>A. leonorae</i>	52514	2.11	21.74	-30	-63	-55	-79	0.73	NA	NA
<i>A. alberta</i>	<i>A. lugens</i>	54462	2.19	5.23	13	11	8	27	7.33	NA	NA
<i>A. alberta</i>	<i>A. munda</i>	45727	1.84	28.26	126	196	184	353	3.19	NA	NA
<i>A. alberta</i>	<i>A. nahuana</i>	906625	36.46	67.54	55	79	72	128	0.73	NA	NA
<i>A. alberta</i>	<i>A. oculata</i>	0	0.00	0.00	0	0	0	0	5.04	NA	NA

<i>A. alberta</i>	<i>A. oenea</i>	420	0.02	0.09	358	89	210	216	4.07	NA	NA
<i>A. alberta</i>	<i>A. pallens</i>	38062	1.53	7.13	157	186	210	293	2.07	NA	NA
<i>A. alberta</i>	<i>A. plana</i>	436328	17.55	50.76	109	120	125	148	2.61	NA	NA
<i>A. alberta</i>	<i>A. pulla</i>	0	0.00	0.00	0	0	0	0	7.84	NA	NA
<i>A. alberta</i>	<i>A. rhoadsi</i>	75	0.00	0.04	-100	-100	247	904	6.03	NA	NA
<i>A. alberta</i>	<i>A. sedula</i>	255925	10.29	10.24	20	28	24	36	7.75	NA	NA
<i>A. alberta</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	4.36	NA	NA
<i>A. alberta</i>	<i>A. tezpi</i>	92	0.00	0.05	-100	-100	-100	-100	4.36	NA	NA
<i>A. alberta</i>	<i>A. tibialis</i>	33481	1.35	1.73	81	88	60	19	5.34	NA	NA
<i>A. alberta</i>	<i>A. tonto</i>	52245	2.10	29.05	39	59	70	53	4.77	NA	NA
<i>A. alberta</i>	<i>A. translata</i>	137981	5.55	7.66	3	-6	-7	3	5.77	NA	NA
<i>A. alberta</i>	<i>A. ulmeca</i>	0	0.00	0.00	617	350	650	513	5.80	NA	NA
<i>A. alberta</i>	<i>A. vivida</i>	1236805	49.74	55.60	10	4	5	-13	2.34	NA	NA
<i>A. anceps</i>	<i>A. apicalis</i>	0	0.00	0.00	0	0	0	0	7.97	NA	NA
<i>A. anceps</i>	<i>A. cuprea</i>	23744	6.62	26.28	-20	-10	-3	6	9.85	NA	NA
<i>A. anceps</i>	<i>A. emma</i>	0	0.00	0.00	185	34	101	0	8.10	NA	NA
<i>A. anceps</i>	<i>A. extranea</i>	272022	75.90	69.93	3	4	7	6	5.86	NA	NA
<i>A. anceps</i>	<i>A. funcki</i>	106628	29.75	81.93	-11	-16	-9	-32	13.68	NA	9.90
<i>A. anceps</i>	<i>A. garrisoni</i>	9668	2.70	96.76	-2	49	50	47	9.76	NA	NA
<i>A. anceps</i>	<i>A. harknessi</i>	55359	15.45	75.39	19	53	48	69	7.68	NA	NA
<i>A. anceps</i>	<i>A. hinei</i>	273	0.08	0.03	-100	-86	-100	-86	8.38	NA	5.61
<i>A. anceps</i>	<i>A. immunda</i>	28930	8.07	3.24	18	14	10	8	11.37	NA	NA
<i>A. anceps</i>	<i>A. lacrimans</i>	162321	45.29	12.86	-2	-5	-4	-14	5.01	NA	NA
<i>A. anceps</i>	<i>A. leonorae</i>	0	0.00	0.00	0	0	0	0	4.86	NA	NA
<i>A. anceps</i>	<i>A. lugens</i>	313659	87.51	30.14	13	12	12	14	11.94	NA	8.38
<i>A. anceps</i>	<i>A. munda</i>	40	0.01	0.02	351	1499	549	1499	7.32	NA	0.16
<i>A. anceps</i>	<i>A. nahuana</i>	223	0.06	0.02	-73	-82	-82	-100	4.81	NA	NA
<i>A. anceps</i>	<i>A. oculata</i>	171304	47.80	62.01	8	13	16	17	9.67	NA	8.09
<i>A. anceps</i>	<i>A. oenea</i>	275625	76.90	56.37	19	28	31	40	8.10	NA	7.07

<i>A. anceps</i>	<i>A. pallens</i>	268018	74.78	50.20	21	25	25	28	5.17	NA	NA
<i>A. anceps</i>	<i>A. plana</i>	20008	5.58	2.33	10	37	28	-14	6.18	NA	6.33
<i>A. anceps</i>	<i>A. pulla</i>	187316	52.26	60.29	12	21	20	30	8.99	NA	6.41
<i>A. anceps</i>	<i>A. rhoadsi</i>	15144	4.23	7.79	36	52	52	80	6.71	NA	7.08
<i>A. anceps</i>	<i>A. sedula</i>	6640	1.85	0.27	9	56	50	52	7.50	NA	8.05
<i>A. anceps</i>	<i>A. tarascana</i>	176737	49.31	80.00	13	22	19	21	7.16	NA	6.94
<i>A. anceps</i>	<i>A. tezpi</i>	150225	41.91	75.76	17	28	25	30	7.97	NA	15.72
<i>A. anceps</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	9.42	NA	NA
<i>A. anceps</i>	<i>A. tonto</i>	101	0.03	0.06	-81	-1	-100	-40	5.68	NA	0.10
<i>A. anceps</i>	<i>A. translata</i>	17008	4.75	0.94	1	28	27	33	7.92	NA	8.93
<i>A. anceps</i>	<i>A. ulmeca</i>	81907	22.85	47.17	-1	-1	9	2	10.23	NA	10.57
<i>A. anceps</i>	<i>A. vivida</i>	0	0.00	0.00	590	387	539	1009	6.25	NA	NA
<i>A. apicalis</i>	<i>A. cuprea</i>	11850	0.43	13.12	-95	-96	-100	-100	8.46	NA	6.18
<i>A. apicalis</i>	<i>A. emma</i>	0	0.00	0.00	108	1090	230	768	6.46	NA	NA
<i>A. apicalis</i>	<i>A. extranea</i>	0	0.00	0.00	0	0	0	0	5.69	NA	NA
<i>A. apicalis</i>	<i>A. funcki</i>	0	0.00	0.00	0	0	0	0	8.76	NA	NA
<i>A. apicalis</i>	<i>A. garrisoni</i>	0	0.00	0.00	0	0	0	0	8.84	NA	NA
<i>A. apicalis</i>	<i>A. harknessi</i>	0	0.00	0.00	0	0	0	0	6.86	NA	NA
<i>A. apicalis</i>	<i>A. hinei</i>	7816	0.28	0.85	35	-47	24	-73	6.05	NA	NA
<i>A. apicalis</i>	<i>A. immunda</i>	450378	16.18	50.40	89	106	95	96	6.91	NA	NA
<i>A. apicalis</i>	<i>A. lacrimans</i>	18	0.00	0.00	-100	-100	203	-100	6.34	NA	NA
<i>A. apicalis</i>	<i>A. leonora</i>	89695	3.22	37.13	9	-17	2	-22	4.70	NA	NA
<i>A. apicalis</i>	<i>A. lugens</i>	0	0.00	0.00	0	0	0	0	7.83	NA	NA
<i>A. apicalis</i>	<i>A. munda</i>	0	0.00	0.00	0	0	0	0	6.36	NA	NA
<i>A. apicalis</i>	<i>A. nahuana</i>	505516	18.16	37.66	56	61	70	84	4.05	NA	NA
<i>A. apicalis</i>	<i>A. oculata</i>	0	0.00	0.00	0	0	0	0	7.11	NA	NA
<i>A. apicalis</i>	<i>A. oenea</i>	0	0.00	0.00	0	0	0	0	6.47	NA	NA
<i>A. apicalis</i>	<i>A. pallens</i>	0	0.00	0.00	0	0	0	0	4.68	NA	NA
<i>A. apicalis</i>	<i>A. plana</i>	644238	23.14	74.94	79	90	77	131	6.02	NA	NA

<i>A. apicalis</i>	<i>A. pulla</i>	0	0.00	0.00	0	0	0	0	10.69	NA	NA
<i>A. apicalis</i>	<i>A. rhoadsi</i>	19582	0.70	10.07	-46	-66	-51	-75	8.62	NA	NA
<i>A. apicalis</i>	<i>A. sedula</i>	2191565	78.72	87.68	25	23	24	33	10.95	NA	NA
<i>A. apicalis</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	0.49	NA	NA
<i>A. apicalis</i>	<i>A. tezpi</i>	0	0.00	0.00	0	0	0	0	0.00	NA	NA
<i>A. apicalis</i>	<i>A. tibialis</i>	1898954	68.21	98.04	25	33	26	43	4.37	NA	NA
<i>A. apicalis</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	7.44	NA	NA
<i>A. apicalis</i>	<i>A. translata</i>	1578730	56.71	87.64	36	29	32	27	8.35	NA	NA
<i>A. apicalis</i>	<i>A. ulmeca</i>	0	0.00	0.00	0	0	0	0	6.38	NA	NA
<i>A. apicalis</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	5.69	NA	NA
<i>A. cuprea</i>	<i>A. emma</i>	0	0.00	0.00	106	574	207	2740	9.09	NA	NA
<i>A. cuprea</i>	<i>A. extranea</i>	51897	57.44	13.34	-12	-23	-12	-20	8.10	NA	0.24
<i>A. cuprea</i>	<i>A. funcki</i>	13473	14.91	10.35	-31	-39	-28	-51	8.70	NA	0.33
<i>A. cuprea</i>	<i>A. garrisoni</i>	9698	10.73	97.07	29	52	74	173	6.38	NA	NA
<i>A. cuprea</i>	<i>A. harknessi</i>	2060	2.28	2.81	-84	-88	-73	-100	2.93	NA	NA
<i>A. cuprea</i>	<i>A. hinei</i>	10747	11.90	1.17	-99	-100	-100	-100	8.09	NA	0.17
<i>A. cuprea</i>	<i>A. immunda</i>	46717	51.71	5.23	-31	-38	-31	-10	9.73	0.05	NA
<i>A. cuprea</i>	<i>A. lacrimans</i>	2899	3.21	0.23	-19	26	38	-7	8.90	NA	NA
<i>A. cuprea</i>	<i>A. leonora</i>	21557	23.86	8.92	-97	-98	-100	-100	6.18	NA	NA
<i>A. cuprea</i>	<i>A. lugens</i>	563	0.62	0.05	-64	-89	-93	-100	6.84	NA	0.23
<i>A. cuprea</i>	<i>A. munda</i>	0	0.00	0.00	0	0	0	0	8.39	NA	NA
<i>A. cuprea</i>	<i>A. nahuana</i>	21595	23.90	1.61	-97	-98	-100	-100	5.76	NA	6.39
<i>A. cuprea</i>	<i>A. oculata</i>	57241	63.36	20.72	-1	-5	1	14	7.13	0.04	0.22
<i>A. cuprea</i>	<i>A. oenea</i>	56976	63.07	11.65	-2	-5	1	16	4.25	0.03	0.21
<i>A. cuprea</i>	<i>A. pallens</i>	1123	1.24	0.21	-77	-89	-61	-100	8.64	NA	NA
<i>A. cuprea</i>	<i>A. plana</i>	17590	19.47	2.05	-90	-77	-76	-78	8.46	NA	0.20
<i>A. cuprea</i>	<i>A. pulla</i>	45254	50.09	14.57	4	-1	10	23	9.59	0.08	0.17
<i>A. cuprea</i>	<i>A. rhoadsi</i>	19886	22.01	10.23	15	29	27	127	9.22	0.07	0.15
<i>A. cuprea</i>	<i>A. sedula</i>	29016	32.12	1.16	-79	-75	-75	-75	9.45	NA	0.28

<i>A. cuprea</i>	<i>A. tarascana</i>	383	0.42	0.17	-47	-84	-37	-100	8.47	NA	0.21
<i>A. cuprea</i>	<i>A. tezpi</i>	2240	2.48	1.13	-86	-89	-75	-100	8.46	0.07	0.22
<i>A. cuprea</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	11.20	NA	NA
<i>A. cuprea</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	9.27	NA	7.29
<i>A. cuprea</i>	<i>A. translata</i>	48921	54.15	2.72	-21	-23	-18	3	6.72	NA	0.22
<i>A. cuprea</i>	<i>A. ulmeca</i>	58348	64.59	33.60	1	-1	2	26	5.58	0.03	0.24
<i>A. cuprea</i>	<i>A. vivida</i>	61	0.07	0.00	563	1539	509	5434	8.10	NA	NA
<i>A. emma</i>	<i>A. extranea</i>	17	0.00	0.00	23060	23545	21881	39956	4.86	NA	NA
<i>A. emma</i>	<i>A. funcki</i>	0	0.00	0.00	67	0	0	0	6.90	NA	NA
<i>A. emma</i>	<i>A. garrisoni</i>	0	0.00	0.00	0	229	0	46	7.82	NA	NA
<i>A. emma</i>	<i>A. harknessi</i>	0	0.00	0.00	0	0	0	0	5.80	NA	NA
<i>A. emma</i>	<i>A. hinei</i>	214181	10.38	23.39	-19	-21	-23	-38	6.24	NA	NA
<i>A. emma</i>	<i>A. immunda</i>	0	0.00	0.00	0	0	0	0	9.08	NA	NA
<i>A. emma</i>	<i>A. lacrimans</i>	338514	16.41	26.82	-20	-26	-25	-45	6.45	NA	NA
<i>A. emma</i>	<i>A. leonorae</i>	0	0.00	0.00	0	0	0	0	4.86	NA	NA
<i>A. emma</i>	<i>A. lugens</i>	95907	4.65	9.22	-34	-45	-46	-54	8.02	NA	NA
<i>A. emma</i>	<i>A. munda</i>	13903	0.67	8.59	-60	-73	-82	-92	5.85	NA	NA
<i>A. emma</i>	<i>A. nahuana</i>	59322	2.88	4.42	-18	-51	-41	-59	4.12	NA	NA
<i>A. emma</i>	<i>A. oculata</i>	0	0.00	0.00	99	49	116	132	6.90	NA	NA
<i>A. emma</i>	<i>A. oenea</i>	17	0.00	0.00	35806	15320	24902	27062	5.43	NA	NA
<i>A. emma</i>	<i>A. pallens</i>	3143	0.15	0.59	28	13	-35	-67	5.17	NA	NA
<i>A. emma</i>	<i>A. plana</i>	48749	2.36	5.67	64	42	39	5	5.16	NA	NA
<i>A. emma</i>	<i>A. pulla</i>	0	0.00	0.00	0	0	0	0	8.17	NA	NA
<i>A. emma</i>	<i>A. rhoadsi</i>	518	0.03	0.27	103	413	97	726	8.28	NA	NA
<i>A. emma</i>	<i>A. sedula</i>	0	0.00	0.00	0	65	16	181	9.19	NA	NA
<i>A. emma</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	6.47	NA	NA
<i>A. emma</i>	<i>A. tezpi</i>	0	0.00	0.00	0	0	0	0	6.46	NA	NA
<i>A. emma</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	6.04	NA	NA
<i>A. emma</i>	<i>A. tonto</i>	74150	3.59	41.23	-24	-41	-40	-70	6.90	NA	NA

<i>A. emma</i>	<i>A. translata</i>	0	0.00	0.00	33	274	212	630	8.19	NA	NA
<i>A. emma</i>	<i>A. ulmeca</i>	333	0.02	0.19	1833	2880	2020	5866	6.96	NA	NA
<i>A. emma</i>	<i>A. vivida</i>	1672140	81.05	75.16	-10	-22	-19	-40	4.86	NA	NA
<i>A. extranea</i>	<i>A. funcki</i>	106436	27.36	81.78	-15	-19	-12	-36	9.47	NA	0.35
<i>A. extranea</i>	<i>A. garrisoni</i>	9738	2.50	97.47	22	43	57	32	6.51	NA	NA
<i>A. extranea</i>	<i>A. harknessi</i>	46266	11.89	63.01	3	29	26	25	5.40	NA	NA
<i>A. extranea</i>	<i>A. hinei</i>	18	0.00	0.00	-100	-100	-100	-100	4.56	NA	0.17
<i>A. extranea</i>	<i>A. immunda</i>	35117	9.03	3.93	4	-12	4	-10	7.39	NA	NA
<i>A. extranea</i>	<i>A. lacrimans</i>	85048	21.86	6.74	5	-1	6	1	4.42	NA	NA
<i>A. extranea</i>	<i>A. leonorae</i>	0	0.00	0.00	0	0	0	0	2.66	NA	NA
<i>A. extranea</i>	<i>A. lugens</i>	197424	50.75	18.97	16	14	16	17	8.55	NA	0.26
<i>A. extranea</i>	<i>A. munda</i>	18	0.00	0.01	-100	-100	-100	-100	3.54	NA	5.74
<i>A. extranea</i>	<i>A. nahuana</i>	18	0.00	0.00	-100	-100	-100	-100	2.05	NA	NA
<i>A. extranea</i>	<i>A. oculata</i>	220393	56.66	79.77	-6	-10	-4	-17	5.36	NA	0.36
<i>A. extranea</i>	<i>A. oenea</i>	327799	84.27	67.04	-5	-7	-2	-5	5.04	NA	0.26
<i>A. extranea</i>	<i>A. pallens</i>	189981	48.84	35.59	14	13	15	13	3.02	NA	NA
<i>A. extranea</i>	<i>A. plana</i>	9966	2.56	1.16	7	46	46	-3	0.24	NA	0.06
<i>A. extranea</i>	<i>A. pulla</i>	217422	55.89	69.98	-1	-1	1	-4	8.28	NA	0.21
<i>A. extranea</i>	<i>A. rhoadsi</i>	27338	7.03	14.06	22	23	29	39	7.12	NA	0.15
<i>A. extranea</i>	<i>A. sedula</i>	12452	3.20	0.50	-20	-10	-8	-22	9.00	NA	0.22
<i>A. extranea</i>	<i>A. tarascana</i>	153388	39.43	69.43	11	16	16	10	5.69	NA	0.21
<i>A. extranea</i>	<i>A. tezpi</i>	138525	35.61	69.86	9	15	15	5	5.69	NA	0.22
<i>A. extranea</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	5.66	NA	NA
<i>A. extranea</i>	<i>A. tonto</i>	18	0.00	0.01	-100	-100	-100	-100	5.17	NA	5.56
<i>A. extranea</i>	<i>A. translata</i>	42555	10.94	2.36	-5	-17	0	-18	6.86	NA	0.22
<i>A. extranea</i>	<i>A. ulmeca</i>	146277	37.60	84.24	-14	-22	-12	-20	6.91	NA	0.32
<i>A. extranea</i>	<i>A. vivida</i>	67	0.02	0.00	11396	10986	11383	18208	0.98	NA	NA
<i>A. funcki</i>	<i>A. garrisoni</i>	1208	0.93	12.09	-40	-18	-7	-63	9.06	NA	NA
<i>A. funcki</i>	<i>A. harknessi</i>	33893	26.04	46.16	0	11	3	-11	5.59	NA	NA

<i>A. funcki</i>	<i>A. hinei</i>	0	0.00	0.00	0	0	0	0	8.26	NA	0.27
<i>A. funcki</i>	<i>A. immunda</i>	4334	3.33	0.49	-19	-23	-26	-37	12.30	NA	NA
<i>A. funcki</i>	<i>A. lacrimans</i>	24141	18.55	1.91	-16	-25	-13	-38	11.43	NA	NA
<i>A. funcki</i>	<i>A. leonorae</i>	0	0.00	0.00	0	0	0	0	8.73	NA	NA
<i>A. funcki</i>	<i>A. lugens</i>	82503	63.39	7.93	1	-5	-1	-25	1.78	NA	0.08
<i>A. funcki</i>	<i>A. munda</i>	0	0.00	0.00	0	0	0	0	9.74	NA	7.16
<i>A. funcki</i>	<i>A. nahuana</i>	0	0.00	0.00	0	0	0	0	7.75	NA	NA
<i>A. funcki</i>	<i>A. oculata</i>	93692	71.99	33.91	-13	-17	-14	-34	7.81	NA	0.29
<i>A. funcki</i>	<i>A. oenea</i>	111337	85.55	22.77	-8	-13	-8	-31	7.66	NA	0.32
<i>A. funcki</i>	<i>A. pallens</i>	97355	74.81	18.24	-7	-11	-7	-30	8.18	NA	NA
<i>A. funcki</i>	<i>A. plana</i>	1708	1.31	0.20	-65	-30	-38	-91	9.85	NA	0.28
<i>A. funcki</i>	<i>A. pulla</i>	101100	77.68	32.54	-5	-9	-5	-28	11.69	NA	0.22
<i>A. funcki</i>	<i>A. rhoadsi</i>	4006	3.08	2.06	-20	-18	-17	-35	11.46	NA	0.23
<i>A. funcki</i>	<i>A. sedula</i>	160	0.12	0.01	-100	-100	-100	-100	12.37	NA	0.33
<i>A. funcki</i>	<i>A. tarascana</i>	80819	62.10	36.58	-3	-5	-1	-22	8.76	NA	0.31
<i>A. funcki</i>	<i>A. tezpi</i>	82471	63.37	41.59	2	0	2	-26	8.76	NA	0.33
<i>A. funcki</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	12.19	NA	NA
<i>A. funcki</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	12.61	NA	9.99
<i>A. funcki</i>	<i>A. translata</i>	4981	3.83	0.28	-10	-10	-11	-29	8.48	NA	0.29
<i>A. funcki</i>	<i>A. ulmeca</i>	39538	30.38	22.77	-24	-30	-24	-46	7.45	NA	0.30
<i>A. funcki</i>	<i>A. vivida</i>	0	0.00	0.00	34	0	0	0	9.47	NA	NA
<i>A. garrisoni</i>	<i>A. harknessi</i>	0	0.00	0.00	0	0	0	0	4.91	NA	NA
<i>A. garrisoni</i>	<i>A. hinei</i>	0	0.00	0.00	0	0	0	0	7.23	NA	NA
<i>A. garrisoni</i>	<i>A. immunda</i>	9391	93.99	1.05	50	94	115	188	9.08	NA	NA
<i>A. garrisoni</i>	<i>A. lacrimans</i>	561	5.61	0.04	-50	93	123	39	8.82	NA	NA
<i>A. garrisoni</i>	<i>A. leonorae</i>	0	0.00	0.00	0	0	0	0	6.64	NA	NA
<i>A. garrisoni</i>	<i>A. lugens</i>	263	2.64	0.03	-100	-100	-100	-100	8.36	NA	NA
<i>A. garrisoni</i>	<i>A. munda</i>	0	0.00	0.00	0	0	0	0	7.74	NA	NA
<i>A. garrisoni</i>	<i>A. nahuana</i>	0	0.00	0.00	0	0	0	0	5.81	NA	NA

<i>A. garrisoni</i>	<i>A. oculata</i>	9693	97.01	3.51	48	107	130	231	5.74	NA	NA
<i>A. garrisoni</i>	<i>A. oenea</i>	9991	100.00	2.04	53	114	129	243	5.05	NA	NA
<i>A. garrisoni</i>	<i>A. pallens</i>	0	0.00	0.00	0	0	0	0	7.40	NA	NA
<i>A. garrisoni</i>	<i>A. plana</i>	6141	61.46	0.71	-22	60	55	55	6.85	NA	NA
<i>A. garrisoni</i>	<i>A. pulla</i>	2929	29.32	0.94	70	78	118	291	10.95	NA	NA
<i>A. garrisoni</i>	<i>A. rhoadsi</i>	9032	90.40	4.64	83	160	171	313	8.84	NA	NA
<i>A. garrisoni</i>	<i>A. sedula</i>	7393	74.00	0.30	22	101	96	115	9.97	NA	NA
<i>A. garrisoni</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	8.84	NA	NA
<i>A. garrisoni</i>	<i>A. tezpi</i>	0	0.00	0.00	0	0	0	0	8.84	NA	NA
<i>A. garrisoni</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	11.28	NA	NA
<i>A. garrisoni</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	8.02	NA	NA
<i>A. garrisoni</i>	<i>A. translata</i>	9974	99.83	0.55	51	110	125	237	6.36	NA	NA
<i>A. garrisoni</i>	<i>A. ulmeca</i>	9694	97.03	5.58	47	107	126	228	4.20	NA	NA
<i>A. garrisoni</i>	<i>A. vivida</i>	0	0.00	0.00	0	244	0	46	6.51	NA	NA
<i>A. harknessi</i>	<i>A. hinei</i>	0	0.00	0.00	0	0	0	0	6.52	NA	NA
<i>A. harknessi</i>	<i>A. immunda</i>	0	0.00	0.00	0	0	0	0	8.46	NA	NA
<i>A. harknessi</i>	<i>A. lacrimans</i>	2408	3.28	0.19	-11	11	-42	-47	6.46	NA	NA
<i>A. harknessi</i>	<i>A. leonora</i>	0	0.00	0.00	0	0	0	0	5.12	NA	NA
<i>A. harknessi</i>	<i>A. lugens</i>	48466	66.00	4.66	44	75	57	90	5.11	NA	NA
<i>A. harknessi</i>	<i>A. munda</i>	0	0.00	0.00	0	0	0	0	6.05	NA	NA
<i>A. harknessi</i>	<i>A. nahuana</i>	0	0.00	0.00	0	0	0	0	4.40	NA	NA
<i>A. harknessi</i>	<i>A. oculata</i>	46530	63.37	16.84	26	57	58	79	5.22	NA	NA
<i>A. harknessi</i>	<i>A. oenea</i>	69355	94.45	14.18	30	60	53	81	3.29	NA	NA
<i>A. harknessi</i>	<i>A. pallens</i>	64810	88.26	12.14	35	66	56	89	5.81	NA	NA
<i>A. harknessi</i>	<i>A. plana</i>	1143	1.56	0.13	-54	-27	-38	-98	5.72	NA	NA
<i>A. harknessi</i>	<i>A. pulla</i>	68079	92.72	21.91	31	62	55	84	8.66	NA	NA
<i>A. harknessi</i>	<i>A. rhoadsi</i>	0	0.00	0.00	360	621	80	520	7.16	NA	NA
<i>A. harknessi</i>	<i>A. sedula</i>	0	0.00	0.00	0	0	0	0	8.65	NA	NA
<i>A. harknessi</i>	<i>A. tarascana</i>	58690	79.93	26.57	30	59	43	75	6.87	NA	NA



<i>A. harknessi</i>	<i>A. tezpi</i>	69769	95.02	35.18	32	63	56	84	6.86	NA	NA
<i>A. harknessi</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	9.00	NA	NA
<i>A. harknessi</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	7.20	NA	NA
<i>A. harknessi</i>	<i>A. translata</i>	0	0.00	0.00	0	0	0	0	4.51	NA	NA
<i>A. harknessi</i>	<i>A. ulmeca</i>	12379	16.86	7.13	21	68	95	90	4.20	NA	NA
<i>A. harknessi</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	5.76	NA	NA
<i>A. hinei</i>	<i>A. immunda</i>	202350	22.10	22.65	33	36	26	17	8.00	NA	NA
<i>A. hinei</i>	<i>A. lacrimans</i>	691523	75.53	54.79	-11	-7	-10	-20	6.12	NA	NA
<i>A. hinei</i>	<i>A. leonorae</i>	84107	9.19	34.81	21	-26	-18	-64	3.63	NA	NA
<i>A. hinei</i>	<i>A. lugens</i>	348105	38.02	33.45	-30	-38	-38	-54	7.39	NA	0.18
<i>A. hinei</i>	<i>A. munda</i>	143080	15.63	88.42	39	77	56	105	4.91	NA	5.11
<i>A. hinei</i>	<i>A. nahuana</i>	435798	47.60	32.47	2	4	-2	-11	3.58	NA	NA
<i>A. hinei</i>	<i>A. oculata</i>	0	0.00	0.00	0	0	0	0	5.80	NA	0.23
<i>A. hinei</i>	<i>A. oenea</i>	3071	0.34	0.63	56	40	41	66	6.64	NA	0.26
<i>A. hinei</i>	<i>A. pallens</i>	148722	16.24	27.86	44	67	59	82	3.58	NA	NA
<i>A. hinei</i>	<i>A. plana</i>	95084	10.39	11.06	2	17	26	7	4.86	NA	0.15
<i>A. hinei</i>	<i>A. pulla</i>	0	0.00	0.00	0	0	0	0	10.39	NA	0.17
<i>A. hinei</i>	<i>A. rhoadsi</i>	4683	0.51	2.41	421	265	280	180	7.51	NA	0.11
<i>A. hinei</i>	<i>A. sedula</i>	73268	8.00	2.93	31	23	27	29	8.53	NA	0.24
<i>A. hinei</i>	<i>A. tarascana</i>	208	0.02	0.09	-100	-100	-100	-100	6.05	NA	0.14
<i>A. hinei</i>	<i>A. tezpi</i>	387	0.04	0.20	-100	-100	-100	-100	6.05	NA	0.27
<i>A. hinei</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	7.86	NA	NA
<i>A. hinei</i>	<i>A. tonto</i>	157631	17.22	87.66	7	10	9	-6	6.98	NA	6.54
<i>A. hinei</i>	<i>A. translata</i>	51400	5.61	2.85	34	5	4	-2	7.16	NA	0.21
<i>A. hinei</i>	<i>A. ulmeca</i>	0	0.00	0.00	0	0	0	0	6.51	NA	0.23
<i>A. hinei</i>	<i>A. vivida</i>	231011	25.23	10.38	-13	-15	-18	-35	4.56	NA	NA
<i>A. immunda</i>	<i>A. lacrimans</i>	130657	14.62	10.35	79	114	98	116	9.19	NA	NA
<i>A. immunda</i>	<i>A. leonorae</i>	221988	24.84	91.88	-8	-30	-27	-53	6.71	NA	NA
<i>A. immunda</i>	<i>A. lugens</i>	18178	2.03	1.75	24	13	-25	-17	10.52	NA	NA

<i>A. immunda</i>	<i>A. munda</i>	24500	2.74	15.14	162	330	272	547	7.38	NA	NA
<i>A. immunda</i>	<i>A. nahuana</i>	601158	67.28	44.78	60	76	74	100	5.86	NA	NA
<i>A. immunda</i>	<i>A. oculata</i>	35215	3.94	12.75	37	36	47	83	8.08	0.05	NA
<i>A. immunda</i>	<i>A. oenea</i>	39036	4.37	7.98	45	46	54	85	7.19	0.04	NA
<i>A. immunda</i>	<i>A. pallens</i>	32005	3.58	5.99	133	183	168	241	7.47	NA	NA
<i>A. immunda</i>	<i>A. plana</i>	276767	30.97	32.20	95	121	114	135	7.74	NA	NA
<i>A. immunda</i>	<i>A. pulla</i>	23637	2.65	7.61	51	44	56	112	12.56	0.08	NA
<i>A. immunda</i>	<i>A. rhoadsi</i>	127383	14.26	65.50	25	19	10	19	10.32	0.07	NA
<i>A. immunda</i>	<i>A. sedula</i>	596998	66.81	23.88	81	101	88	95	12.60	NA	NA
<i>A. immunda</i>	<i>A. tarascana</i>	1946	0.22	0.88	-55	-65	-57	-75	6.91	NA	NA
<i>A. immunda</i>	<i>A. tezpi</i>	0	0.00	0.00	0	0	0	0	6.91	0.08	NA
<i>A. immunda</i>	<i>A. tibialis</i>	159104	17.81	8.21	125	175	132	104	7.95	NA	NA
<i>A. immunda</i>	<i>A. tonto</i>	2284	0.26	1.27	53	194	65	364	9.27	NA	NA
<i>A. immunda</i>	<i>A. translata</i>	468382	52.42	26.00	94	107	94	107	9.00	NA	NA
<i>A. immunda</i>	<i>A. ulmeca</i>	35727	4.00	20.57	37	34	46	81	8.00	0.05	NA
<i>A. immunda</i>	<i>A. vivida</i>	9806	1.10	0.44	-77	-26	-55	177	7.39	NA	NA
<i>A. lacrimans</i>	<i>A. leonorae</i>	36585	2.90	15.14	2	-25	-12	-74	4.42	NA	NA
<i>A. lacrimans</i>	<i>A. lugens</i>	543613	43.07	52.24	-20	-27	-26	-41	9.97	NA	NA
<i>A. lacrimans</i>	<i>A. munda</i>	148119	11.74	91.54	47	85	65	116	5.39	NA	NA
<i>A. lacrimans</i>	<i>A. nahuana</i>	472987	37.48	35.24	13	19	16	7	3.75	NA	NA
<i>A. lacrimans</i>	<i>A. oculata</i>	20388	1.62	7.38	-5	-12	2	-31	7.50	NA	NA
<i>A. lacrimans</i>	<i>A. oenea</i>	59630	4.72	12.20	33	33	45	57	5.36	NA	NA
<i>A. lacrimans</i>	<i>A. pallens</i>	271299	21.50	50.82	31	48	46	43	4.73	NA	NA
<i>A. lacrimans</i>	<i>A. plana</i>	146084	11.58	16.99	50	78	83	56	4.73	NA	NA
<i>A. lacrimans</i>	<i>A. pulla</i>	27287	2.16	8.78	-3	2	12	-3	9.35	NA	NA
<i>A. lacrimans</i>	<i>A. rhoadsi</i>	2062	0.16	1.06	-20	30	43	20	7.80	NA	NA
<i>A. lacrimans</i>	<i>A. sedula</i>	29211	2.31	1.17	-22	84	91	109	8.75	NA	NA
<i>A. lacrimans</i>	<i>A. tarascana</i>	81636	6.47	36.95	-8	-5	-3	-16	6.34	NA	NA
<i>A. lacrimans</i>	<i>A. tezpi</i>	19892	1.58	10.03	10	14	14	-6	6.34	NA	NA

<i>A. lacrimans</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	7.40	NA	NA
<i>A. lacrimans</i>	<i>A. tonto</i>	162242	12.86	90.22	4	4	6	-16	3.50	NA	NA
<i>A. lacrimans</i>	<i>A. translata</i>	10957	0.87	0.61	-41	-14	-16	-3	7.51	NA	NA
<i>A. lacrimans</i>	<i>A. ulmeca</i>	9710	0.77	5.59	23	0	31	-25	7.54	NA	NA
<i>A. lacrimans</i>	<i>A. vivida</i>	382218	30.29	17.18	-12	-17	-16	-40	3.77	NA	NA
<i>A. leonora</i>	<i>A. lugens</i>	4825	2.00	0.46	61	23	-43	-69	7.83	NA	NA
<i>A. leonora</i>	<i>A. munda</i>	11979	4.96	7.40	117	58	60	-58	3.54	NA	NA
<i>A. leonora</i>	<i>A. nahuana</i>	194818	80.64	14.51	1	-19	-16	-47	0.99	NA	NA
<i>A. leonora</i>	<i>A. oculata</i>	0	0.00	0.00	0	0	0	0	4.73	NA	NA
<i>A. leonora</i>	<i>A. oenea</i>	19	0.01	0.00	-100	-100	-100	-100	4.42	NA	NA
<i>A. leonora</i>	<i>A. pallens</i>	7756	3.21	1.45	106	63	50	-58	2.39	NA	NA
<i>A. leonora</i>	<i>A. plana</i>	70102	29.02	8.15	-16	-53	-52	-53	2.94	NA	NA
<i>A. leonora</i>	<i>A. pulla</i>	0	0.00	0.00	0	0	0	0	7.87	NA	NA
<i>A. leonora</i>	<i>A. rhoadsi</i>	43983	18.20	22.62	-45	-43	-59	-75	5.69	NA	NA
<i>A. leonora</i>	<i>A. sedula</i>	161999	67.05	6.48	4	-16	-12	-37	7.39	NA	NA
<i>A. leonora</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	4.71	NA	NA
<i>A. leonora</i>	<i>A. tezpi</i>	0	0.00	0.00	0	0	0	0	4.70	NA	NA
<i>A. leonora</i>	<i>A. tibialis</i>	8902	3.68	0.46	-91	-100	-83	-100	6.02	NA	NA
<i>A. leonora</i>	<i>A. tonto</i>	863	0.36	0.48	515	320	58	-24	4.46	NA	NA
<i>A. leonora</i>	<i>A. translata</i>	150581	62.33	8.36	6	-16	-13	-33	5.44	NA	NA
<i>A. leonora</i>	<i>A. ulmeca</i>	0	0.00	0.00	0	0	0	0	6.24	NA	NA
<i>A. leonora</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	2.66	NA	NA
<i>A. lugens</i>	<i>A. munda</i>	112350	10.80	69.43	-3	-3	-13	-41	8.83	NA	6.75
<i>A. lugens</i>	<i>A. nahuana</i>	99298	9.54	7.40	-5	-13	-14	-37	6.92	NA	NA
<i>A. lugens</i>	<i>A. oculata</i>	102900	9.89	37.25	22	21	20	20	6.84	NA	0.36
<i>A. lugens</i>	<i>A. oenea</i>	205562	19.75	42.04	38	45	46	63	5.85	NA	0.27
<i>A. lugens</i>	<i>A. pallens</i>	399914	38.43	74.91	24	29	27	27	7.32	NA	NA
<i>A. lugens</i>	<i>A. plana</i>	54179	5.21	6.30	-18	1	-7	-24	8.91	NA	0.23
<i>A. lugens</i>	<i>A. pulla</i>	145585	13.99	46.86	27	34	29	44	10.72	NA	0.24

<i>A. lugens</i>	<i>A. rhoadsi</i>	389	0.04	0.20	161	-100	-45	-95	10.97	NA	0.19
<i>A. lugens</i>	<i>A. sedula</i>	203	0.02	0.01	175	334	689	1766	10.84	NA	0.28
<i>A. lugens</i>	<i>A. tarascana</i>	180047	17.30	81.50	17	25	24	26	7.83	NA	0.26
<i>A. lugens</i>	<i>A. tezpi</i>	131097	12.60	66.11	33	44	38	50	7.83	NA	0.25
<i>A. lugens</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	12.06	NA	NA
<i>A. lugens</i>	<i>A. tonto</i>	101045	9.71	56.19	12	15	11	-11	11.55	NA	9.31
<i>A. lugens</i>	<i>A. translata</i>	0	0.00	0.00	0	0	0	0	8.28	NA	0.23
<i>A. lugens</i>	<i>A. ulmeca</i>	31851	3.06	18.34	4	-13	-4	-30	6.12	NA	0.30
<i>A. lugens</i>	<i>A. vivida</i>	85647	8.23	3.85	-24	-34	-36	-41	8.55	NA	NA
<i>A. munda</i>	<i>A. nahuana</i>	108581	67.10	8.09	67	107	89	146	2.89	NA	NA
<i>A. munda</i>	<i>A. oculata</i>	0	0.00	0.00	0	0	0	0	6.46	NA	7.02
<i>A. munda</i>	<i>A. oenea</i>	2445	1.51	0.50	-96	-85	-84	-79	6.05	NA	7.91
<i>A. munda</i>	<i>A. pallens</i>	80324	49.64	15.05	113	156	158	215	4.16	NA	NA
<i>A. munda</i>	<i>A. plana</i>	23912	14.78	2.78	89	162	182	273	3.50	NA	5.31
<i>A. munda</i>	<i>A. pulla</i>	0	0.00	0.00	0	0	0	0	8.96	NA	8.38
<i>A. munda</i>	<i>A. rhoadsi</i>	0	0.00	0.00	0	0	0	463	7.44	NA	7.98
<i>A. munda</i>	<i>A. sedula</i>	5678	3.51	0.23	38	155	151	517	9.28	NA	7.82
<i>A. munda</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	6.36	NA	7.94
<i>A. munda</i>	<i>A. tezpi</i>	553	0.34	0.28	-100	-100	-100	-100	6.36	NA	10.40
<i>A. munda</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	7.08	NA	NA
<i>A. munda</i>	<i>A. tonto</i>	72067	44.54	40.08	27	34	35	28	6.18	NA	0.10
<i>A. munda</i>	<i>A. translata</i>	90	0.06	0.01	61	-100	-100	2495	6.47	NA	6.21
<i>A. munda</i>	<i>A. ulmeca</i>	0	0.00	0.00	0	0	0	0	6.41	NA	8.22
<i>A. munda</i>	<i>A. vivida</i>	13179	8.14	0.59	-44	-60	-78	-81	3.22	NA	NA
<i>A. nahuana</i>	<i>A. oculata</i>	0	0.00	0.00	0	0	0	0	4.70	NA	NA
<i>A. nahuana</i>	<i>A. oenea</i>	2287	0.17	0.47	-62	-86	-63	-94	3.75	NA	NA
<i>A. nahuana</i>	<i>A. pallens</i>	84670	6.31	15.86	100	137	134	140	2.36	NA	NA
<i>A. nahuana</i>	<i>A. plana</i>	499653	37.22	58.12	99	117	116	161	2.32	NA	NA
<i>A. nahuana</i>	<i>A. pulla</i>	0	0.00	0.00	0	0	0	0	7.44	NA	NA

<i>A. nahuana</i>	<i>A. rhoadsi</i>	29526	2.20	15.18	-7	9	-16	-18	5.67	NA	NA
<i>A. nahuana</i>	<i>A. sedula</i>	540415	40.26	21.62	42	43	48	64	7.33	NA	NA
<i>A. nahuana</i>	<i>A. tarascana</i>	0	0.00	0.00	61	0	41	0	4.05	NA	NA
<i>A. nahuana</i>	<i>A. tezpi</i>	553	0.04	0.28	-100	-100	-100	-100	4.05	NA	NA
<i>A. nahuana</i>	<i>A. tibialis</i>	81175	6.05	4.19	107	72	90	49	5.32	NA	NA
<i>A. nahuana</i>	<i>A. tonto</i>	84113	6.27	46.78	20	23	32	-2	4.42	NA	NA
<i>A. nahuana</i>	<i>A. translata</i>	417596	31.11	23.18	37	27	33	49	5.40	NA	NA
<i>A. nahuana</i>	<i>A. ulmeca</i>	0	0.00	0.00	0	0	0	0	5.43	NA	NA
<i>A. nahuana</i>	<i>A. vivida</i>	157801	11.76	7.09	68	84	86	89	2.05	NA	NA
<i>A. oculata</i>	<i>A. oenea</i>	262499	95.01	53.69	18	27	30	42	4.50	0.03	0.22
<i>A. oculata</i>	<i>A. pallens</i>	131157	47.47	24.57	20	24	22	27	5.39	NA	NA
<i>A. oculata</i>	<i>A. plana</i>	4118	1.49	0.48	-34	28	21	11	5.68	NA	0.32
<i>A. oculata</i>	<i>A. pulla</i>	198025	71.68	63.74	26	35	32	54	9.92	0.08	0.28
<i>A. oculata</i>	<i>A. rhoadsi</i>	28486	10.31	14.65	94	122	120	256	8.77	0.06	0.24
<i>A. oculata</i>	<i>A. sedula</i>	13978	5.06	0.56	4	28	33	32	10.53	NA	0.35
<i>A. oculata</i>	<i>A. tarascana</i>	97675	35.35	44.21	19	23	21	24	7.11	NA	0.23
<i>A. oculata</i>	<i>A. tezpi</i>	130599	47.27	65.86	16	22	21	24	7.11	0.07	0.29
<i>A. oculata</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	9.38	NA	NA
<i>A. oculata</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	8.38	NA	6.86
<i>A. oculata</i>	<i>A. translata</i>	45917	16.62	2.55	77	95	94	147	6.07	NA	0.24
<i>A. oculata</i>	<i>A. ulmeca</i>	148755	53.84	85.66	18	22	30	36	5.59	0.04	0.22
<i>A. oculata</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	5.36	NA	NA
<i>A. oenea</i>	<i>A. pallens</i>	235641	48.19	44.14	33	47	46	66	5.44	NA	NA
<i>A. oenea</i>	<i>A. plana</i>	5861	1.20	0.68	-39	21	4	1	5.36	NA	0.31
<i>A. oenea</i>	<i>A. pulla</i>	284450	58.18	91.56	27	40	32	60	7.44	0.07	0.28
<i>A. oenea</i>	<i>A. rhoadsi</i>	27321	5.59	14.05	115	155	156	311	7.16	0.05	0.24
<i>A. oenea</i>	<i>A. sedula</i>	12503	2.56	0.50	8	44	41	62	8.22	NA	0.34
<i>A. oenea</i>	<i>A. tarascana</i>	171589	35.09	77.67	22	32	29	41	6.47	NA	0.23
<i>A. oenea</i>	<i>A. tezpi</i>	191481	39.16	96.56	20	31	29	38	6.47	0.06	0.28

<i>A. oenea</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	7.78	NA	NA
<i>A. oenea</i>	<i>A. tonto</i>	493	0.10	0.27	73	162	97	327	6.04	NA	6.03
<i>A. oenea</i>	<i>A. translata</i>	46548	9.52	2.58	89	116	99	165	5.37	NA	0.26
<i>A. oenea</i>	<i>A. ulmeca</i>	150280	30.74	86.54	23	31	33	55	4.91	0.04	0.24
<i>A. oenea</i>	<i>A. vivida</i>	114	0.02	0.01	4023	2029	3188	4411	5.04	NA	NA
<i>A. pallens</i>	<i>A. plana</i>	38679	7.24	4.50	57	99	113	108	3.30	NA	NA
<i>A. pallens</i>	<i>A. pulla</i>	182001	34.09	58.58	26	35	31	47	8.34	NA	NA
<i>A. pallens</i>	<i>A. rhoadsi</i>	19	0.00	0.01	801	-100	500	3	6.77	NA	NA
<i>A. pallens</i>	<i>A. sedula</i>	7462	1.40	0.30	-40	62	37	95	7.32	NA	NA
<i>A. pallens</i>	<i>A. tarascana</i>	205604	38.51	93.06	18	26	24	29	4.69	NA	NA
<i>A. pallens</i>	<i>A. tezpi</i>	170590	31.95	86.03	30	44	39	52	4.68	NA	NA
<i>A. pallens</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	6.73	NA	NA
<i>A. pallens</i>	<i>A. tonto</i>	54825	10.27	30.49	44	65	63	61	5.48	NA	NA
<i>A. pallens</i>	<i>A. translata</i>	54	0.01	0.00	67	-100	-100	-100	6.14	NA	NA
<i>A. pallens</i>	<i>A. ulmeca</i>	28511	5.34	16.42	5	-1	6	-12	5.43	NA	NA
<i>A. pallens</i>	<i>A. vivida</i>	3043	0.57	0.14	5	0	-41	-81	3.58	NA	NA
<i>A. plana</i>	<i>A. pulla</i>	2251	0.26	0.72	-53	35	55	-13	8.33	NA	0.17
<i>A. plana</i>	<i>A. rhoadsi</i>	5028	0.58	2.59	-52	-5	5	-8	7.16	NA	0.14
<i>A. plana</i>	<i>A. sedula</i>	482773	56.16	19.31	35	44	28	71	9.10	NA	0.19
<i>A. plana</i>	<i>A. tarascana</i>	20588	2.39	9.32	-16	1	3	-47	6.02	NA	0.16
<i>A. plana</i>	<i>A. tezpi</i>	1703	0.20	0.86	-69	-47	-58	-99	6.02	NA	0.23
<i>A. plana</i>	<i>A. tibialis</i>	226433	26.34	11.69	116	157	93	171	6.00	NA	NA
<i>A. plana</i>	<i>A. tonto</i>	20510	2.39	11.41	61	120	126	135	5.48	NA	5.89
<i>A. plana</i>	<i>A. translata</i>	391092	45.50	21.71	15	14	5	35	6.92	NA	0.23
<i>A. plana</i>	<i>A. ulmeca</i>	3404	0.40	1.96	-32	38	34	34	6.97	NA	0.29
<i>A. plana</i>	<i>A. vivida</i>	63916	7.44	2.87	38	42	18	-9	0.99	NA	NA
<i>A. pulla</i>	<i>A. rhoadsi</i>	11326	3.65	5.82	225	268	232	615	2.94	0.03	0.10
<i>A. pulla</i>	<i>A. sedula</i>	3818	1.23	0.15	-23	-4	24	-6	7.66	NA	0.25
<i>A. pulla</i>	<i>A. tarascana</i>	144726	46.58	65.51	25	36	32	45	9.80	NA	0.25

<i>A. pulla</i>	<i>A. tezpi</i>	179211	57.68	90.37	25	35	33	43	10.69	0.11	0.32
<i>A. pulla</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	12.38	NA	NA
<i>A. pulla</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	9.81	NA	8.18
<i>A. pulla</i>	<i>A. translata</i>	34033	10.95	1.89	120	132	106	186	9.15	NA	0.24
<i>A. pulla</i>	<i>A. ulmeca</i>	103002	33.15	59.32	32	40	30	58	9.66	0.08	0.24
<i>A. pulla</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	7.87	NA	NA
<i>A. rhoadsi</i>	<i>A. sedula</i>	102288	52.60	4.09	83	68	54	52	6.13	NA	0.24
<i>A. rhoadsi</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	7.81	NA	0.16
<i>A. rhoadsi</i>	<i>A. tezpi</i>	80	0.04	0.04	849	1024	198	724	8.62	0.10	0.27
<i>A. rhoadsi</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	10.62	NA	NA
<i>A. rhoadsi</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	7.83	NA	7.22
<i>A. rhoadsi</i>	<i>A. translata</i>	61401	31.57	3.41	114	139	112	184	6.06	NA	0.19
<i>A. rhoadsi</i>	<i>A. ulmeca</i>	29826	15.34	17.18	102	140	134	267	7.67	0.07	0.20
<i>A. rhoadsi</i>	<i>A. vivida</i>	776	0.40	0.03	35	248	31	447	6.43	NA	NA
<i>A. sedula</i>	<i>A. tarascana</i>	0	0.00	0.00	0	0	0	0	9.99	NA	0.22
<i>A. sedula</i>	<i>A. tezpi</i>	129	0.01	0.07	-100	-100	-100	-100	10.95	NA	0.27
<i>A. sedula</i>	<i>A. tibialis</i>	1763316	70.55	91.04	16	16	16	17	12.20	NA	NA
<i>A. sedula</i>	<i>A. tonto</i>	600	0.02	0.33	221	223	382	1021	7.54	NA	6.94
<i>A. sedula</i>	<i>A. translata</i>	1596645	63.88	88.64	38	34	35	34	9.14	NA	0.27
<i>A. sedula</i>	<i>A. ulmeca</i>	14912	0.60	8.59	6	25	27	32	8.74	NA	0.23
<i>A. sedula</i>	<i>A. vivida</i>	591	0.02	0.03	-26	-8	17	184	8.27	NA	NA
<i>A. tarascana</i>	<i>A. tezpi</i>	139119	62.97	70.16	27	42	35	47	0.49	NA	0.23
<i>A. tarascana</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	5.04	NA	NA
<i>A. tarascana</i>	<i>A. tonto</i>	0	0.00	0.00	0	61	0	61	7.44	NA	10.55
<i>A. tarascana</i>	<i>A. translata</i>	0	0.00	0.00	0	0	0	0	8.35	NA	0.19
<i>A. tarascana</i>	<i>A. ulmeca</i>	13448	6.09	7.74	17	12	16	-15	6.38	NA	0.23
<i>A. tarascana</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	5.69	NA	NA
<i>A. tezpi</i>	<i>A. tibialis</i>	0	0.00	0.00	0	0	0	0	4.37	NA	NA
<i>A. tezpi</i>	<i>A. tonto</i>	535	0.27	0.30	-100	-100	-100	-100	7.44	NA	14.15

<i>A. tezpi</i>	<i>A. translata</i>	731	0.37	0.04	-100	-100	-100	-100	8.35	NA	0.23
<i>A. tezpi</i>	<i>A. ulmeca</i>	33687	16.99	19.40	9	6	12	-5	6.38	0.09	0.30
<i>A. tezpi</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	5.69	NA	NA
<i>A. tibialis</i>	<i>A. tonto</i>	0	0.00	0.00	0	0	0	0	7.41	NA	NA
<i>A. tibialis</i>	<i>A. translata</i>	1201609	62.04	66.71	35	32	33	21	9.83	NA	NA
<i>A. tibialis</i>	<i>A. ulmeca</i>	0	0.00	0.00	0	0	0	0	9.85	NA	NA
<i>A. tibialis</i>	<i>A. vivida</i>	0	0.00	0.00	0	0	0	0	6.38	NA	NA
<i>A. tonto</i>	<i>A. translata</i>	0	0.00	0.00	0	0	0	0	8.40	NA	5.98
<i>A. tonto</i>	<i>A. ulmeca</i>	0	0.00	0.00	0	0	0	0	8.01	NA	9.68
<i>A. tonto</i>	<i>A. vivida</i>	85592	47.60	3.85	-22	-36	-35	-66	5.17	NA	NA
<i>A. translata</i>	<i>A. ulmeca</i>	46754	2.60	26.92	94	117	103	158	5.36	NA	0.25
<i>A. translata</i>	<i>A. vivida</i>	0	0.00	0.00	0	45	65	493	6.19	NA	NA
<i>A. ulmeca</i>	<i>A. vivida</i>	1084	0.62	0.05	855	1180	887	2196	6.24	NA	NA



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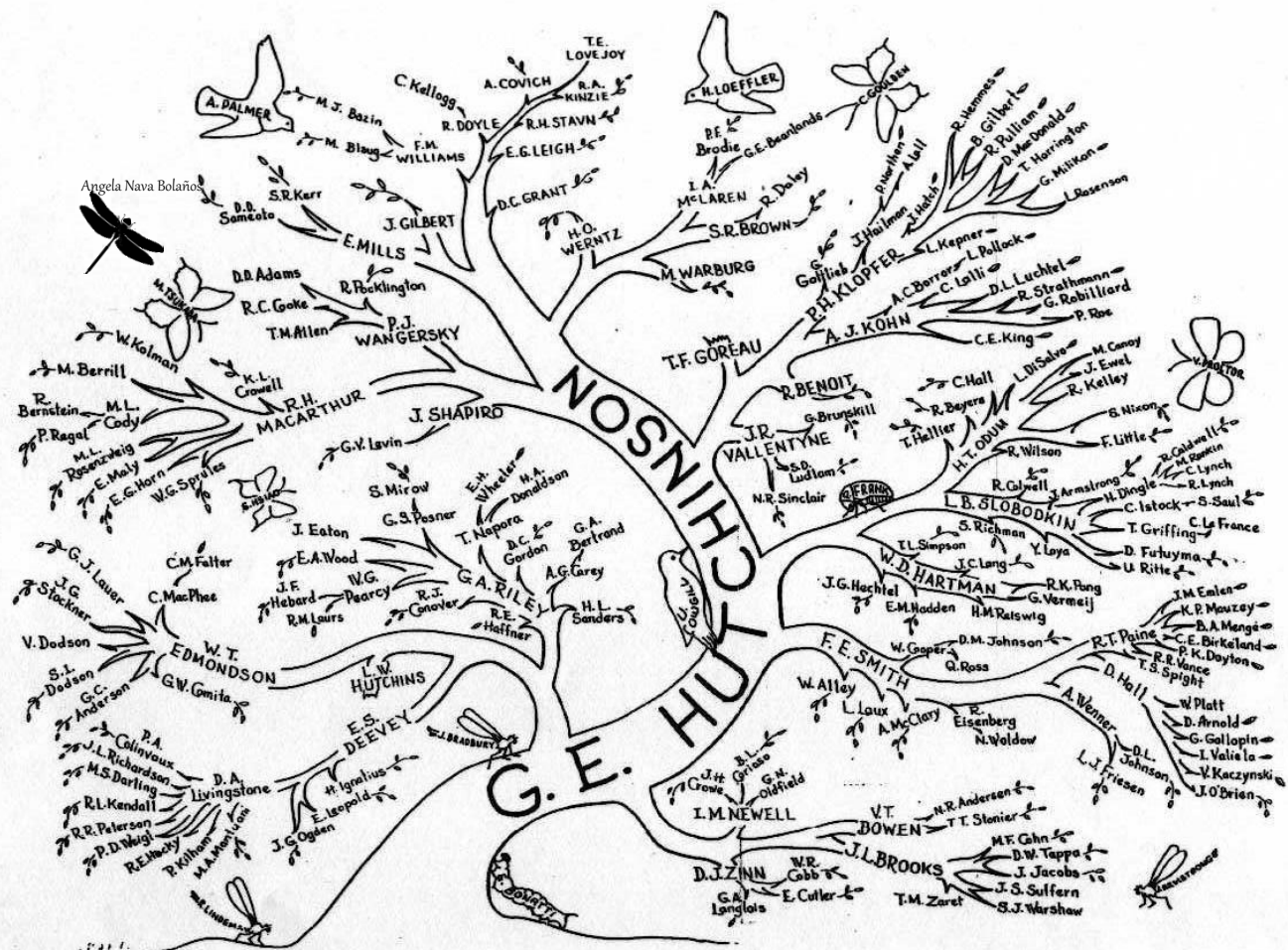
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Árbol filogenético de descendientes intelectuales de Hutchinson,

Modificado de [http://www.chebucto.ns.ca/ccn/info/Science/SWCS/PEOPLE/PIC/hutchinson\\_tree.jpg](http://www.chebucto.ns.ca/ccn/info/Science/SWCS/PEOPLE/PIC/hutchinson_tree.jpg)

# CAPÍTULO II

## Niche conservatism and hybridization

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## **Abstract**

The study of ecological niche patterns as niche conservatism or niche evolution in hybridization process can help us to have a better understand of evolutionary processes derived by hybridization. Niche conservatism is the tendency of species to preserve ancestral ecological traits (Wiens & Graham, 2005). This concept has brought strong scientific debate as to whether niches of the species are really preserved. The evolution is mirrored in the diversity of ecological strategies and causes of both are fundamental focus. Currently, we can see a great diversification of niches and species, because ecological niche had been evolving. However, niches had been also preserving, and there is wide evidence of niche conservatism where niches or niche attributes can be in same conditions through extended periods of stasis. However, no one has addressed the role of hybridization processes into the ecological niche theory, niche conservatism or niche evolution. Although, hybridization is a relevant evolutionary process, which is increasing their importance into evolutionary studies, because is recognized their relevance in processes such as gene flow, diversification, variation, speciation and extinction. This is a review of the criticisms and evidence for and against niche conservatism, and we propose a position from the perspective of the species hybridization. That is, what happens with the ecological niche species and their evolutionary patters when species are in hybrid process?

## **Ecological Niche Patterns**

Ecological niche is a fundamental concept in evolutionary ecology. Multiple definitions have been proposed, one relevant definition was proposed by Hutchinson (Hutchinson, 1957), who defined it as the set of biotic and abiotic conditions in which species are able to persist and maintain stable population sizes. Hutchinson has also defined the fundamental niche as the abiotic conditions under which species are able to persist. He also has defined the realized niche as the conditions where species persists given the presence of other interacting species.

The tendency of a species to preserve aspects of their fundamental niche through time is called niche conservatism (Wiens & Graham, 2005). Preserved ecological niche attributes, such as climatic tolerance of the species, can limit the range of geographic expansion. Therefore, it may represent a critical role in processes such as allopatric speciation, historical biogeography, community structure and function, spread of invasive species, responses of species to climate change, and even human evolution (Wiens & Graham, 2005). The phylogenetic niche conservatism has expanded the vision of niche conservatism to a phylogenetic scale, referring to the species tendency to

conserve ancestral ecological traits. The descendant species tend to share a substantial proportion of the biological and physiological characteristics, which determine that the adaptations of the ancestral species (to a set of ecological niche conditions) tend to be conserved by the descendant species.

A better understanding of the conditions under which niche conservatism occur and operate may be important for a better understanding of ecological and evolutionary patterns and processes, such as speciation patterns species richness and community structure. As well, the inability of organisms to adapt to different conditions that exist in their ancestral niche can be important in the structure and distribution of communities. If niche conservatism prevails on highly heterogeneous environment, diversification could occur through a process of fragmentation in the range, caused by the inability of the species to adapt to new environmental conditions (Rangel, Diniz-Filho, & Colwell, 2007).

### **Does niche conservatism, makes sense?**

Some authors argue that the evidence of niche conservatism is equivocal, and that it should be test rather than assumed *a priori* (Wiens & Brook, 2008). The ecological niches evolve, and their diversification has lead the diversification of the life on the earth. Understanding the evolution and diversification of the ecological niche is a key in studies of ecology, biogeography and biodiversity and adaptive radiations (Peterson *et al.*, 2011).

There are wide evidence that documented that niche evolution has occurred (Holt & Gaines, 1992; Gomulkiewicz & Holt, 1995; Holt, 1996; Holt & Gomulkiewicz, 1997, 2004). Outside niche populations of a species are sink populations, that eventually will go extinct without immigration if adaptation does not take place. The spatial structure of the selective pressure is also expected to be an important factor in the evolution of ecological niche (Peterson *et al.*, 2011).

A particularly interesting study was done with the spotted hyena *Crocuta crocuta* (Varela, Rodríguez, & Lobo, 2009). The current distribution of spotted hyena was accurately predicted but failed to predict their historical distribution. Although the projection of niche model to the last interglacial period could not predict the spatial distribution of the records across western Eurasia, extensive fossil records were found for this period in this area, suggesting that their current occupation range represents a subset of its escenopoetic fundamental niche (Peterson *et al.*, 2011).



There is a wide debate in recent literature on whether niche is or is not preserved. This debate is not very fruitful, because niches are not presumably identical or completely different between species, and the answer simply could depend on the scale and details of the test (Wiens & Graham, 2005). Thus, certain attributes of niche can be maintained over time, but also there are periods of niche developments go hand in hand with the species evolution.

A more useful approach in the study of niche patterns may be to study the patterns of evolution or conservation attributes niche that can help create hypotheses, for example to understand the speciation, species richness, community structure, spread of invasive species, or responses to global warming. Niche conservatism is difficult to generalize, conservatism require a specific context, under which it makes sense.

### **Testing Niche Patterns**

The simplest and most frequently test applied in niche conservatism is to check if the site characteristics are consistent across geographical distribution of species. Under this simple premise, niche models are based on a sector of occupied area of distribution of a species, and tested whether ecological niche models are predictive with respect to distributions are built in other sectors. But this is only possible to the ecological niche is preserved (Letters, 2003).

There is considerable evidences documenting ecological niche conservatism. We now know that niches can remain stable for long periods, encouraging restrictions on developmental physiological tolerances of species and their distribution (Peterson & Navarro-Sigüenza, 1999; Martı́ & Navarro-sigu, 2004; Nogués-Bravo *et al.*, 2008). Evidence of niche conservatism is documented in studies of variation in ecological niche distribution in geographical range, with comparisons of ecological niche characteristics between invasive and native species over time (Martı́nez-Meyer, Peterson, & Hargrove, 2004a; Martı́nez-Meyer & Peterson, 2006; Waltari *et al.*, 2007; Nogués-Bravo *et al.*, 2008), and comparisons of ecological niche along a phylogenetic lineage and between phylogenies (Ackerly, 2003; Martı́nez-Meyer, Peterson, & Navarro-Sigüenza, 2004b), and transplantation experiments showing that fitness subordinates margin distributions (Crozier, 2004; Angert & Schemske, 2005).

The analysis of the historical biogeography (Bradshaw, 1991) and predictive models of invasions (Peterson & Vieglais, 2001) suggest that niches of the species are relatively conserved. Theoretical studies of evolution in heterogeneous environments (Holt & Gaines, 1992; Kawecki, 1995, 2000; Holt & Gomulkiewicz, 1997; Kirkpatrick &

Barton, 1997; Tufto, 2001; Kawecki & Holt, 2002; Proulx, 2002; Holt, 2003) provide a framework for understanding how niche conservatism may reflect interactions between dispersal, selection and demographic asymmetries (e.g., spatial variation of abundance). For example, in the capacity along environmental gradients, gene flow can lead to keep species ranges evolutionary conserved (Kirkpatrick & Barton, 1997), especially when the competitors are present (Case & Taper, 2000). Various analyzes have shown that conservatism seems to be dominant in birds, mammals and butterflies (Peterson & Vieglais, 2001; Anderson, Peterson, & Go, 2002).

Ecological niche conservatism is strong among sister species. But fades in longer time scales. Warren (Warren, Glor, & Turelli, 2008) has confirmed that niche of these species is usually more like what you would expect at random, although not completely identical (Peterson *et al.*, 2011). Maybe the focus should be on phenotypically or genetically definable lineages composed of a mixture of genetic material of parental lineages and that has invaded new environmental niches. For these cases hybridization between closely related taxa could help to understand in a better manner evolutionary process. Also, hybridization is increasingly being recognized as a potentially creative force in evolution because it can lead to mixture of novel genotypes (Nolte & Tautz, 2010), some of which have the potential for rapid adaptation to new environmental conditions. It could be related with the ecological niche patterns like niche evolution or niche conservatism.

### **Hybridization as a creative force**

Hybridization (mating between heterospecific individuals) is an important factor in evolutionary processes, such as: gene flow, speciation, variation and even local extinction (Rhymer & Simberloff, 1996; van Gossum, Sánchez, & Rivera, 2003; Melo *et al.*, 2009; Arnold & Martin, 2010; Sánchez-Guillén *et al.*, 2013a,b, 2014). While adaptation to novel niches by hybrid species which have trait values that differ from those of both parent species have not wide documented. We should understand which mechanisms can reduce or enhance the niche patterns or partitioning, increasing the fitness of hybrids. For example, adaptive introgression of alleles could reduce sexual conflict or enhancing intersexual niche partitioning may facilitate local adaptation, and it could favour the colonization of novel habitats. Furthermore, in nowadays many species have altered their geographical distribution patterns by rising global temperatures, giving rise to patterns of hybridization ( Kelly, Whiteley, & Tallmon, 2010; Crispo *et al.*, 2011; Abbott *et al.*, 2013; Mallet, Wynne, & Thomas, 2011; Sánchez-Guillén *et al.*, 2013a it is wide explained in: From allopatry to sympatry). So far, the role of evolutive patterns, like niche conservatism or niche evolution, in so important process

as hybridization has not yet been addressed. In the hybrid process, individuals of two established species are found in an ecological space that is not part of the normal ecological range of their parent species. There, they can form a hybrid swarm, so that the contributions of the progenitor species will normally be unequal. Hybrid lineages can arise from these hybrid swarms and are expected to occupy different ecological niches than their parent species. The genetic flow can continue to some extent between them and their progenitor species. The processes that occur within the hybrid swarm to the formation of a hybrid lineage are largely unknown, but are expected to differ genetically from other speciation processes (Nolte & Tautz, 2010).

A hybrid zone occurs where the range of two species overlap, and individuals from the two species hybridize and produce offspring of mixed ancestry (Taylor, Larson, & Harrison, 2015) (Figure 1A). This interspecific gene flow can increase the genetic variation by introducing new genetic variants into the population that can then be selected to increase the fitness. However, when too much gene flow occurs, the effects of selection can be overwhelmed by an influx of non-adapted genotypes. Gene flow can then act as constrain to adaptive evolution. A growing body of evidences show that hybridization of previously allopatric lineages is a common phenomenon in nature (Bell & Travis, 2007; Roman & Darling, 2007; Dlugosch & Parker, 2008; Lawson Handley & et al., 2011). Thus, such hybridization generates novel allelic combinations that can be beneficial when certain conditions are met. Potential benefits resulting from admixture include increased population fitness like heterosis (Facon, 2005) and the increase of the adaptive potential (Verhoeven, 2011). These benefits parallel in many ways the observed benefits of interspecific hybridization, which have been dealt with elsewhere (Arnold & Hodges, 1995; Ellstrand & Schierenbeck, 2000; Seehausen, 2004; Rieseberg & et al., 2007). The role of genetic interchange in increasing overall genetic variance has been broadly discussed in terms of overcoming the potentially deleterious influences of founder effects (Roman & Darling, 2007; Dlugosch & Parker, 2008), and several recent studies suggest that enhanced genetic diversity associated with introgression does indeed provide the raw material for local adaptation in newly colonized ecological niche (Rius & Darling, 2014).

### **Hybridization incurs benefits or negative effects?**

One of the most widely recognized benefits of hybridization is heterosis or hybrid vigor (Facon, 2005), broadly defined as the phenotypic superiority of hybrids over their parents (Lippman & Zamir, 2006) (Figure 2B). Heterosis can also lead inbreeding depression. Thus, reducing the negative effects of genetic bottlenecks by increasing the frequency of heterozygotes. Which can mask deleterious mutations or allow expression

of over dominance (Figure 1C). Also another important benefit might be the emergence of novel genotypes not found in parental populations (Roman & Darling, 2007). In some cases, these new genotypic combinations can produce transgressive phenotypes well outside parental norms (Johansen-Morris & Latta, 2006; Bell & Travis, 2007; Rius & Darling, 2014).

However, hybridization can also have immediate negative impacts over the fitness. Such as reduced viability or fertility in the case of serious genetic incompatibilities (Johansen-Morris & Latta, 2006). Although, such costs are expected more frequently with interspecific hybridization. More commonly, outbreeding depression can result from a selective disadvantage of intermediate genotypes or loss of advantageous parental traits, resulting in reduced population fitness (Figure 1B).

In hybridization, the ecological niche is similar to the parental ecological niche when there is niche conservatism, and populations might be preadapted to the new system (Lenormand, 2002) (Figure 1B). Otherwise, selection should act against the hybridization of ecologically divergent subpopulations. Selection for parental types can constrain locally adapted gene pools. Thus, hybridization could have substantial fitness costs (Angert, 2008; Rius & Darling, 2014). This clearly has implications for range expansions. If hybrid populations are adapted to the ecological niche conditions, by increasing their ecological capacities, they could conserve all niche attributes of one or both parental species and get higher physiological tolerances ranges (Figure 1B and C). Marginal or colonizing populations could experience no parental selective pressures. In such circumstances, gene flow from core populations could have maladaptive effects (Holt, 2003; Sexton & et al., 2009; Hardie & Hutchings, 2010), and alleles that could increase fitness at the ecological niche margins can incur fitness costs, against those alleles that were combined with gene flow. Which could hinder adaptation to marginal ecological niche conditions and hinder adaptation and divergence. However, when populations have conserved only a few attributes of parental niche and face a sudden change in environmental and/or ecological conditions, that were previously only found outside the species range, rapid adaptation should occur, by selection pressure otherwise could occur local extinction. Such conditions likely could occur for example under climate change scenarios and when species colonize other regions, by ecological niche patterns like niche conservatism or niche evolution. In such cases (niche conservatism of only a few ecological attributes), the increase of the genetic variation results from multiple introductions and admixture, that can further enhance adaptive responses (Cothran, 2008; Rius & Darling, 2014). Thereby, hybrid zones are an evolutive watershed by process like adaptation and novel genetic combinations, either through of

divergent ecological niche (until hybrid speciation and diversification), or niche conservatism (Figure 1D). The emergence of novel phenotypes by hybridization can also have an important role in post-colonization adaptation and niche divergence (Rieseberg & et al., 1999). To range expansion and novel environmental conditions associated to selective pressures, the emergence of non-parental genotypes might provide opportunities for local adaptation that were previously inaccessible (Figure 1C). Models of evolution at environmental range limits predict that local adaptation is unlikely to result from *in situ* emergence of beneficial alleles in marginal ecological niche (Bridle & Vines, 2007). This suggests that the appearance of novel allelic combinations through multiple introductions by introgression provides unique opportunities for colonizing populations to respond to selection pressures in novel environments (Figure 1C). Some studies suggest that the availability of genotypic variation is especially important when colonized ecological niche are particularly novel or challenging (Ahloth & Al., 2003; Hufbauer, 2013; Rius & Darling, 2014).

### **Limits to niche evolution**

In hybrid and parental populations genetically based trade-offs, can occur through pleiotropy when simultaneous effects influence traits closely related to fitness but in opposing directions. These trade-offs may result in different traits being favored in different environments. Although, trade-offs due to trait interactions are often regarded as essential for evolutionary constraints by limits to the ecological niche of species, other types of genetic interactions among traits can also prevent selection responses. If directional selection for increased expression of a trait persists for many generations, the alleles favored by selection is expected to increase and eventually fixation. Once this occurs across all the loci affecting a trait, genetic variation in the trait is expected to decrease toward zero, preventing any further selection response until the strong directional selection is alleviated, and new mutations can accumulate (Hoffmann, 2014). Lack of evolution in most species may be due more to lack of appropriate variability than to other causes, a condition for which the term “genostasis” has been proposed. In those situations where appropriate genetic variation is available for one reason or another, evolution is found to be very rapid (Bradshaw, 1991). This may help to explain why species are restricted in their physiological capabilities and distribution, and why an evolutionary limit exists, based on a lack of genetic variation preventing them moving out of their warm and moist ecological niche (Hoffmann, 2014).

### **From allopatry to sympatry**

A good model situation to investigate the consequences for studying hybridization process and evolutionary patterns of niche evolution like niche conservatism and niche evolution would be the case for those species that have recently diverged, they are genetically close, but they were also allopatric their niche divergence could increase. However, climate change could promote coexistence of secondary sympatry. As allopatric populations become reciprocally adapted to their local environments they are also less likely to successfully colonize the geographical range of their sister species. An increasing number of climate-induced range shifts have now been associated with hybridization (Kelly, Whiteley, & Tallmon, 2010; Crispo *et al.*, 2011; Abbott *et al.*, 2013; Mallet, Wynne, & Thomas, 2011). One well known hybridization example is that of the polar bear *Ursus maritimus* and grizzly bear *U. arctos* (Kelly, Whiteley, & Tallmon, 2010). Observations over the last decade indicate that brown and black bears are shifting northward into the Arctic with hybridization between these lineages in their new sympatric areas (Crispo *et al.*, 2011; Abbott *et al.*, 2013). Hybrid zones as a by-product of increasing temperatures also include invertebrates, such as brown Argus butterflies (*Polyommatus agestis* and *P. Artaxerxes* (Mallet, Wynne, & Thomas, 2011). These species have been shifting their range northwards, potentially threatening the long-term survival of the northern species (Mallet, Wynne, & Thomas, 2011). Another example comes from damselflies, where hybridization and introgression events have been documented in a number of species. A particularly well documented example is that of the hybridization between *Ischnura elegans* and *I. graellsii*, with introgressive hybridization of *I. graellsii* genes into recently established *I. elegans* populations (Sánchez-Guillén *et al.*, 2011, Wellenreuther *et al.*, 2018). One consequence is a rapid species turnover in sympatric regions in favour of *I. elegans* (Sánchez-Guillén *et al.*, 2011).

Adaptation to ecological niches beyond the ancestral range may result in range expansion, but local adaptation may result in strong niche divergence. Moreover, large-scale environmental change may cause range shifts that erase local adaptation to ancestral ecological niches, with coexistence achieved because of prior niche divergence. Secondary sympatry results in coexistence when competitive displacement reduce niche overlap and subsequent adaptation, or hybridization when reinforcement is possible (Mittelbach & Schemske, 2015). A clearer understanding of the factors that influence secondary sympatry and species coexistence is needed to better link the concept of ecological niche evolution, and their patterns like niche conservatism and niche evolution.

## **Conclusion**

Biological diversity is generated ultimately by evolution, and much attention has been focused on the rapid development of ecological features. However, the tendency of many ecological features to be maintained over time can have interesting consequences on the ecological and evolutionary patterns and processes not just academic or theoretical interest, but also in applied issues as conservation, invasive species of economic importance, among others. The importance of niche conservatism does not depend on ecological features remain indefinitely in the species, if not different patterns that can be explained by niche conservatism in different time scales. Niche has evolved until an optimal fitness of species, if species are adapted then this optimal, niche species will be preserved. In this review, we focus on evolutionary patterns of niche evolution, like niche conservatism and niche evolution, in a very narrow sense, emphasizing the effects when a process of hybridization between species occurs, and the evolutive consequences that this has on the ecological niche of the species.

## **Glossary**

**Adaptive radiation:** the diversification by adaptation of a single phylogenetic lineage into multiple taxa occupying different ecological niches.

**Admixture:** the result of interbreeding between two or more previously isolated populations within a species.

**Allopatric speciation:** speciation following the division of a large population into at least two new populations that are separated by a geographic barrier.

**Allopatry:** geographical separation between species or populations.

**Assortative mating:** the tendency for individuals with similar phenotypes to make preferentially with each other.

**Fundamental niche:** the requirements of a species to maintain a positive population growth rate, disregarding biotic interactions.

**Genetic drift:** change in allele frequencies caused by the random sampling of alleles between generations; its effects grow stronger as population size decreases.

**Hybrid zone:** a parapatric margin where two or more populations or species exchange genes through hybridization. Regions where genetically distinct groups of individuals meet and mate resulting in at least some offspring of mixed ancestry.

**Hybrid speciation (homoploid):** origin of new species through the production of a stable population of recombinant individuals that are reproductively isolated from the parent species.

**Hybridization:** interbreeding of individuals from two populations or species, which are distinguishable on the basis of one or more heritable characters.

**Introgression:** incorporation of alleles of one entity into the gene pool of another, typically through hybridization and backcrossing. Adaptive introgression refers to the introgression of advantageous alleles.

**Inbreeding depression:** the reduction of fitness caused by mating between close relatives, often resulting from the unmasking of recessive deleterious alleles or the loss of heterozygote advantage (overdominance).

**Introgression:** the movement of alleles between differentiated genomes.

**Niche:** the requirements of a species to maintain positive population growth rates (see fundamental and realized niche).

**Niche conservatism:** the tendency of species to preserve ancestral ecological features.

**Linkage disequilibrium:** the tendency for alleles at different loci to be associated within a population. These non-random associations are broken up by recombination between the loci but are generated and maintained by selection or assortative mating.

**Outbreeding depression:** a reduction of fitness in offspring of parents from different populations, possibly due to disruption of co-adapted gene complexes.

**Realized niche:** the portion of the fundamental niche in which a species has positive population growth rates, given the constraining effects of biological interactions.

**Secondary sympatry:** the reestablishment of overlap in species ranges after allopatric speciation.

**Sympatry:** geographical overlap between species or populations.



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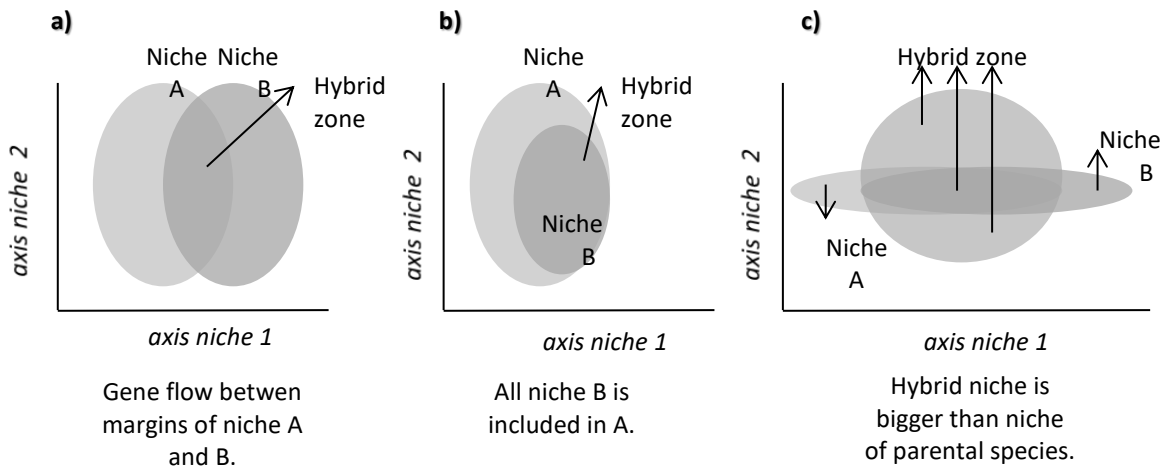
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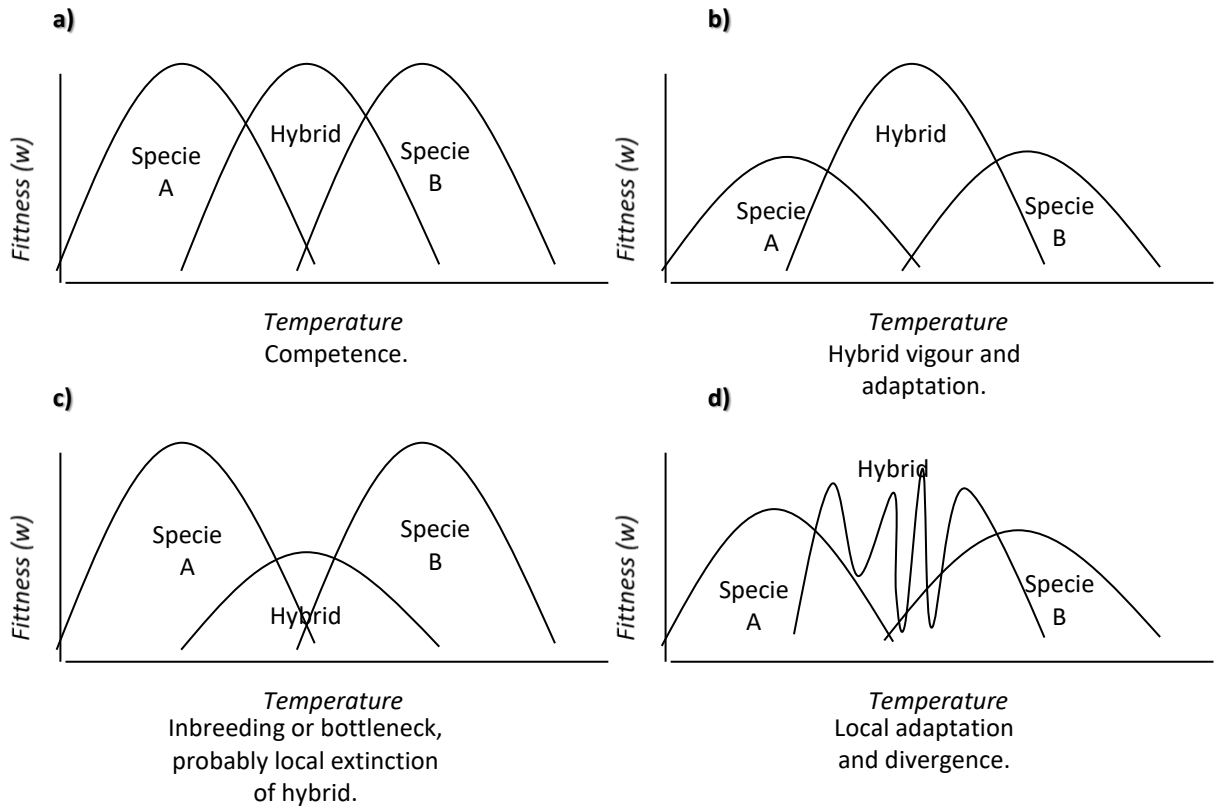
**Figure 1. Ecological niche with different scenarios of hybridization.**

Niche A and niche B indicate the ecological niche of different species, but when that species hybridize share ecological conditions, here are presented how species could share ecological niche condition in different sceneries of hybridization and when gene flow only happens on marginal conditions in a), when niche of hybrids are completely merge in a parental niche in b), and when the hybrid niche is bigger than some of both parental niche.



## Figure 2. Evolutionary scenarios of hybridization

Here is presented a feature of ecological niche, represented by temperature, and different possibilities of fitness of this feature of ecological niche, in different sceneries. And how it is affected by competence in a), hybrid vigor and adaptation of hybrid in b), inbreeding or bottleneck, probably local extinction of hybrid in c), and local adaptation and divergence in d).





### **CAPÍTULO III**



## Isolation barriers and genetic divergence in non-territorial *Argia* damselflies

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Isolation barriers work at different instances during the mating process in odonate insects. In territorial damselflies, heterospecific interactions are mainly precluded by sexual (visual) isolation, while in non-territorial damselflies, heterospecific interactions are mostly precluded by mechanical isolation and sexual (tactile) isolation. In this study we investigated the strength of three premating barriers (visual, mechanical and tactile), genetic divergence and degree of sympatry (on their entire distribution) between four non-territorial *Argia* damselflies (*A. anceps*, *A. extranea*, *A. oenea* and *A. tezpi*). Our results are explained in the light of learned mating preferences and Kaneshiro's hypothesis. We detected a strong reproductive isolation between all pairs of species by the joint action of the three studied barriers [visual (90.6%), mechanical (8.7%) and tactile (0.7%)]. Sexual (visual) isolation was the most important barrier, perhaps driven by learning mating preferences. One of the studied species, *A. extranea*, which is the most derived of the studied species, showed a highly asymmetric isolation in reciprocal crosses, which is consistent with Kaneshiro's hypothesis. Moreover, we detected a negligible ecological niche differentiation between the studied species (70% of shared distribution). Our results suggest that sexual (visual) selection may be an important force driving speciation in non-territorial species. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 00, 000–000.

**KEYWORDS:** *Argia* damselflies – genetic divergence – mating preference – mechanical isolation – niche conservatism – non-territorial – radiation – sexual isolation – sympatric patterns.

### INTRODUCTION

A central question in evolutionary biology concerns the accumulation of reproductive barriers during speciation. One of the greatest contributions to the modern synthesis was the concept of reproductive isolation mechanisms (barriers) by Dobzhansky (1937). Reproductive barriers can prevent gene flow before (pre-mating barriers) or after mating (post-mating barriers), and before (prezygotic) or after (postzygotic) fertilization (Dobzhansky, 1937; Coyne & Orr, 2004). In relation to this, one long-standing

information gap concerns the order at which the reproductive barriers accumulate at the initial steps of diversification, and the intensity with which these barriers prevent genetic exchange. Numerous studies in as diverse taxa as plants, insects, fishes, birds or mammals (for references see Sánchez-Guillén *et al.*, 2014a) have been done to address the importance of pre- and postzygotic barriers in the accumulation of reproductive isolation. Moreover, some of these studies (e.g. angiosperms, Moyle, Olson & Tiffin, 2004; frogs, Sasa, Chippindale & Johnson, 1998; birds, Price & Bouvier, 2002; butterflies, Presgraves, 2002; and damselflies, Sánchez-Guillén *et al.*, 2014a) are in line with the 'speciation clock' phenomenon, i.e. the idea that reproductive isolation is a by-product of

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gradual genetic divergence. However, an increased sexual selection can provide more opportunities for speciation (Gage *et al.*, 2002) and thus can accelerate the accumulation of reproductive isolation (see Darwin, 1871).

Sexual selection is a key driver of divergence, speciation, and radiation in odonate insects (Wellenreuther & Sánchez-Guillén, 2016). Visual, olfactory and tactile signals are involved in both intraspecific and interspecific recognition (sexual isolation) in these insects (Wellenreuther & Sánchez-Guillén, 2016). Intraspecific sexual selection *via* learned mating preferences has been recently found to be an important process in three different odonates genera (*Calopteryx*, *Enallagma* and *Ischnura*, Fincke, Fargevielle & Schultz, 2007; Svensson *et al.*, 2007; Sánchez-Guillén *et al.*, 2013). However, the role of learning in the interspecific sexual isolation context comes only from calopterygid damselflies, whose males learn to discriminate against heterospecific mates following courtship interactions (Waage, 1979; Wellenreuther, Tynkkynen & Svensson, 2010). Learning in these species could occur during such interactions as males have the time to ‘inspect’ and differentiate individual females. Thus, one key issue here is whether learning can still occur in species where individuals do not engage in such premating interactions (which is the case of non-territorial species and without courtship), which is pivotal to establish if such mechanism is widespread during sexual isolation in odonates.

*Argia* is a good model genus to investigate the role of learning ability in sexual isolation in non-territorial odonates and without courtship. This monophyletic genus is one of the most speciose Nearctic genus from the New World (Caesar & Wenzel, 2009), with around 120 described species (Garrison, 1994). For instance, up to six *Argia* species have been found in sympatry (e.g. Central Mexico; Peralta-Vázquez, 2009). Additionally, *Argia* is a non-territorial genus which lacks courtship behaviour (Bick & Bick, 1982) and whose populations occur usually at high densities. Importantly, two classical studies of reproductive isolation in damselflies (Paulson, 1974; Robertson & Paterson, 1982) have highlighted the importance of both visual and tactile signals in sexual isolation in *Argia* species.

The aim of this study was to test the role of learning in interspecific sexual isolation and the ‘speciation clock’ phenomenon in odonates. To this end, we investigated the strength of visual, mechanical and tactile isolation between four pairs of closely related species of the non-territorial genus *Argia* (Fig. 1) in one community with high density for the four species, but with different species proportions. First, to

establish a basis for isolation barriers, we estimated the strength of sexual isolation in terms of visual, mechanical and tactile isolation. Second, we linked the strength of such isolation with the presumed genetic divergence among all four species by using a previously sequenced ribosomal gene (see Caesar & Wenzel, 2009). This link is fundamental to see whether isolation is a consequence of interspecific genetic differences. Third, based on previous findings that showed the importance of the degree of sympatry (Wellenreuther *et al.*, 2010) in the intensity of the sexual isolation, we tested the correlation between degree of sympatry and reproductive isolation strength. For this, we measured the degree of sympatry between the four studied species in their complete distribution, by using modern techniques of distributional modelling.

## METHODS

### STUDY SPECIES AND POPULATIONS

We used four sympatric *Argia* species (*Argia anceps*, *A. extranea*, *A. oenea* and *A. tezpi*; Fig. 1) which are common in Mexico, and usually show overlapping distributions (all authors’, unpubl. data). *A. anceps* males are completely blue (thorax and abdomen) with slightly amber wings, while females are dark brown and blue (Fig. 1A). *A. extranea* males show violaceous-blue thorax coloration and prominent ventrolateral black streaks on the 8<sup>th</sup>–10<sup>th</sup> abdominal segments. *A. extranea* females have light-blue thorax coloration and almost complete light-brown abdominal coloration, except for the 8<sup>th</sup> and 9<sup>th</sup> abdominal segments which are blue (Fig. 1B). *A. oenea* males have red eyes, and blue thorax and abdomen with black stripes, while females have brown thorax and abdomen (Fig. 1C). Males of *A. tezpi* have a solid metallic thorax, while females have light dark brown thorax and abdomen (Fig. 1D; see Garrison, Ellenrieder & Louton, 2009 for further descriptions). Our four studied species are related within the genus, and vary in relative abundance in our study site, Tetlama river (18°46′05″N, 99°14′17″W), in Xochitepec, Mexico. To obtain actual measures of population density of each species, we counted adults of the four species in separate days at the beginning and at the end of the experiment of reproductive isolation, which started at early November and ended at late December of 2012. Density measures were estimated as the number of adults captured per minute, on a 100 m transect on the river. Such countings were done at noon, when adult activity was at its highest level (all authors’, unpubl. data).



**Figure 1.** Males and females of all *Argia* species studied: *A. anceps* in A, *A. extranea* in B, *A. oenea* in C and *A. tezpi* in D. Pictures were courtesy of Keith D. P. Wilson (A), and Erland Refling Nielsen (B and C). Photo D taken by A ngela Nava-B olaños.

#### REPRODUCTIVE ISOLATION: SEXUAL (VISUAL AND TACTILE) AND MECHANICAL BARRIERS

Reproduction in non-territorial odonate species without courtship, such as *Argia* damselflies, starts when a male finds a female and attempts to mate (*attempt to tandem*) without any behavioural input from her. To achieve copulation, the male must grasp the female with his anal appendages by holding her mesostigmal plates (*tandem*) and after that, the female must accept the mating by bending her abdomen, thus allowing the contact between male's and female's primary genitalia (reaching the so-called *wheel position*). Based on the reproductive biology of odonates, copulation can be firstly precluded by the lack of sexual attraction of the male by the female, so that males do *not attempt the tandem* (visual sexual isolation). If the male is sexually attracted by the female and she attempts to form the tandem, the *tandem* position can be impeded by the incompatibility between male cerci and female mesostigmal plates (mechanical isolation). Thirdly, if the *tandem*

is formed, *wheel position* can be prevented by tactile isolation because of male's inappropriate stimulation of the female, so that the female does not bend her abdomen (tactile sexual isolation) to form the *wheel position*, and if the female accept to bend her abdomen, by mechanical incompatibility between male and female primary genitalia (mechanical isolation) (see Wellenreuther & Sánchez-Guillén, 2016).

To avoid handling the focal organisms (males) we manipulated the females. Only sexually mature females showing bright body colours and rigid body structure (Corbet, 1999) were used for the reproductive isolation experiment, which was done on windless and sunny days, from 10:00 to 15:00 h, in the field. However, we could not control whether females were virgin or not, as there was no way to have tracked their mating history. Alive female models of each species were tied with a fine thread to allow them to move freely (see Cordero, 1999; Cordero-Rivera & Sánchez-Guillén, 2007, for similar methodologies). These models were presented repeatedly to

males of the four species, to record male responses. Females were presented on average four times for species (this is, some females were presented as less as 1 time, but as many as 12 times). Females were presented to males as these were found along the river. It is highly likely that we did not 'use' the same male as we moved away several meters to find another new male. Only those females that were in good physical condition were considered. Male responses were categorized as: (1) *no sexual response*, which included males that did not have sexual response but show responses like: from faces off and/or approaches without physical contact, to flies away or moves around the perch; (2) *attempt of tandem*, which was when the male approached the female and *attempted the tandem* by curving his abdomen; (3) *tandem*, which was when the male grasped the female with his anal appendages; and (4) *mating*, which was when female does genital contact with the male. However, in the case of conspecific couples, we did not let them to reach *wheel position*, to prevent harming the female models that would occur when disconnecting a mating pair. Those males that did not show any type of response to female models were not considered in the analysis. This methodology allows deducing mating formation, based on the premise that males reached copulation in 95% of tandem events among conspecifics (Cordero-Rivera & Sánchez-Guillén, 2007). In total, 147 (*A. anceps*), 54 (*A. extranea*), 211 (*A. oenea*) and 192 (*A. tezpi*) male responses were recorded.

Because of each reproductive step is relative to the previous one, the percentages of responses were estimated as following: *attempt to tandem* was estimated respect to the total number of presented females, and *tandem* was estimated respect to the total number of *attempts to tandem*. The absolute contribution of both reproductive barriers [sexual (visual and tactile) and mechanical] were estimated following a previously published methodology (see Sánchez-Guillén, Wellenreuther & Cordero-Rivera, 2012; Sánchez-Guillén *et al.*, 2014a). Visual (sexual) reproductive isolation was estimated as  $(RI_{\text{sexual}}) = 1 - (\text{tandem attempts that a heterospecific female was presented}) / (\text{tandem attempts that a conspecific female was presented})$ . Mechanical reproductive isolation was estimated as  $(RI_{\text{mechanical}}) = 1 - (\text{tandems/attempts to tandem in heterospecific interactions}) / (\text{tandems/attempts to tandem in conspecific interactions})$ . Tactile (sexual) isolation  $(RI_{\text{tactile}}) = 1 - (\text{copula/tandem in heterospecific interactions}) / (\text{copula/tandem in conspecific interactions})$ . All indexes ranged from -1 to 1 with negative values indicating higher success in heterospecific than conspecific combinations. In order to test whether the reproductive isolation indexes

(sexual and mechanical recognition) are statistically significant we used a chi-square test.

Alternatively, we also computed cumulative link mixed models (CLMM; (Fox, 2002; Agresti & Maria, 2011) to test significance of the strength of the studied reproductive barriers (sexual, mechanical and tactile). CLMM link cumulative probabilities to linear predictors and thus can be used for ordinal data with mixed effects. In our experiment, we have three cumulative probabilities (*attempt to tandem*, *tandem* and *copula*), and mixed effects because females were involved in more than one presentation, thus data are not independent from each other. We assigned 0 to no sexual responses, 1 to *attempts tandem* and 2 to *tandems*. We did not observe any copulation, so this response was not included in the analyses. We constructed one model for each studied species and male's responses were evaluated. CLMM were fitted with the `clmm2` function in package `ordinal` (Christensen, 2015). Statistical analyses were done using R version 3.0.2 (R Core Development Team 2012) software. Maximum likelihood estimates of the parameters are provided using the Laplace approximation to estimate the likelihood function (Supporting Information, Table S2). The *P*-values for the location coefficients provided are based on *Wald* statistics, and a more accurate test was provided by likelihood ratio tests, obtained with deviance analyses.

#### CORRELATION BETWEEN REPRODUCTIVE ISOLATION AND THE GENETIC DIVERGENCE AND DEGREE OF SYMPATRY

The phylogenetic relationship between the North American *Argia* species, including our four studied *Argia* species have been resolved by Caesar & Wenzel (2009) using the mtDNA gene 16S. Genetic distances between each pair of species have been estimated using the best evolutionary model, which was inferred, by using MEGA V. 6. The evolutionary model Tamura 3-parameters considers differences between transitions and transversions, and provides equal substitution rates among sites. The correlation between the strength of sexual and mechanical isolation and genetic divergence and degree of sympatry were estimated by using the Pearson's correlation.

#### SPECIES DISTRIBUTION: OVERLAPPING RANGES AND ESTIMATION OF GEOGRAPHICAL SYMPATRY

Two parameters were used to measure the potential and observed sympatry. To measure the former, ecological niche models for each pair of species were used, and the potential overlapping range (between each pair of species) was estimated. To measure observed sympatry, frequency counts of each species

in each locality were done, and thus, the total degree of observed sympatry was derived from the relation between the number of sympatric localities vs. the total number of localities in which each species was found.

Our study area included North and Central America and, covering land between the latitudes 53.00–0.00N, and the longitudes –130.00 to –55.00W. As bioclimatic variables, we used WorldClim 1.4 (www.worldclim.org) data set (Hijmans *et al.*, 2005) at 0.041666669 cell size. To establish a set of uncorrelated climatic variables, we intersected the variables with target group points, and with 10 000 points randomly selected in the extension of the study area (*M*), and with special extent delineating *M* for each particular species, we eliminated some variables with an exploratory data analysis and a Pearson correlation analysis (i.e. any value > 0.7). After this, the final data set included mean diurnal range (bio 02), temperature seasonality (bio 04), mean temperature of warmest quarter (bio 10), precipitation of wettest month (bio 13), precipitation of driest month (bio 14), and precipitation seasonality (bio 15).

Presences of the four species were compiled from literature records, confirmed records in Odonata Central (<http://www.odonatacentral.org>), CONABIO records ([www.conabio.gob.mx](http://www.conabio.gob.mx)), and GBIF ([www.gbif.org](http://www.gbif.org)) (accessed October 2014) records which were carefully checked for geographic accuracy. Some records are registered to county, which are rather heterogeneous in size, ranging from small and climatically homogeneous in the US Great Plains, to large and heterogeneous at the US Pacific coast. To avoid inaccurate geo-referencing, only county records from the Great Plain distributions were used. The final data set included 372 unique presences, 68 for *A. anceps*, 114 for *A. extranea*, 112 for *A. oenea* and 78 for *A. tezpi*.

Species distribution models were generated with Maxent 3.3.3k (Phillips, Anderson & Schapire, 2006), to choose the best background. Models were constructed setting several parameters to default ('Auto features', convergence = 10–5, maximum number of iterations = 500, background = 10 000). However, we used random seed (with a test percentage of 30), 10 replicates, removed duplicate records, ran bootstrap replicated type, and no extrapolation and no clamping. All this to find which combination of settings and variables generated the best outcomes (highest area under the curve, or AUC) while minimizing the number of model parameters, as well as producing 'closed', bell-shaped response curves which guaranteed model calibration (Elith, Michael & Steven, 2010). The best background was 10 000 points randomly selected in the extension of the study area. Final models were built with BIOMOD (Biodiversity

Modelling) package in R software. This package is a platform for predicting species' distribution, including the ability to model the distribution using various techniques and test patterns. We trained models using four widely used algorithms (Maxent, RF, GBM and MARS). From individual models obtained with these different algorithms, we generated a 'consensus model' where the final model indicated the degree of overlap between models. This was done to reduce biases and limitations of use only individual models. The final validation of models was performed with TSS (True Skill Statistics), average net rate of successful prediction for sites of presence and absence (Liu, White & Newell, 2009), ranging from –1 to 1, where the more positive values indicate a higher degree of accuracy and discrimination model (Allouche, Tsoar & Kadmon, 2006).

A total of 52 models were generated, and whose performance was assessed by mean of the AUC and TSS statistics, while minimizing the number of model parameters, and the best presence/absence models using the '10 percentile-training presence' are presented. The degree of sympatry between each pair of species was estimated in terms of the observed sympatry (the proportion of locations in which one of the studied species coexisted with at least another of the studied species). Additionally, potential sympatry was estimated as the proportion of potential locations in which one of the studied species overlaps with the potential distribution of at least another of the studied species.

## RESULTS

### STRENGTH OF THE REPRODUCTIVE ISOLATION

Male responses, to conspecific and heterospecific females are shown in Table 1, Figure 2. Because of the high but not complete sexual isolation (Table 2), *tandem* position was reached in three out of the 12 possible heterospecific combinations (Table 1, Fig. 2) but copulation was strongly precluded by sexual (tactile) isolation, i.e. in any case the female bent her abdomen to form the *wheel position* (Table 1).

Sexual (visual) isolation of *A. anceps* males was stronger with *A. oenea* (0.91) and *A. tezpi* (0.73) than with *A. extranea* (0.55) (Table 1, Fig. 2). Although mechanical isolation was complete (1.00) with *A. extranea* females, preventing completely the gene flow, it was not with *A. oenea* (–0.38) and *A. tezpi* (0.54) as it allowed the formation of heterospecific *tandems* (Table 1, Fig. 2). In the case of *A. extranea* males, sexual (visual) isolation was complete (1.00) (Table 1, Fig. 2, Supporting Information, Tables S1, S2) with the three heterospecific females, and thus, mechanical and tactile isolation could not be

**Table 1.** Male responses to conspecific and heterospecific female presentations and indexes of reproductive isolation (visual, mechanical, tactile and total)

Species combination	Type	N	Attempt to	Tandem	Copula	RI	RI	RI	RI
			N	(%)		(%)	Visual	Mechanical	Tactile
♂ <i>A. anceps</i> – ♀ <i>A. anceps</i>	Conspecific	32	11 (34.34)	8 (72.7)	–	–	–	–	–
♂ <i>A. extranea</i> – ♀ <i>A. extranea</i>	Conspecific	11	3 (27.3)	1 (33.3)	–	–	–	–	–
♂ <i>A. oenea</i> – ♀ <i>A. oenea</i>	Conspecific	66	32 (48.5)	12 (37.5)	–	–	–	–	–
♂ <i>A. tezpi</i> – ♀ <i>A. tezpi</i>	Conspecific	71	23 (32.4)	10 (43.5)	–	–	–	–	–
♂ <i>A. anceps</i> – ♀ <i>A. extranea</i>	Heterospecific	20	5 (25.0)	0	0	0.545	1.000		1.000
♂ <i>A. anceps</i> – ♀ <i>A. oenea</i>	Heterospecific	53	1 (1.9)	1 (100.0)	0	0.909	–0.375	1.000	1.000
♂ <i>A. anceps</i> – ♀ <i>A. tezpi</i>	Heterospecific	42	3 (7.1)	1 (33.3)	0	0.727	0.542	1.000	1.000
♂ <i>A. extranea</i> – ♀ <i>A. anceps</i>	Heterospecific	13	0	0	0	1.000			1.000
♂ <i>A. extranea</i> – ♀ <i>A. oenea</i>	Heterospecific	22	0	0	0	1.000			1.000
♂ <i>A. extranea</i> – ♀ <i>A. tezpi</i>	Heterospecific	8	0	0	0	1.000			1.000
♂ <i>A. oenea</i> – ♀ <i>A. anceps</i>	Heterospecific	42	3 (7.1)	1 (33.3)	0	0.906	0.111	1.000	1.000
♂ <i>A. oenea</i> – ♀ <i>A. extranea</i>	Heterospecific	24	5 (20.8)	0	0	0.844	1.000		1.000
♂ <i>A. oenea</i> – ♀ <i>A. tezpi</i>	Heterospecific	79	7 (8.9)	0	0	0.781	1.000		1.000
♂ <i>A. tezpi</i> – ♀ <i>A. anceps</i>	Heterospecific	39	2 (5.1)	0	0	0.913	1.000		1.000
♂ <i>A. tezpi</i> – ♀ <i>A. extranea</i>	Heterospecific	29	5 (17.2)	0	0	0.783	1.000		1.000
♂ <i>A. tezpi</i> – ♀ <i>A. oenea</i>	Heterospecific	53	9 (17.0)	0	0	0.609	1.000		1.000

First and second column represent the species combination and type of interaction. *N* represent the total number of presentations in each species combination. Fourth column represent the total number and the percentage (in brackets) of males that sexually responded to the female model. Fifth column represents total number and percentage (in brackets) of males that reached the tandem. Sixth column represents total number and percentage (in brackets) of males that reached the copula (*wheel position*). Strength of the sexual (visual), mechanical, tactile and total isolation are given in the 8<sup>th</sup>–10<sup>th</sup> columns. Strength values ranged from zero (no isolation) to one (complete isolation), (–) denotes data not available.

measured because it was prevented by the previous reproductive stage (sexual isolation). Sexual (visual) isolation of *A. oenea* males was strong but not complete with any heterospecific female [*A. tezpi* = 0.78, *A. anceps* = 0.91 or *A. extranea* = 0.84; (Table 1, Fig. 2)]. In all cases, the degree of sexual isolation measured by behavioural response of sexual interest was significant (Supporting Information, Table S1). Moreover, mechanical isolation was only complete (1.00) with *A. tezpi* and *A. extranea* females, allowing heterospecific *tandems* with *A. anceps* (0.11) (Table 1, Fig. 2, Supporting Information, Table S2). Sexual (visual) isolation of *A. tezpi* males was strong but not complete with any of the three heterospecific *Argia* females [*A. oenea* = 0.61; *A. tezpi* = 0.91; and *A. anceps* = 0.78], while mechanical isolation was complete (1.00) with the three heterospecific females (Table 1, Fig. 2).

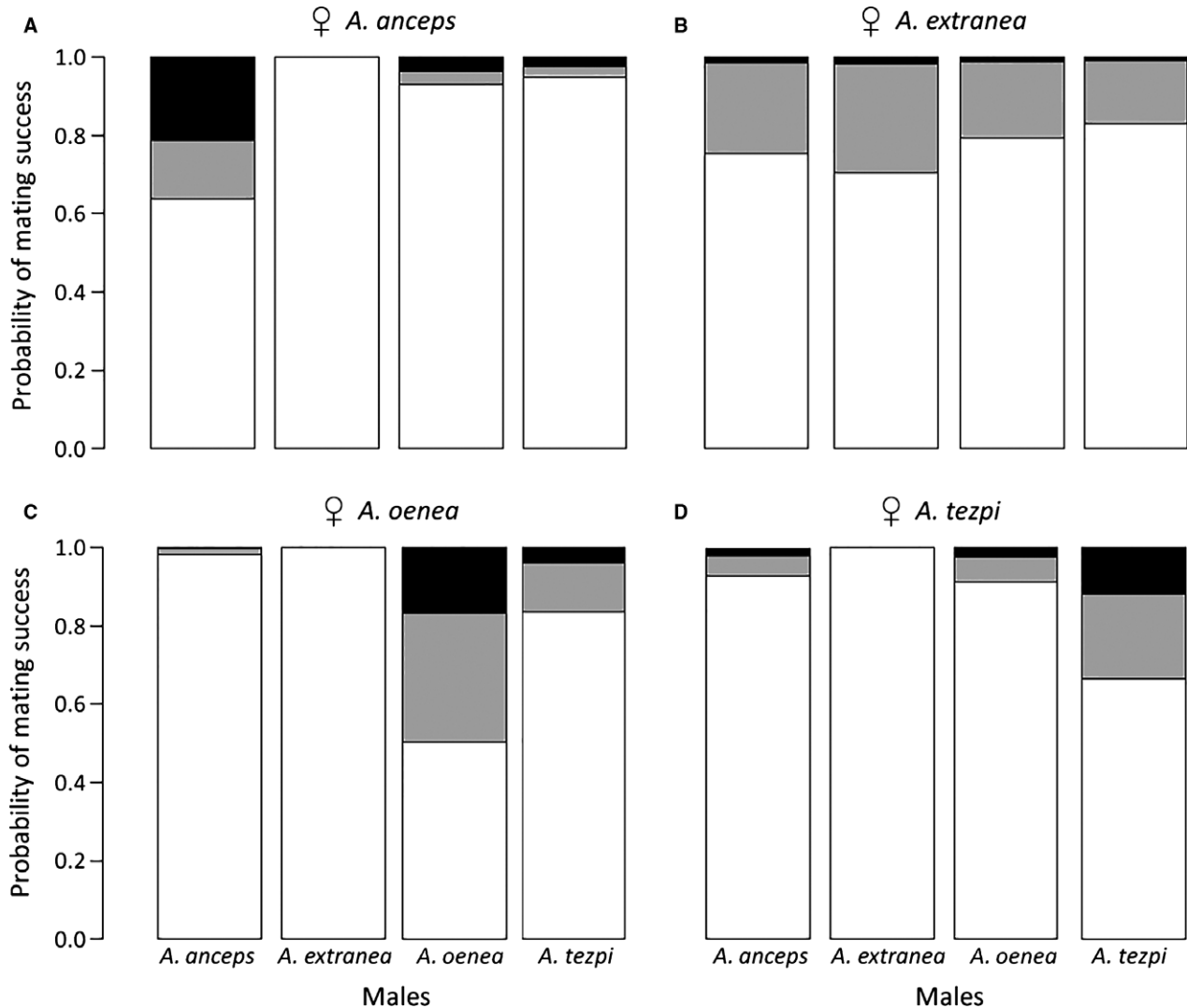
Sexual (tactile) isolation was strong, as none of the three females (two of *A. tezpi*, one of *A. oenea* and one of *A. anceps*) that formed a heterospecific *tandem* bent her abdomen to form the wheel position, i.e. none of them accepted to mate.

Our results by the CLMM model indicated that species recognition in terms of sexual isolation was

greater between conspecifics than between heterospecific (Table 2, Supporting Information, Table S2, S3). In some heterospecific cases, these species could produce *tandem* (Supporting Information, Table S2). The likelihood ratio test testing the effect of male *sexual response* to females of different species is shown in Figure 2, and indicated the predicted probability by models for each behavioural response or indicators of sexual isolation (visual) and mechanical isolation (Supporting Information, Table S2). Although heterospecific recognition occurs, species recognition in total terms (species recognition and mechanical compatibility) was significantly higher in conspecific than in heterospecific combinations in three out of the four species: in *A. anceps* [deviance = 18.6 Pr( $\chi$ ) = < 0.001], *A. oenea* [deviance = 54.5 Pr( $\chi$ ) = < 0.001] and *A. tezpi* [deviance = 22.1 Pr( $\chi$ ) = < 0.001; Table 2, Supporting Information, Table S3].

#### POPULATION DENSITY, SPECIES DISTRIBUTION AND DEGREE OF SYMPATRY

Species proportions in the studied community was highly dissimilar in the year of study: *A. extranea*



**Figure 2.** Maximum likelihood estimated by cumulative link mixed models for reproductive isolation. Intensity of the predicted values of reproductive isolation barriers estimated from the predicted values of the probability of male responses: not sexually responses (NSR) (white), *attempt of tandem* (A T) (grey), and *tandem* (T) (black) in conspecific vs. heterospecific combinations for the four studied species: (A) *A. anceps*; (B) *A. extranea*, (C) *A. oenea*; and (D) *A. tezpi* females.

was the less abundant species (7%) followed by *A. anceps* (26%), *A. oenea* (30%) and *A. tezpi*, which was the most abundant *Argia* damselfly species (37%).

Degree of sympatry and allopatry between each pair of species in terms of the observed sympatry (number of overlapped locations) and observed allopatry (number of non-overlapped locations) is shown in Table 3. Potential distributions were estimated by using niche models. Best models were those with the best performance levels, i.e. with the highest TSS values (*A. anceps* = 0.889, *A. extranea* = 0.88, *A. oenea* = 0.879 and *A. tezpi* = 0.897). Potential distributions in terms of potential sympatry (overlapped km<sup>2</sup>) and observed

allopatry (non-overlapped km<sup>2</sup>) are supplied in Table 3, Figure 3 and Supporting Information, Table S4. In summary, the four *Argia* studied species showed a wide potential range in North America and shared (between each other) more than 80% of their territory.

#### CORRELATION BETWEEN REPRODUCTIVE ISOLATION AND THE GENETIC DIVERGENCE AND DEGREE OF SYMPATRY

Figure 4A, B show phylogenetic relations between the four *Argia* studied species. Pairwise of genetic distances between the *Argia* damselflies ranged from

**Table 2.** Likelihood ratio test of the male's efficiency in conspecific vs. heterospecific female presentations

	Deviance	Pr ( $\chi$ )
<i>A. anceps</i>	18.578	< <b>0.001</b>
<i>A. extranea</i>	0.840	0.840
<i>A. oenea</i>	54.481	< <b>0.001</b>
<i>A. tezpi</i>	22.091	< <b>0.001</b>

Table shows deviance values and significance values ( $P < 0.05$ ) in bold. The intensity of male responses, in terms of visual (sexual) recognition, was significantly higher in conspecific than heterospecific interactions for *A. anceps*, *A. oenea* and *A. tezpi*.

(4.60–7.76%) for the mtDNA 16S. *Argia anceps* showed the highest levels of genetic divergence with the other three species (5.75%, 7.20% and 7.76% with *A. extranea*, *A. oenea* and *A. tezpi*, respectively; Supporting Information, Table S5). The degree of genetic divergence between the other three pair of species: *A. extranea* and *A. oenea* (4.60%); *A. extranea* and *A. tezpi* (4.84%); and *A. oenea* and *A. tezpi* (5.99%) were much lower (Supporting Information, Table S5).

A significant negative correlation ( $r = -0.678$ ,  $P < 0.05$ ; Fig. 4C) between observed sympatry and

sexual (visual) isolation was detected in the *Argia* studied species. However, we did not detect a significant correlation between genetic divergence and degree of sexual (visual) isolation ( $r = -0.116$ ,  $P = 0.72$ ; Fig. 4D). Mechanical isolation was not included in these correlational analyses because of this barrier was almost complete in nine out of the 12 species combinations.

## DISCUSSION

In territorial damselflies and with courtship, heterospecific interactions are mainly precluded by visual sexual isolation, while in non-territorial damselflies and without courtship, heterospecific interactions are mostly precluded by mechanical or tactile sexual isolation (see Wellenreuther & Sánchez-Guillén, 2016). For example, in territorial calopterygids, male wing melanisation is used during intra- and interspecific recognition (Waage, 1979; Wellenreuther *et al.*, 2010). To date, in the studied non-territorial damselflies with learned mate preferences, such as *Ischnura* and *Enallagma* genera, intra- and interspecific recognition was mainly through mechanical (physical contact of primary and secondary genitalia) or sexual (tactile) barriers

**Table 3.** Observed and potential distribution ranges: sympatric and allopatric degrees

Species	Current (observed) distribution		Potential distribution	
	Absolute-sympatric (locations)	Relative-sympatry	Absolute-sympatry (km <sup>2</sup> )	Relative-sympatry
<i>A. anceps</i> –all species	42	0.70	264 101	0.77
<i>A. extranea</i> –all species	53	0.54	328 864	0.86
<i>A. oenea</i> –all species	65	0.71	334 050	0.71
<i>A. tezpi</i> –all species	40	0.63	207 808	0.91
<i>A. anceps</i> – <i>A. extranea</i>	28	0.47	245 272	0.72
<i>A. anceps</i> – <i>A. oenea</i>	29	0.48	240 768	0.70
<i>A. anceps</i> – <i>A. tezpi</i>	19	0.32	170 253	0.50
<i>A. extranea</i> – <i>A. anceps</i>	28	0.28	245 272	0.64
<i>A. extranea</i> – <i>A. oenea</i>	34	0.34	299 225	0.78
<i>A. extranea</i> – <i>A. tezpi</i>	21	0.21	159 719	0.42
<i>A. oenea</i> – <i>A. anceps</i>	29	0.32	240 768	0.51
<i>A. oenea</i> – <i>A. extranea</i>	34	0.37	299 225	0.63
<i>A. oenea</i> – <i>A. tezpi</i>	35	0.38	200 736	0.42
<i>A. tezpi</i> – <i>A. anceps</i>	19	0.30	170 253	0.74
<i>A. tezpi</i> – <i>A. extranea</i>	21	0.33	159 719	0.70
<i>A. tezpi</i> – <i>A. oenea</i>	35	0.55	200 736	0.88

Table shows current distribution in terms of the absolute and relative number of sympatric locations. Potential distribution areas (km<sup>2</sup>) were estimated by using ecological niche models; absolute values represent the extent in km<sup>2</sup> of sympatry areas, and the relative values represent the proportion of the area of sympatry. Data are given for each species respect to the other three species (*A. anceps*–all species, *A. extranea*–all species, *A. oenea*–all species and *A. tezpi*–all species) and for each pair of species combination.

(Robertson & Paterson, 1982; Fincke *et al.*, 1997; Sánchez-Guillén, Van Gossum & Cordero-Rivera, 2005; McPeck, Shen & Farid, 2009; Sánchez-Guillén *et al.*, 2012, 2014b). In our study, reproductive isolation was symmetrical in all species except *A. extranea* (which was the most derivative of the studied species). *Argia extranea* males showed complete visual isolation with the other three studied species, while *A. extranea* females were the most preferred females in heterospecific combinations, although they were completely isolated by the action of the mechanical barriers. Reproductive isolation was complete by the joint action of the three studied barriers (visual, mechanical and tactile). Sexual (visual) isolation was the most important barrier in all species, precluding the 90.6% of the matings. Although mechanical isolation was almost complete, only precluded the remaining 8.7% of the matings. Finally, isolation was very strong via female tactile recognition, precluding the last 0.7% of the matings, due to that in none of the three heterospecific *tandems*, females accepted the copula. However, since we recorded male responses only, future research should also focus on female responses.

A salient result of our study showed that *A. extranea* showed highly asymmetry in reciprocal crosses with the other three studied species. Males of *A. extranea* were completely isolated by sexual (visual) isolation, while *A. extranea* females were the most preferred females in heterospecific combinations, but were completely isolated by mechanical incompatibilities. Interestingly, in relation to the four studied species, *A. extranea* is the less abundant species in the community; the most derivate species (Fig. 4A, B); and shows the lowest degree of pairwise genetic divergence. Although *A. tezpi* males were partially attracted to the three heterospecific females, *tandems* were in all cases mechanically precluded, and thus *A. tezpi* males are also reproductively isolated to the other three species. Moreover, although *A. oenea* and *A. anceps* males are involved in heterospecific *tandems* with *A. anceps* and *A. tezpi* females in the case of *A. oenea*, and with *A. oenea* females in the case of *A. anceps*, they are also reproductively isolated by sexual (tactile) isolation, which was complete on the basis that none of the three females that formed a *tandem* (with a heterospecific male) bent her abdomen to form the *wheel position*, i.e. none of them accepted the copulation.

#### REPRODUCTIVE ISOLATION, GENETIC DIVERGENCE AND PHYLOGENETIC RELATIONS

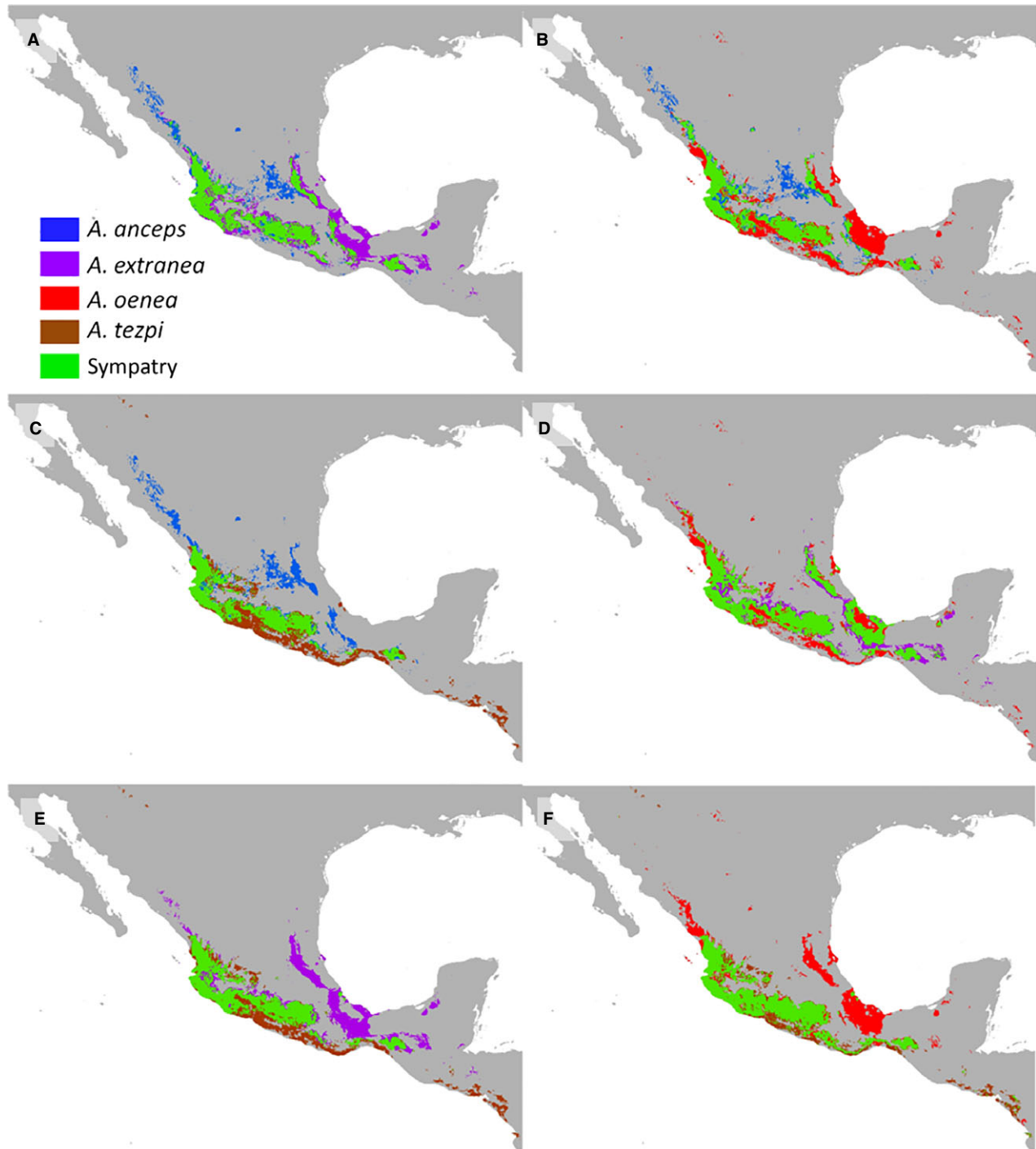
Males of the four *Argia* species were discriminative and preferred to mate with their conspecific females, although only one species (*A. extranea*) was

completely isolated to the other three *Argia* species by sexual (visual) isolation. Because of reproductive barriers act in a sequential way, the contribution of each barrier (to total reproductive isolation) depends on the contribution of previous barriers. For instance, if sexual isolation precludes 85% of matings between males of *A. tezpi* and females of *A. anceps*, although the absolute intensity of the mechanical isolation is complete (100%), it can only prevent the remaining 15% (relative contribution), being thus, less important precluding isolation than sexual isolation.

Our observations are in contrast with previous studies in other coenagrionid non-territorial damselflies such as *Ischnura* or *Enallagma* (Robertson & Paterson, 1982; Fincke *et al.*, 1997; Sánchez-Guillén *et al.*, 2005, 2012, 2014b; McPeck *et al.*, 2009), but are in line to what has been observed in the territorial damselfly genus *Calopteryx* (Waage, 1979; Weltenreuther *et al.*, 2010). Evidence from these previous studies suggested that in territorial taxa with courtship, sexual (visual) isolation is the most important reproductive barrier, while in non-territorial taxa, without courtship, mechanical isolation is the most important reproductive barrier. However, based on the positive correlation detected between genetic divergence and degree of the reproductive isolation in damselflies (Sánchez-Guillén *et al.*, 2014a), our results are not contradictory with previous observations, but also complementary. In our study, all four *Argia* species were more genetically divergent between each other (from 4.84% to 7.76%) than the previously studied *Ischnura* damselflies, whose genetic divergence was lower than 1% in all pairwise combinations (Monetti, Sánchez-Guillén & Rivera, 2002; Sánchez-Guillén *et al.*, 2012, 2014b). Thus, assuming the 'speciation clock' phenomenon, and based on evidences from previous studies with *Ischnura*, *Enallagma* and *Calopteryx* damselflies and our results with *Argia* species, our results suggest that mechanical barriers appeared earlier than the sexual (visual) barriers in the speciation process in odonates, but since mechanical barriers can only prevent the gene flow allowed by sexual isolation, their importance is lower in more divergent species, such as the case of the studied *Argia* species.

The relative strength of the isolating barriers depends on the direction of gene exchange (Arnold & Hodges, 1995; Tiffin, Olson & Moyle, 2001; Takami *et al.*, 2007), and in this context, asymmetries in pre-mating (sexual and mechanical) barriers have been discussed intensively (e.g. Kaneshiro, 1980). The Kaneshiro (1980) hypothesis proposed that when one species is derived from another via a founder event, females from the ancestral species are more likely to reject males from the derived species which would

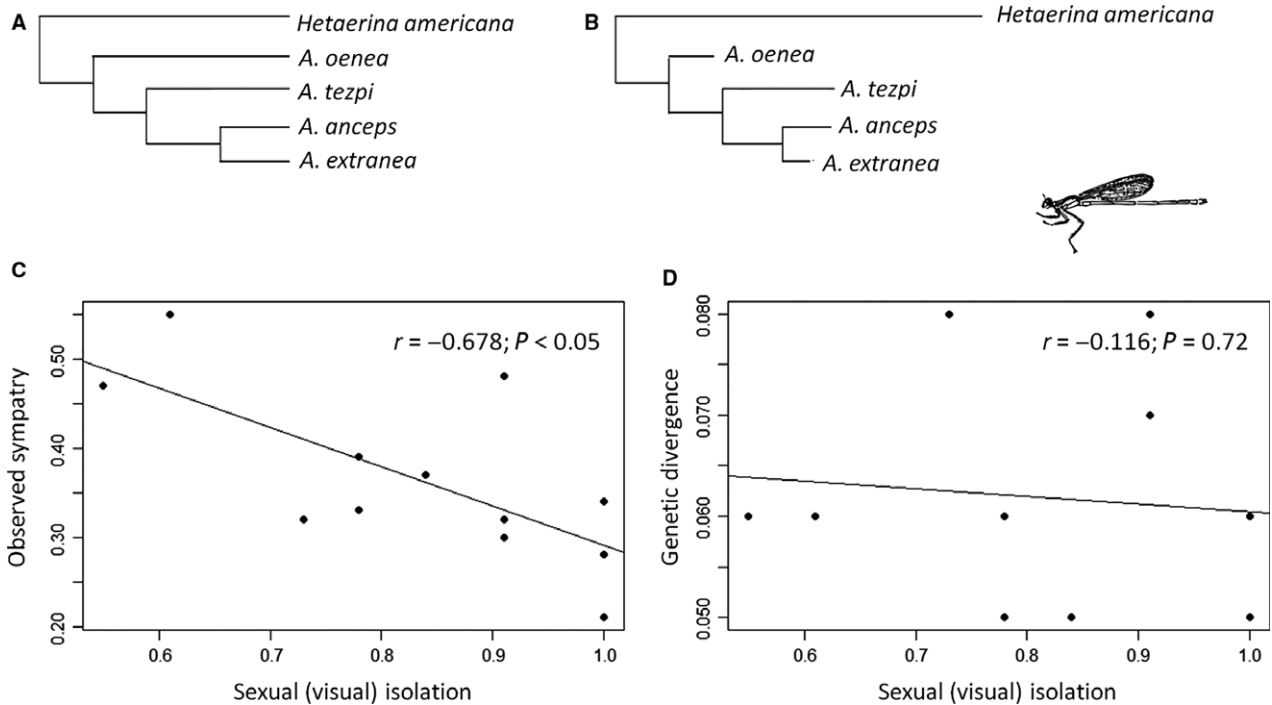




**Figure 3.** Degree of potential sympatry between the six pairs of species combinations. Overlapping areas between the four *Argia* studied species were predicted by using potential distributional models: green colour represents areas in which suitable conditions converge between each pair of species: (A) *A. anceps* and *A. extranea*; (B) *A. anceps* and *A. oenea*; (C) *A. anceps* and *A. tezpi*; (D) *A. extranea* and *A. oenea*; (E) *A. extranea* and *A. tezpi*; and (F) *A. oenea* and *A. tezpi*.

have changed more [especially due to the loss of behavioural (mating) traits] than males from the ancestral species. Several examples of the

asymmetry in reproductive isolation come from the genus *Drosophila* (Watanabe & Kawanishi, 1979; Kaneshiro, 1988) as well as from salamanders



**Figure 4.** Evolutionary trees and correlation between genetic divergence and degree of sympatry with the strength of sexual (visual) isolation. A, strict consensus parsimonious tree from combined rDNA 16S. B, best maximum likelihood tree from the rDNA 16S. Both trees have been re-drawn from C aesar & Wenzel (2009), and indicate a simplified relative relationships. C, correlation between the degree of observed sympatry and the strength of sexual (visual) isolation ( $r = -0.678$ ,  $P < 0.05$ ). D, correlation between genetic divergence with the degree of sexual (visual) isolation ( $r = -0.116$ ,  $P = 0.72$ ).

(Arnold & Hodges, 1995), parasitic wasps (Bordenstein, Drapeau & Werren, 2000), snakes (Shine *et al.*, 2002) and more recently from *Ischnura* damselflies (Sánchez-Guillén *et al.*, 2012). Both, mechanical (tactile) and sexual (visual) isolation are part of the mechanisms of species recognition in odonates. According to the Kaneshiro (1980) hypothesis, sexual asymmetrical isolation involves preferential mating with females of the most derivative species by males of the ancestral species. The reciprocal asymmetrical isolation observed in *A. extranea* with the other *Argia* studied species, is a candidate species to test this Kaneshiro hypothesis.

#### REPRODUCTIVE ISOLATION AND DEGREE OF SYMPATRY AND THE ROLE OF LEARNING MATING PREFERENCES

Our findings are in line with the role of learning mating preferences in interspecific sexual isolation, which is consistent with a positive frequency dependent selection. Males learn to prefer the conspecific females, and this learning depends on the frequency of the heterospecific females in the community, i.e. the higher the density of the heterospecific female is in the community, the higher the degree of the

sexual (visual) isolation is in between these pair of species. Our study has also showed evidences of the role of learning in heterospecific sexual recognition in non-territorial and without courtship coenagrionid damselflies. Previous studies had supported its role in territorial odonates (see Waage, 1979; Wellenreuther *et al.*, 2010). Thus, sexual (visual) isolation can be driven by learning mate preferences in a non-territorial and without courtship genus (*Argia*), suggesting that visual recognition and learning ability may be widespread in odonates, including non-territorial species.

Our results showed that all four *Argia* species have a high degree of sympatry (their ecological characteristics have been preserved), sharing more than 70% of their potential distribution. We hypothesize that the observed finding in sexual (visual) isolation in the studied locality may be a general pattern in other sympatric localities of these species based on the high degree of sympatry between the four studied species (around 70%). It is possible that learning has allowed these four congeners to share the same habitats. Take for example the case of *A. oenea*. Although this species shares the fewest environmental characteristics, it still shows a high

degree of niche conservatism. One explanation for such patterns may lie on the speciation process in this genus. It is known that when speciation processes are driven by sexual selection, there is low niche differentiation. This may be the case for the four *Argia* species studied. Radiation *via* sexual selection is not related to resource exploitation, and thus reproductive isolation is not linked to the build-up of ecological niche diversification (Gittenberger, 1991; Rundell & Price, 2009). Other explanations for such radiation are those mediated by chromosomal architecture, gene duplication or ploidy levels, or through processes arising from mating male-female interactions, such as sexual conflict and learning (Gittenberger, 1991; Rundell & Price, 2009). The low interest in radiations *via* sexual selection have limited the number of studies that have dealt with the concept so that only a few examples exist (Mendelson & Shaw, 2005; Kozak & Wiens, 2006; Pereira & Wake, 2009; Wilke *et al.*, 2010).

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## SUPPORTING INFORMATION

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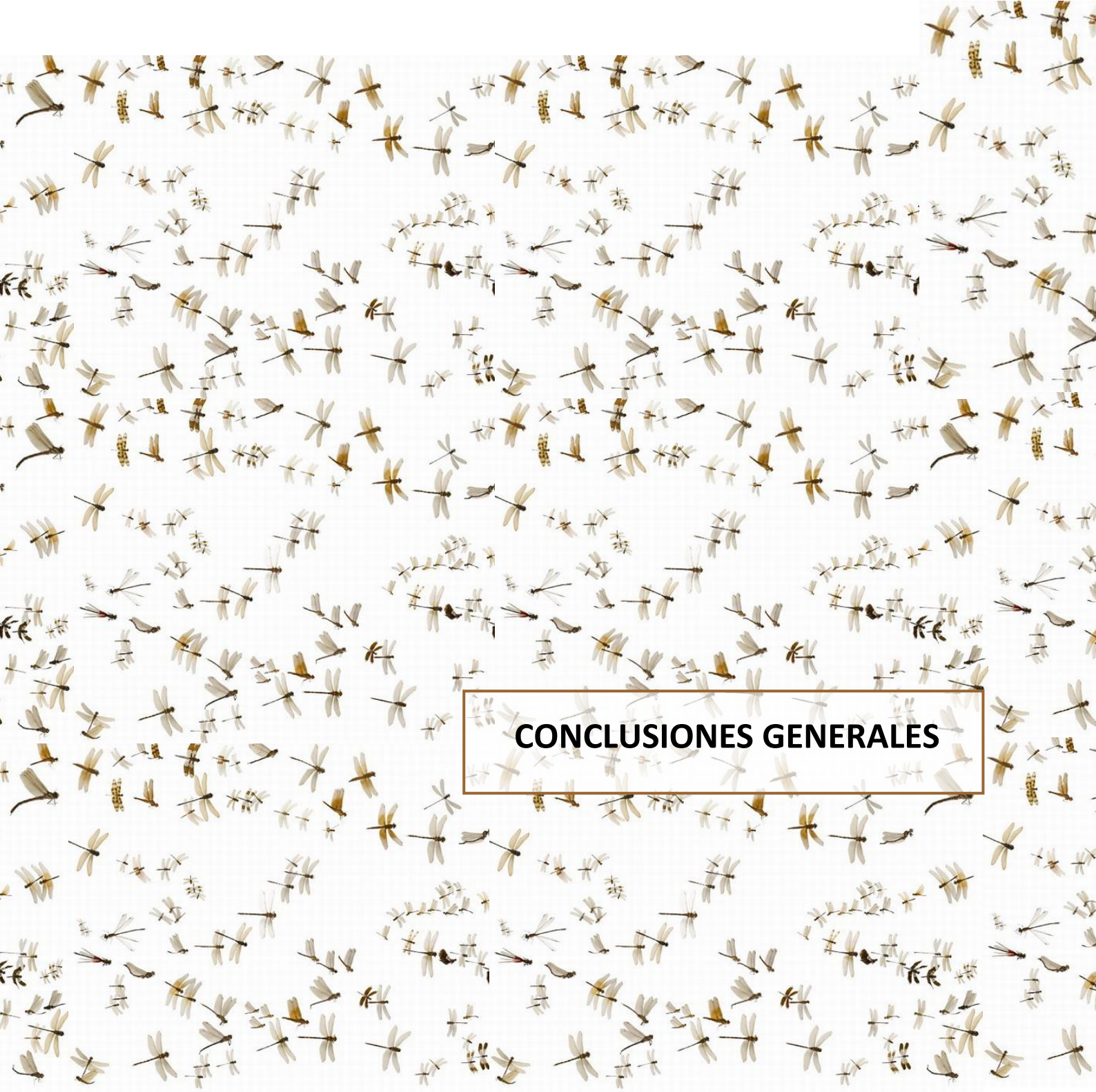
**Table S1.** Chi-squared tests of reproductive isolation (sexual and mechanical) between *Argia* species.

**Table S2.** Maximum likelihood provided by cumulative link mixed models.

**Table S3.** Cumulative link mixed models used to analyse the reproductive success measured in the different components of fitness.

**Table S4.** Potential distribution of *Argia* species.

**Table S5.** Estimates of evolutionary divergence between *Argia* species.



## **CONCLUSIONES GENERALES**

## Conclusiones generales

Uno de los principales resultados que observamos en este trabajo es la tendencia de las especies de libélulas a incrementar su área potencial de distribución, como un aumento en las proyecciones de idoneidad de hábitat favorable para las especies, en respuesta al cambio climático. Si bien hay especies cuya área de idoneidad se verá más restringida, el patrón general que se observa en las proyecciones es de incremento. De acuerdo con nuestros escenarios de cambio climático, la mayoría de las especies (20 de 30) aumentarán sus límites de distribución potencial.

Nuestras proyecciones indican que los aumentos en las áreas de distribución ocurrirán con mayor intensidad en latitudes al norte, como se ha documentado en otras especies de Odonata en el centro y norte de Europa. Estos incrementos de distribución potencial de las especies del género *Argia*, estarán acompañados por incrementos en extensas áreas de simpátricas donde convergan las distribuciones potenciales de las especies. A su vez las nuevas áreas de distribución potencial donde coexistan las especies en simpatría estarán acompañadas por nuevas interacciones ecológicas entre especies. Esto puede llevar a la formación de zonas de posible hibridación donde es probable que se produzcan encuentros sexuales interespecíficos en especies.

Nuestros resultados indican que 94 combinaciones de pares de especies con hibridación altamente predicha muestran un grado de superposición en sus áreas de distribución potencial. Curiosamente, para las condiciones actuales, las especies *Argia* se superponen en gran medida en su distribución potencial proyectada al presente, lo que significa que estas especies comparten en gran medida características de nicho ecológico, mostrando pequeñas diferencias interespecíficas de nicho. En odonatos, se ha reportado que estas pequeñas diferencias de nicho pueden conducir a frecuentes interacciones sexuales interespecíficas a escala local con alta intensidad de selección sexual, lo cual podría estar dirigiendo la especiación este género. De hecho, este patrón puede parecer común en los odonatos que generalmente muestran grandes

diferencias en los caracteres sexuales secundarios, pero a menudo tienen diferencias de nicho interespecífico más pequeñas.

Así, la hibridación podría ocurrir como consecuencia del cambio climático en especies simpátricas con baja diferencia genética del género *Argia*. Detectamos 108 pares de especies con bajas distancias genéticas con respecto al umbral de hibridación de 1,78% de diferencia genética para el marcador de COI. La superposición espacial a su vez, podría promover la formación de zonas híbridas y esto aumentaría según lo observado en nuestras proyecciones de cambio climático. Además, el cambio climático puede inducir a que las zonas híbridas actuales sean inestables, como se observa en algunas especies de aves. Ignoramos en gran medida las consecuencias de formación de los nuevos híbridos. Un escenario posible es el que se ha observado en híbridos del género *Ischnura*, los cuales son más exitosos que sus especies parentales, y han desplazado por lo tanto a las especies parentales de sus hábitats originales.

Nuestros resultados pueden ser útiles para proyectar y predecir cómo las especies del género *Argia* podrían responder al cambio climático y estudiar como aumentarán las zonas de posible hibridación entre especies *Argia* estrechamente relacionadas, en consecuencia, a los cambios de distribución potencial ante el cambio climático. Nuestros resultados, a su vez, pueden ayudar a dirigir los esfuerzos de conservación en este grupo de organismos, para comprender los patrones de riqueza y biodiversidad, por ejemplo, como lo hemos propuesto para la especie *A. garrisoni*, la cuál presenta una distribución potencial sumamente reducida y en áreas donde es acentuada la pérdida de hábitat, o bien, estudiando la función de las áreas naturales protegidas que se crearon para proteger la biodiversidad, y si esto se vera modificado bajo escenarios de cambio climático.



## **APÉNDICE I**

**Estimating distribution area in six *Argia* damselflies (Insecta:  
Odonata: Coenagrionidae) including *A. garrisoni*, a threatened  
species**

**Estimando el área de distribución de seis caballitos del diablo  
del género *Argia* (Insecta: Odonata: Coenagrionidae)  
incluyendo *A. garrisoni*, una especie amenazada**

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## **Abstract.**

Similar to other organisms, damselflies and dragonflies (Insect: Odonata) are currently facing a number of threats. One tool to provide a straightforward assessment of how much at risk a species is, is distribution area. Here we have used ecological niche modeling to estimate distribution range for six species of *Argia* damselflies distributed in North America: *A. cuprea*, *A. funcki*, *A. garrisoni*, *A. harknessi*, *A. munda* and *A. rhoadsi*. These species are not included in the International Union for the Conservation of Nature (IUCN) Red List, except for *A. garrisoni* which has been categorized as Least Concern. Our results indicated large distributions areas for all species, except for *A. garrisoni*, (8,038 km<sup>2</sup> after a refinement analysis looking for suitable habitat). Large distribution can be explained by similar niche properties shared by all study species, as has also been detected for other *Argia* species. This is not the case for *A. garrisoni* whose situation seems worrying. This species was found in the Mexican state of San Luis Potosí in 1999 and there have been no further observations so it deserves further inspection to see whether current populations are at risk. In the meantime, and according to the IUCN criteria, *A. garrisoni* should be placed under a vulnerable category.

Resumen. Similar a lo que pasa con otros organismos, los caballitos del diablo y las libélulas (Insecta: Odonata) enfrentan actualmente una serie de amenazas. Una herramienta que provee una evaluación inmediata de cuánto una especie está en riesgo, es el área de distribución. Hemos usado aquí el modelado de nicho ecológico para estimar el rango de distribución de seis especies de caballitos del diablo del género *Argia*, distribuidas en Norte América: *A. cuprea*, *A. funcki*, *A. garrisoni*, *A. harknessi*, *A. munda* y *A. rhoadsi*. Estas especies no están incluidas en la Lista Roja de la Unión Internacional para la Conservación de la Naturaleza (UICN), excepto por *A. garrisoni* la cual se categorizó como de poca importancia ("Least Concern"). Nuestros resultados indicaron distribuciones amplias para todas las especies, excepto para *A. garrisoni* (8,038 km<sup>2</sup> después de un análisis refinado que buscaba hábitat disponible).

Una distribución amplia puede explicarse por propiedades de nicho similares compartidas por todas las especies, tal como se ha detectado para otras especies del género *Argia*. Este no es el caso para *A. garrisoni* cuya situación es preocupante. Esta especie se encontró en el estado mexicano de San Luis Potosí en 1999 y desde entonces no se ha vuelto a observar por lo que debería buscarse para saber si sus poblaciones actuales están en riesgo. Mientras tanto, y de acuerdo a los criterios de la UICN, *A. garrisoni* debería ser incluida en la categoría de vulnerable.

**Key words:** Distribution range, ecological niche modeling, damselflies, *Argia*, *Argia garrisoni*, IUCN

**Palabras clave:** Rango de distribución, modelado de nicho ecológico, caballitos del diablo, *Argia*, *Argia garrisoni*, UICN

## Introduction

The biodiversity crisis that has been documented in detail for several taxa (e.g. Dirzo et al., 2014), includes insects of the Odonata order (dragonflies and damselflies) with one in ten odonate species being threatened with extinction (Clausnitzer et al., 2009). This despite their fairly high dispersal ability and flexibility to occupy different habitats (Stoks & Córdoba-Aguilar, 2012). Similar to other aquatic insects, deforestation and susceptibility of anthropogenic change for their freshwater habitats (especially lentic waters; Clausnitzer et al., 2009) seem major drivers underlying threats for odonate viability (Dijkstra et al., 2014). However, these are major, general causes but we are largely ignorant of the particular threats most odonate species face.

*Argia* damselflies are a highly speciose genus that includes up to 144 species (Garrison, 1994; Caesar & Wenzel, 2009), with a large occurrence throughout the American continent. Some generalities of their ecology are that they use both lentic and lotic freshwaters, and occur in different habitats that include tropical and non-tropical areas (Garrison, 1994). *Argia* species are mostly small-sized odonates with males of some species being either territorial (e.g. Guillermo-Ferreira & Del Claro, 2012) or nonterritorial (Sánchez-Guillén et al., 2014). The International Union for Conservation of Nature (from now on, IUCN) has included 36 species of *Argia* in their Red List, of which only *A. sabino* appears threatened (“vulnerable” category; IUCN, 2017). This reduced number implies that further efforts are badly needed to uncover more *Argia* species. One criteria to be included as part of IUCN assessment is the extent of occurrence (criterion B; IUCN, 2017) which can be gathered using ecological niche models (e.g. Guisan & Zimmermann, 2000; Gouveia et al., 2011).

With the aim of providing biological information of odonates that have not been included in the IUCN Red List, in this work we have assessed the distribution area of six *Argia* species: *Argia cuprea* (Hagen, 1861), *A. funcki* (Selys, 1854), *A. garrisoni* Daigle, 1991, *A. harknessi* Calvert, 1899, *A. munda* Calvert, 1902 and *A. rhoadsi* Calvert, 1902.

Except for *A. garrisoni* which appears as Least Concern (IUCN, 2017), the remaining five species have not been assessed.

## **Materials and methods**

### ***Species distributional data***

Presences of the six *Argia* species were compiled from literature records, confirmed records in OdonataCentral (<http://www.odonatacentral.org>), CONABIO records ([www.conabio.gob.mx](http://www.conabio.gob.mx)), GBIF records ([www.gbif.org](http://www.gbif.org)), and published papers, thesis and data provided by experts in odonates. All data were checked carefully for geographic accuracy which, when in doubt, included asking specialists (e.g. Rosser Garrison).

Notice that most records were gathered from OdonataCentral, which is a repository of odonate record information. We are confident that the biases of identification should be minimal because these data were provided by experts in these animals. Still, we limited our analyses to the six species indicated above as these were the ones we were fully confident in regards to the quality of their records.

Distribution models were built only for species with more than 30 localities. Thus, the final data set included 1585 unique presences of the six species, which were predominantly adult specimens: 759 records for *A. cuprea*, 261 for *A. funcki*, 255 for *A. garrisoni*, 185 for *A. harknessi*, 86 for *A. munda* and 39 for *A. rhoadsi*. Although these records spanned the last 40 years, most came from the last 15 years. The database is available upon request.

### ***Ecological Niche Models***

#### ***Environmental predictors***

We used the WorldClim 1.4 ([www.worldclim.org](http://www.worldclim.org)) data set (Hijmans et al., 2005) at 0.041666669 degrees (or 5 km) of resolution to set predicting bioclimatic variables for our models. To establish a set of uncorrelated climatic variables, we intersected the variables with target group points, and with 10,000 points randomly selected in the

extension of the study area (M). We eliminated some variables with an exploratory data analysis of contribution of variables using jackknife and Pearson correlation analyses (i.e. any value >0.7). Thus, we selected variables with low correlation and high contribution to reduce the parametrization of the models. After this, the final data set included uncorrelated variables which had more biological importance for *Argia* species, and more contribution according to the jackknife analysis. Such variables were: mean diurnal temperature range (bio 02), temperature seasonality (bio 04), mean temperature of warmest quarter (bio 10), precipitation of wettest month (bio 13), precipitation of driest month (bio 14), and precipitation seasonality (bio 15). Our study area included North America where our study species occur.

### ***Background selection***

Our study area is included in North and Central America where members of the genus *Argia* occur, covering land between the latitudes 53.00 to 0.00N, and the longitudes -130.00 to -55.00W. To choose the best background, preliminary species distribution models were generated with Maxent 3.3.3k (Phillips et al., 2006) with target group points, 10,000 points randomly selected in the extension of the study area (M), and with special extent delineating M for each particular species with ecoregions (World Wildlife Fund; [www.worldwildlife.org/](http://www.worldwildlife.org/)) for North America, and biogeographical provinces (CONABIO) for Mexico. Models were constructed setting several parameters to default ('Auto features', convergence = 10<sup>-5</sup>, maximum number of iterations = 500). However, we used random seed (with a 30 test percentage), 10 replicates, removed duplicate records, ran bootstrap replicated type, with no extrapolation and no clamping. All this to find which combination of settings and variables generated the best outcomes (highest area under the curve, or AUC) while minimizing the number of model parameters, as well as producing 'closed', bell-shaped response curves guaranteeing model calibration and transferability (Elith et al., 2010). The best background was 10,000 points randomly selected in the extension of the study area.

### ***Training ENM***



Final models were built with BIOMOD (Biodiversity Modelling) package in R software. This package is a platform for predicting species' distribution, including the ability to model the distribution using various techniques and test patterns (Thuiller et al., 2010). When using BIOMOD, we trained models using four widely used algorithms: Maximum Entropy (Maxent), Random Forest (RF), Generalized Boosting Methods (GBM) and Multivariate Adaptive Regression Splines (MARS). These models have shown good performance in terms of predictive power (Pliscoff & Fuentes-Castillo, 2011; Reiss et al., 2011; Broennimann et al., 2012). From individual models obtained with these different algorithms, we generated a "consensus model". Such model combination is the best logistic compromise to avoid either overfitting and overpredicting (Merow et al., 2014). In other words, this reduces biases and limitations of using only individual models. 70 % of data was used for training, and 30 % for validation with 10 replicates. The final validation of models was performed with TSS (True Skills Statistics), average net rate of successful prediction for sites of presence and absence (Liu et al., 2009), ranging from -1 to 1, where the more positive values indicate a higher degree of accuracy and discrimination model (Allouche et al., 2006) (Table 1). It is noteworthy that the result of these models is not the area that species occupies absolutely, because these models do not consider population dynamics, dispersibility, interactions with other species and human impacts. However, these models can make right transferences where species can be potentially found given their environmental conditions. This is based on the assumption that the distribution known of species provides enough information to characterize its environmental requirements.

A total of 420 models were generated, whose performance was assessed by means of the AUC and TSS statistic, while minimizing the number of model parameters. The best presence/absence models using the "10 percentile training presence" are presented. This threshold was used because we prefer to err in the side of caution accepting that a 10% of our presences could be problematic (for a similar rationale, see Sánchez-

Guillén et al., 2013). The best models of current climatic conditions of species were used to generate projections into the geography.

### **The case of *Argia garrisoni***

Our results indicated that *A. garrisoni* showed a distribution area of less than 20,000 km<sup>2</sup> (see below). Since a distribution area smaller than 20,000 km<sup>2</sup> is one of those criteria that allows categorizing whether a species may be threatened (IUCN, 2017), we took a step further to characterize the habitat of this species for any field assessment and/or future protection effort (for a similar effort, see Cuevas-Yáñez et al., 2015). For this characterization, all types of land use corresponding to the habitat of the species were selected and trimmed using both the soil cover layer and the vegetation cover of the Instituto Nacional de Estadística y Geografía, INEGI (Union of Layers, Series V: INEGI, 2011), removing, for example, urbanized or arid zones that do not correspond to *A. garrisoni*'s habitat, rendering a “clean” layer with this selection. From the overlap of this layer on the distribution map of the species obtained in the BIOMOD package, a reduced distribution area was provided compared to the initial distribution, but more adjusted to the actual location of the species. The map shown in the results is the one obtained after making the land use cut-off corresponding to the habitat for this species. We finally obtained the area in km<sup>2</sup> by calculating the number of cells to a size of 0.041666669, with ArcMap® software.

### **Results**

ENM predicted considerably large distribution for all species small distribution ranges except for *A. garrisoni* which had 9,991 km<sup>2</sup> (Fig. 1; Table 1). Our trimming exercise to predict a more suitable habitat for this species, rendered a more reduced area of 8,038 km<sup>2</sup>

### **Discussion**

Most *Argia* species that we modelled showed fairly large distribution areas (over 70,000 km<sup>2</sup>). This coincides with large area (previously estimated by Nava-Bolaños et al., 2017) for other *Argia* species such as *A. anceps*, *A. extranea*, *A. oenea* and *A. tezpi* that mainly occur in central Mexico. It seems that the niche properties of the entire genus is highly conserved as assessed by their large degree of area overlapping (i.e. sympatry; Nava-Bolaños et al., 2017). Such similar niche properties even imply frequent copulations with heterospecifics. This is the case of *A. rhoadsi* and *A. munda* which are found mainly in northeastern Mexico and its distribution extends from the southern part of Texas to the lower part of San Luis Potosí (Dunkle, 2004), inhabiting lagoons, streams and riversides (Abbott, 2001). Both species can be frequently found in heterospecific matings (A. Córdoba-Aguilar, unpub. obs.). As a matter of fact, possibly the radiation of the entire genus may not necessarily be related to their ecological pressures but to sexual selection forces as has been proposed for other damselflies (Wellenreuther & Sánchez-Guillén, 2016). This non-adaptive radiation may render species to be reduced habitat-specific. Perhaps the exceptions are those species that are at risk such as *A. sabino* (IUCN, 2017) and *A. garrisoni*.

Our results can be used to fulfill current IUCN Red List knowledge. As indicated before, no risk assessment has been carried out for *A. cuprea*, *A. funcki*, *A. harknessi*, *A. munda* and *A. rhoadsi*. Given the large area of occurrence estimation, these five species should be placed in a low risk category by not meeting the criteria of a reduced extension of occurrence. However, this is not the case of *A. garrisoni*, which is an endemic species from Mexico (González-Soriano & Novelo-Gutiérrez, 2014). Despite the reduced distribution estimated by our methodology, this species was abundant in the Mexican state of San Luis Potosí in 1999, in the roots of citrus along rivers and perched in banks of partially dry sludge (R. Garrison pers. comm.). However, since 1999 there have been no more recent records for this species. According to the IUCN criteria, the fulfillment of even a single criterion, in this case the B1, allows the change from least concern to vulnerable which is when an area of less than 20,000 km<sup>2</sup> is

estimated (IUCN, 2016). Even when one may see such criteria as too vague, restricted distribution areas correlate well with the probability of extinction in odonates (the smaller the area, the more likely that a species will disappear; Korkeamäki & Suhonen, 2002), so our risk concerns for *A. garrisoni* are likely to be founded.

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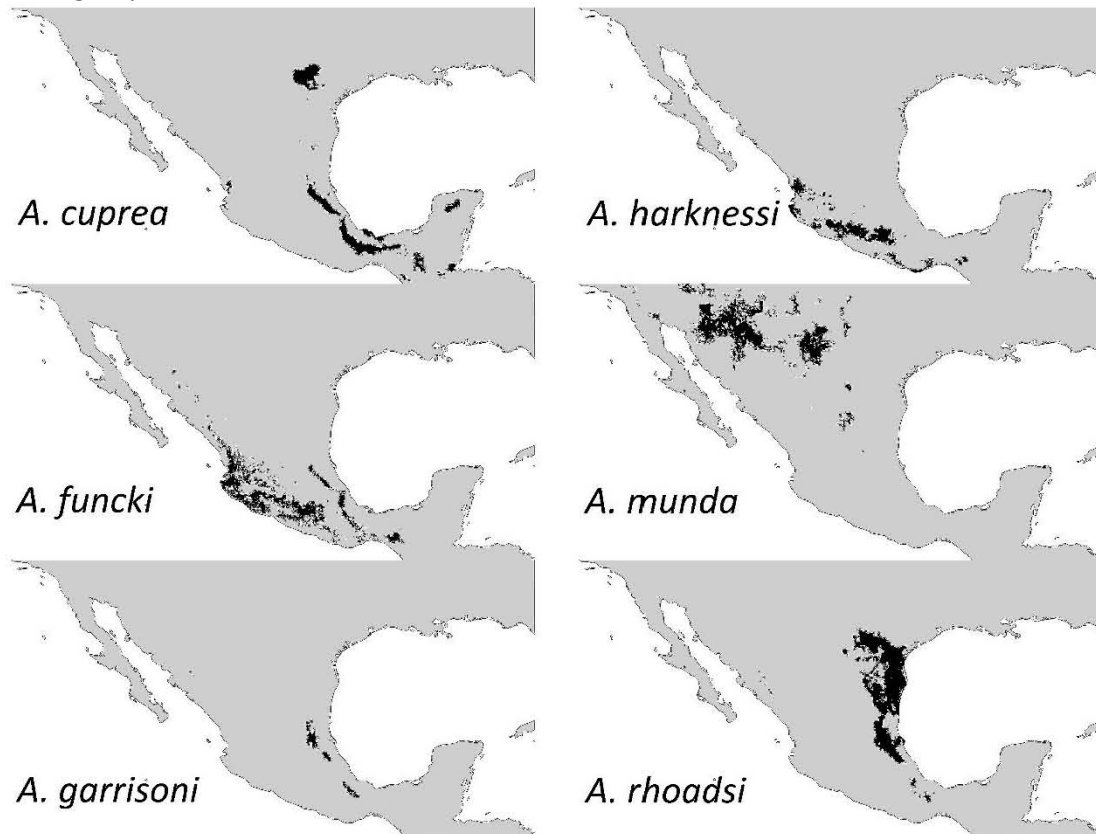
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**Table 1.** Estimates of distribution areas (in km<sup>2</sup>) for six species of the genus *Argia* according to and ecological niche models (ENM) and their TSS values.

Species	ENM area (km <sup>2</sup> )	TSS value
<i>Argia cuprea</i>	113,725	0.905
<i>Argia funcki</i>	130,143	0.853
<i>Argia garrisoni</i>	9,991	0.845
<i>Argia harknessi</i>	73,428	0.886
<i>Argia munda</i>	161,815	0.893
<i>Argia rhoadsi</i>	194,479	0.892



**Figure 1.** Distribution estimates and maps generated according to ecological niche modeling for six *Argia* species.



## APENDICE II

# **Argentinian odonates (dragonflies and damselflies): current and future distributions**

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## Abstract

In terms of conservation, Argentinian odonates have not been assessed using a quantitative approach. To reach this aim, we modelled the current and future (projected year 2050) distribution of 33 odonate (Anisoptera and Zygoptera) species that occur in Argentina. Our models of current times indicate a fairly wide distribution for most species but some exceptions are relevant for conservation purposes: *five of our modeled* species have less than 20,000 km<sup>2</sup> and, according to the IUCN Red List criteria, would be categorized as vulnerable. *Acanthagrion floridense*, *Lestes undulatus* and *Rhionaeschna pallipes* show slightly larger areas than 20,000 km<sup>2</sup> and so can be categorized as near threatened. Future distribution estimates indicate that other two species would face a conservation risk by also reducing their area by less than 20,000 km<sup>2</sup>: *Acanthagrion hidegarda* and *Rhionaeschna viginpunctata*. Although current protected areas embrace most odonate species in Argentina, it is still premature to conclude whether this situation will remain in the future given the physiological tolerance and dispersal abilities of the study species among other driving factors of distribution.

Key words: Odonata, Argentina, IUCN, status, current distribution, global change

## Introduction

Species distribution models have been widely used to assess the potential area where a species occurs as predicted by environmental variables (Peterson, 2006). Odonates have not been an exception to this practice with at least 30 different studies in different world regions (reviewed by Collins & McIntyre, 2015). Such interest is partly understood on the basis of the intrinsic threat that humankind has posed to freshwater bodies (e.g. Sala et al., 2000) related to the direct dependence of odonates on these bodies. Furthermore, a more recent analysis indicated that odonates can be used as the indicators of global change given their practicality as study models (i.e. large body size), well-described macro-ecological responses, key role as predators in aquatic and terrestrial habitats and their trend of becoming field-animal models for temperature-mediated responses (Hassall, 2015). Paradoxically, our current knowledge of the extinction risk for most odonates in the planet is extremely reduced. For example, the IUCN (IUCN, 2016) shows a shortage of species with strong geographical biases, with country-based assessments frequently lacking firm quantitative-supporting data (see for example, Paulson, 2004). One case is that of Argentina: 86 species are listed of which 1 is endangered, 1 is vulnerable, 2 are near threatened, 4 are data deficient and 78 are least concern (IUCN, 2016). This implies that a proper assessment is badly needed for this and other countries.

Distribution models of odonates have provided cues of how current distribution will be affected by increases in temperature (reviewed by Collins & McIntyre, 2015). These studies have covered up to 25% of the total world odonate diversity, and have shown that in general there will be shifts in distribution with lotic species, and narrow-distribution species (e.g. endemic) showing a tendency to have their areas reduced (reviewed by Collins & McIntyre, 2015). In this paper, we have carried out an exercise of calculating current and future distribution models for the case of Argentinian odonates species for two reasons: a) there has not been any distribution assessment so far for these species; and, b) southern temperate forests are predicted to experience the

highest changes in biodiversity due to climate change (Sala et al., 2010). Our analysis is based on a fraction of the 271 species currently known to occur in Argentina (Muzón & von Ellenrieder, 1999; von Ellenrieder & Muzón, 2008). Our aim is to use our assessment to update the current IUCN risk categories for Argentinian odonates.

## **Material and Methods**

### **Species distributional data**

Presences of odonate species were compiled from literature records, GBIF records ([www.gbif.org](http://www.gbif.org) as for 14th October 2015; GBIF Occurrence Download <http://doi.org/10.15468/dl.mf6nh7>), and odonate specialists (Rosser Garrison and Natalia von Ellenrieder). All data were checked carefully for geographic accuracy. Most records were gathered by odonate specialists. We are confident that the biases of identification should be minimal because these data were provided by experts in these animals. Niche models were built only when more than 10 records per species were available. Thus, the final data set included 1734 unique presences of 32 species (see Table 1) which were those species with enough collecting data (range 11 – 158, see Table 1). The database is available upon request.

### **Study area, background and environmental predictors**

Our study area included the covering land between latitudes -55.08 to -21.55S, and longitudes -75.30 to -53.13W. As bioclimatic variables, we used the WorldClim 1.4 ([www.worldclim.org](http://www.worldclim.org)) data set (Hijmans et al., 2005) at 0.041666669 cell size. To establish a background and a set of uncorrelated climatic variables, we intersected the variables with target group points, and with 10,000 points randomly selected in the extension of the study area (M), and with special extent delineating M for each particular species with ecoregions. We eliminated some variables with an exploratory data analysis and Pearson correlation analysis (values >0.7). The final data set included mean diurnal range (bio 02), isothermality (bio 03), temperature seasonality (bio 04), mean

temperature of driest quarter (bio 09), mean temperature of warmest quarter (bio 10), precipitation of wettest month (bio 13), precipitation seasonality (bio 15), precipitation of driest quarter (bio 17), precipitation of warmest quarter (bio 18) and precipitation of the coldest quarter (bio 19).

### **Niche models**

To choose the best background first, species distribution models were generated with Maxent 3.3.3k (Phillips, Anderson, & Schapire, 2006). Models were constructed setting several parameters to default ('Auto features', convergence = 10<sup>-5</sup>, maximum number of iterations = 500, background = 10,000). However, we used random seed (with a test percentage of 30), 10 replicates, removed duplicate records, ran bootstrap replicated type, and no extrapolation and no clamping. All this to find which combination of settings and variables generated the best outcomes (highest area under the curve, or AUC) while minimizing the number of model parameters, as well as producing 'closed', bell-shaped response curves which guaranteed model calibration (Elith et al., 2010). The best background was 10,000 points randomly selected in the extension of the study area.

Final models were built with BIOMOD (Biodiversity Modelling) package in R software. This package is a platform for predicting species' distribution, including the ability to model the distribution using various techniques and test patterns. We trained models employing four widely used algorithms (Maxent, RF, GBM and MARS). From individual models obtained with these different algorithms, we generated a "consensus model" where the final model indicated the degree of overlap between models. This was done to reduce biases and limitations of use only individual models. Final model validation was performed with TSS (True Skill Statistics), average net rate of successful prediction for sites of presence and absence (Liu, White, & Newell, 2009), ranging from -1 to 1, where the more positive values indicate a higher degree of accuracy and discrimination model (Allouche et al., 2006).

A total of 104 final models were generated, whose performance was assessed by mean of the AUC and TSS statistics (Table 2), while minimizing the number of model parameters, and the best presence/absence models using the “10 percentile-training presence” are shown.

## **Projections**

The best models of current climatic conditions of species were used to generate projections in the climatic change scenarios. Data for the future projections were the most recent data from Global Climate Models (GCM) (CNRM-CM5, HadGEM2-ES and MPI-ESM-LR), these climate projections were used in the Fifth Assessment (CMIP5) report of The Intergovernmental Panel on Climate Change (IPPC). The representative concentration pathways used (RCP) were 4.5 and 8.5, to 2050 and 2070 years.

We calculated the percentage of loss or gain of areas with respect to current potential distribution for each future projection (2050 and 2070) under RCP 4.5 and 8.5 (Table 3). Each year was represented by a consensus model where only pixels-predicted-present by all models were considered as representing the presence of the species.

We predicted potential current and future ranges, i.e. we simulated the areas with current and future climate conditions equivalent to those of its present range. This does not mean that the species will occupy all these areas as we did not take into account their population dynamics, dispersal abilities or habitat availability (Sanchez-Guillén et al., 2013).

## **Results**

Table 2 shows the area calculated as a potential current distribution in km<sup>2</sup> by the ecological niche models for each species, and the summary of the performance of the best models (with TSS). This table also shows the current IUCN red List categories and the new categories we suggest on the basis of their distribution area. The geographic distribution can be appreciated in Figure 1.



In regards to the climate changes projections (see Table 3), for the year 2070 with the RCP 8.5, our most pessimistic scenario (that considers emissions would continue to rise), 3 species will maintain their distribution with loss or gain of only around 10% of change, 11 species will increase their distribution, and 13 species will lose their distribution. These changes, in general, were consistent with the other years and scenarios. The interspecific variation in change in area is depicted in Fig. 2 and two examples in the geographic space are provided in the supplementary material Fig. 1. According to our results, the species with the largest increase of potential area was *Erythemis peruviana* with an increase of 761.2%, while the species with the largest lost was *Acanthagrion hidegarda* with a reduction of 98.4% of the area of present distribution.

## Discussion

One benefit species distribution models can bring about is the conservation emphasis. These models and emphasis are badly needed in odonates especially for the neotropics where very few species have been modelled (reviewed by Collins & McIntyre, 2015). In this extent, our results suggest that although most Argentinian species have relatively large distributions, several species would deserve some attention. According to the current IUCN Red List (IUCN, 2016), the following species face some risk: *Andinagrion garrisoni* and *Progomphus kimminsi* (near threatened), *Phyllogomphoides joaquina* (vulnerable) and *Staurophlebia bosqui* (endangered). The remaining 82 are categorized as data deficient (4 species) or least concern (78 species). The threatened four species were classified as such given both the paucity of collecting records and the restricted areas of distribution. We were not able to locate enough collecting points of neither of the four species. However, our work suggests that the species *Acanthagrion gracile*, *A. aepiolum* (with an area below 20,000 km<sup>2</sup>) would have to be considered as vulnerable according to the IUCN criteria of threat. Furthermore, *Acanthagrion floridense* (23,868 km<sup>2</sup>), *Lestes undulatus* (22,255 km<sup>2</sup>) and possibly *Rhionaeschna pallipes* (45,987 km<sup>2</sup>) would deserve some attention as they are close to be vulnerable, so they can be categorized as near threatened. The remaining 21 species can be categorized as least

concern. Future projections would not help some of these species as *A. floridense* would reduce its area from 35 to 63% while *A. aepiolum* would have a more dramatic reduction from 50 to 75 %. Some other species not currently in apparent danger would also face threat: *Acanthagrion hidegarda* and *Rhionaeschna viginpunctata*. These two species may reduce their area to less than 20,000 km<sup>2</sup> under global change scenarios. Essential to these changes is the fact that 70% of Argentinian species are currently present in protected areas (Muzón & von Ellenrieder, 1999). However, given that global change will lead to shifts in current distribution (Sánchez-Guillén et al., 2016), a necessary step is to define whether current Argentinian protected areas will still embrace future odonate geographical distributions. A key issue here is to carry out more intensive collections especially given that we were only able to construct models for 21 out of the 271 species currently described for the country (von Ellenrieder & Muzón, 2008). Moreover, research should analyze whether dispersal abilities can allow odonates catch up with different habitats located at different temperature regimes (Bush et al., 2014).

Related to global change scenarios, it is not surprising to find an inter-specific variation in projected responses to raising temperatures in odonates. Our explanations for this are incomplete yet but may have to do with physiological abilities that affect thermoregulatory responses (e.g. Corbet & May, 2008) and development (especially at egg and larval stages; Pritchard & Leggot, 1987). Given this, it is also not surprising that the largest species turnover will occur at intermediate altitudes where drastic changes in temperature currently occur (Maes et al., 2010). The case of Argentina is actually very relevant to this altitude phenomenon given its sharp changes in elevation. Thus, special attention should be given to these areas.

Apart from North America (Canada and USA; Hassall, 2012) and Brazil (Nóbrega & De Marco, 2011), our study adds a substantially high number of odonate species with projected distributions for America. Considering that there exist around 5,680 described odonate species, of which 25 % had been modelled (Collins & McIntyre, 2015), our study makes an outstanding global contribution at least for the Southern Hemisphere. This

importance can be seen not only in terms of numbers but also in terms of biogeography given the south extreme location of our study species (currently, the southern extreme was Brazil with mainly tropical species; Nóbrega & De Marco, 2011; De Marco et al., 2015).

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**Figures are available, please request to [anb@ciencias.unam.mx](mailto:anb@ciencias.unam.mx)**

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Table 1. Argentinian species and their number of collections used in the study.

<b>Species</b>	<b>No. data points</b>
<i>Acanthagrion aepiolum</i>	13
<i>Acanthagrion cuyabae</i>	26
<i>Acanthagrion floridense</i>	32
<i>Acanthagrion gracile</i>	14
<i>Acanthagrion hidegarda</i>	27
<i>Acanthagrion lancea</i>	41
<i>Erythemis attala</i>	23
<i>Erythemis peruviana</i>	20
<i>Erythemis plebeja</i>	40
<i>Erythemis vesiculosa</i>	37
<i>Ischnura capreolus</i>	42
<i>Ischnura fluviatilis</i>	106
<i>Ischnura ultima</i>	32
<i>Lestes spatula</i>	26
<i>Lestes undulatus</i>	14
<i>Micrathyria hesperis</i>	16
<i>Micrathyria hypodidyma</i>	16
<i>Micrathyria longifasciata</i>	46
<i>Rhionaeschna absoluta</i>	106
<i>Rhionaeschna bonariensis</i>	101
<i>Rhionaeschna confusa</i>	23
<i>Rhionaeschna diffinis</i>	42
<i>Rhionaeschna pallipes</i>	20
<i>Rhionaeschna planaltica</i>	44
<i>Rhionaeschna variegata</i>	43

**Table 2.** Potential distribution of species in km<sup>2</sup> and TSS values.

Species	Present	TSS	Current IUCN status	Suggested IUCN status
<i>Acanthagrion aepiolum</i>	11,492	0.817	NA	Vulnerable
<i>Acanthagrion cuyabae</i>	127,797	0.893	Least concern	Least concern
<i>Acanthagrion floridense</i>	23,868	0.889	NA	Near threatened
<i>Acanthagrion gracile</i>	17,419	0.798	NA	Vulnerable
<i>Acanthagrion hidegarda</i>	87,596	0.898	NA	Least concern
<i>Acanthagrion lancea</i>	96,071	0.886	NA	Least concern
<i>Erythemis attala</i>	59,853	0.898	NA	Least concern
<i>Erythemis peruviana</i>	66,906	0.870	NA	Least concern
<i>Erythemis plebeja</i>	125,529	0.871	Least concern	Least concern
<i>Erythemis vesiculosa</i>	232,533	0.839	Least concern	Least concern
<i>Ischnura capreolus</i>	116,658	0.872	NA	Least concern
<i>Ischnura fluviatilis</i>	267,607	0.844	Least concern	Least concern
<i>Ischnura ultima</i>	64,010	0.878	NA	Least concern
<i>Lestes spatula</i>	159,368	0.885	NA	Least concern
<i>Lestes undulatus</i>	22,255	0.842	NA	Near threatened
<i>Micrathyria hesperis</i>	161,860	0.838	NA	Least concern
<i>Micrathyria hypodidyma</i>	79,474	0.861	NA	Least concern
<i>Micrathyria longifasciata</i>	176,629	0.872	NA	Least concern
<i>Rhionaeschna absoluta</i>	286,941	0.843	NA	Least concern
<i>Rhionaeschna bonariensis</i>	155,439	0.869	NA	Least concern
<i>Rhionaeschna confusa</i>	131,541	0.883	NA	Least concern
<i>Rhionaeschna diffinis</i>	85,158	0.887	NA	Least concern
<i>Rhionaeschna pallipes</i>	45,987	0.881	NA	Least concern/Near threatened
<i>Rhionaeschna planaltica</i>	69,315	0.890	NA	Least concern

<i>Rhionaeschna variegata</i>	259,698	0.828	NA	Least concern
<i>Rhionaeschna viginpunctata</i>	46,063	0.844	Na	

**Table 3.** Relative changes in suitable area per species according to different climatic changes scenarios. Losses are shown as negative values while gains are shown as positive values.

Species	2050	2050	2070	2070
	RCP4.5	RCP8.5	RCP4.5	RCP8.5
<i>Acanthagrion aepiolum</i>	-74.4	-50.5	-59.1	-61.5
<i>Acanthagrion cuyabae</i>	250.2	303.1	307.2	417.6
<i>Acanthagrion floridense</i>	-54.6	-36.5	-61.2	-63.8
<i>Acanthagrion gracile</i>	395.1	339.1	368.4	561.1
<i>Acanthagrion hidegarda</i>	-80.7	-77.5	-91.9	-98.4
<i>Acanthagrion lancea</i>	-55.7	-51.4	-53.5	-72.2
<i>Erythemis attala</i>	7.5	66.4	85.8	77.0
<i>Erythemis peruviana</i>	384.8	513.4	507.8	761.2
<i>Erythemis plebeja</i>	122.8	135.1	202.8	198.3
<i>Erythemis vesiculosa</i>	-51.0	-57.3	-52.3	-87.9
<i>Ischnura capreolus</i>	-42.1	-44.0	-47.0	-61.9
<i>Ischnura fluviatilis</i>	-0.2	0.0	17.3	-0.2
<i>Ischnura ultima</i>	-33.6	-29.8	-34.1	-33.6
<i>Lestes spatula</i>	-30.8	-24.0	-21.8	-30.2
<i>Lestes undulatus</i>	59.2	99.5	154.8	191.4
<i>Micrathyria hesperis</i>	106.8	182.2	197.2	283.3
<i>Micrathyria hypodidyma</i>	197.9	304.7	426.6	749.6
<i>Micrathyria longifasciata</i>	129.0	157.8	178.6	231.9
<i>Rhionaeschna absoluta</i>	41.8	35.7	34.8	40.8
<i>Rhionaeschna bonariesis</i>	-13.3	13.1	11.9	44.8
<i>Rhionaeschna confusa</i>	-53.0	-42.7	-58.8	-65.0
<i>Rhionaeschna diffinis</i>	2.1	2.8	-0.1	-9.6
<i>Rhionaeschna pallipes</i>	-16.2	-4.1	-19.2	-13.8
<i>Rhionaeschna planaltica</i>	-50.4	-45.8	-47.4	-67.2
<i>Rhionaeschna variegata</i>	-6.2	-6.7	-5.4	-10.8
<i>Rhionaeschna viginpunctata</i>	-42.3	-33.0	-43.0	-45.9





## APENDICE III

# Isometric patterns for male genital allometry in four damselfly species

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**Abstract** Recent studies have found that insect genitalic traits show negative allometry, i.e., are relatively small in relation to body size. One interpretation of this is that males use their genitalia to stimulate females. Thus, given the nature of damselfly copulation in which males physically reach the rival sperm that females have stored from previous matings, male genitalic traits are not expected to show negative allometry. To test this idea, we assessed (a) the rival sperm displacement function by the mating male and (b) allometry of aedeagal length of four damselfly species (*Argia anceps*, *Argia tezpi*, *Argia extranea*, and *Enallagma praevarum*). Sperm displacement was assessed by inspecting whether the aedeagus reached the rival sperm during copulation in mating pairs for the four species. To have a standard for comparing allometric patterns, allometry of aedeagal was compared to that of two non-genital traits, tibial, and fourth abdominal segment length. In all cases, the aedeagus was found to reach the rival sperm which supports the idea that stimulation is not the mechanism for sperm displacement but physical displacement. Aedeagal length was isometric, and its slope was lower in general compared to that of tibial length and fourth abdominal segment. Given that this isometric pattern is not common for other odonate species, our interpretation of these varying aedeagal scaling patterns in this insect order is that males' and females' sexual interests are in conflict (males are evolving an elongated aedeagus to reach rival sperm while females are evolving unreachable sperm storage organs to prevent displacement of stored sperm). This sexual conflict scenario would favor varying scaling patterns for aedeagal length in odonates. A final interpretation

is that the risk of interspecific matings in damselflies, may also explain different species-specific, aedeagal allometries.

**Keywords** Sperm displacement · Allometry · Genitalia · Male · Damselfly

## Introduction

Genitalia are highly variable structures even in closely related taxa. Current evidence is strongly in favor of sexual selection as the primary force driving such diversity (reviewed by Leonard and Córdoba-Aguilar 2010), albeit it is less clear how selection mechanisms operate during male–female interaction (Hosken and Stockley 2004). One attempt to understand this is a recent line of research that has looked at the static allometry of genitalia. Static allometry (from now on, allometry) refers to variation of ornaments, structures, organs, body parts, and other quantitative aspects of the body, related to a reference measure such as body size, weight, or volume, and how such relation varies among conspecific individuals (Eberhard et al. 2009; 2010). When trait size increases slower than body size, allometry takes slope values  $<1$ , namely negative allometry; when trait size decreases faster than body size, allometry takes slope values  $>1$ , namely positive allometry. When the slope value = 1, it refers to isometry. A number of studies mainly focused on insects showing that genitalia have relatively low allometric values, i.e., they show negative allometry (e.g., Eberhard et al. 1998; Bernstein and Bernstein 2002; Hosken et al. 2005). For example, male genitalia of a taxonomically diverse sample of insects and spiders have shown a consistent pattern of negative allometry (Eberhard et al. 1998). One explanation for such values is that males with intermediate sizes (i.e., about the population mean) of genitalia, would be favored to copulate with as many females (regardless of female's size), a hypothesis known as “one size

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fits all" (Eberhard et al. 1998). This hypothesis applies if there is a courtship role for male genitalia and so their function has to do with female stimulation rather than physical coercion (Eberhard et al. 1998). A working hypothesis is that if a stimulatory ability is used by a particular species, male genital negative allometry must be expected.

Odonata is a well studied animal order in terms of genital function and morphology (Cordero-Rivera and Córdoba-Aguilar 2010). These insects, like other arthropods, mate multiply and males have the ability to manipulate rival sperm stored in the female sperm storage organs (reviewed by Cordero-Rivera and Córdoba-Aguilar 2010). Male genitalia have evolved mechanisms to reach such rival sperm and place it in sites of the female sperm storage organs where its use for fertilization will be less likely (Miller and Miller 1981; Córdoba-Aguilar et al. 2003; Córdoba-Aguilar and Cordero-Rivera 2008). The aedeagus is a long, heavily sclerotized structure with spine-like pieces on its distal region while the sperm storage organs are sperm containers located at the end of the vagina, of variable size and volumetric capacity (Córdoba-Aguilar and Cordero-Rivera 2008). Since in most species, the spermatheca is an elongated process that emerges from the bursa, the aedeagus has to penetrate both organs to have access to rival sperm. This is why aedeagal length has been interpreted as being sexually selected in several odonate species (Córdoba-Aguilar et al. 2003; Córdoba-Aguilar and Cordero-Rivera 2008). Given this, one would expect selection to favor aedeagal length (to reach the vagina; for supporting evidence see Nava-Bolaños et al. 2012). Thus, in terms of genital allometry, one would expect that the sperm manipulation ability in odonates should lead to non-negative allometric patterns. Studies of genital allometry in odonates are not new (e.g., Córdoba-Aguilar et al. 2010; Nava-Bolaños et al. 2012; Outomuro and Cordero-Rivera 2012) and have indeed shown that the pattern of genital negative allometry found in other insects and spiders (e.g., Eberhard et al. 1998; 2009), may not apply to odonates.

To test whether odonates do not show negative allometry patterns, we have carried out a study using males of four damselfly species (*Argia anceps*, *Argia tezpi*, *Argia extranea*, and *Enallagma praevarum*). After testing whether mating males physically reach and remove rival sperm that females have stored from previous copulations (so that no stimulatory function is claimed to explain sperm displacement), we predicted that the length of this trait should not show negative allometry patterns.

## Material and methods

Males of *A. anceps*, *A. tezpi*, and *A. extranea* were studied and collected in the Tetlama river (18° 46' 05" N, 99° 14' 17" W),

Xochitepec, Morelos, Mexico, while *E. praevarum* males were collected in the Cantera Oriente (19° 19' 7" N, 99° 10' 21" W), Mexico City. Animals were sexually mature as assessed by their vivid body color and rigid body structure (Corbet 1999).

We used a first set of animals to test whether mating males reach the sperm females have stored from previous copulations. For this, we used eight, six, seven, and eight tandem pairs of *A. anceps*, *A. tezpi*, *A. extranea*, and *E. praevarum*, respectively, just before the male introduced his genitalia into those of the female. After approximately ten abdominal movements that each mating couple produced, which is characteristic of the sperm displacement stage in odonates (Córdoba-Aguilar et al. 2003), the abdominal region where both sexes were connected was cut with fine scissors and preserved in 70 % ethanol. After re-hydrating with distilled water for 24 h, the still engaged abdominal pieces were dissected to document where in the female genitalia, the aedeagus was inserted. These dissection and inspection steps were done using fine scissors and a stereoscopic microscope (Olympus®). If the aedeagus was found inserted in either female sperm storage organ (bursa copulatrix or spermatheca), we assumed that physical sperm removal takes place (see also Córdoba-Aguilar et al. 2003). Although desirably, this method should be complemented with measures of stored sperm volumes, our approximation is still a robust one given other studies (e.g., Córdoba-Aguilar 2003).

We captured a second set of males for allometric measurements (see sample sizes in Table 1). All animals were preserved in 70 % ethanol to be later rehydrated with distilled water. Then, body parts were dissected using fine scissors and examined under a stereoscopic microscope (Olympus®). The following traits were measured (using IMAGEJ version 3.00 software, National Institutes of Health, MD, USA) per individual: aedeagus length (from its basis to the anterior site of the distal head; see Fig. 1), wing length (from its point of insertion to the distal site), head width (the longest distance between the eyes), length of tibia of right foreleg, and length of the fourth abdominal segment. Wing length and head width were used as body size indicators as there is always a controversy around which trait is the best indicator of body size (Eberhard et al. 2009), and actual differences in allometric patterns may emerge for each body size indicator (Wheeler et al. 1993; Nava-Bolaños et al. 2012). In our case, we were able to measure wing length for all species, but head width only for *A. anceps* and *A. tezpi*. This was because heads in *A. extranea* and *E. praevarum* got deformed after ethanol preservation. Eberhard et al. (2009) suggested that the steepness of allometric slopes of the genitalia should be compared with those of other similar-sized body parts that are believed to be non-sexually selected. Thus, the validity of, for example, isometry for a genital trait can be compared to a trait that is

**Table 1** Measurement errors, coefficients of variation (CV) and number of measured individuals for all male traits in four damselfly species

Species	Trait	Measurement error (%)	CV (%)	Number
<i>Argia anceps</i>	Wing length	1.375	3.752	32
	Head width	2.537	2.481	32
	Aedeagus	2.645	2.822	32
	Tibial length	7.635	5.446	32
	4th Abdominal segment	4.702	4.480	32
<i>A. tezpi</i>	Wing length	9.843	2.865	33
	Head width	2.329	2.316	33
	Aedeagus	4.687	2.503	33
	Tibial length	3.220	6.148	33
	Fourth abdominal segment	5.067	4.978	33
<i>A. extranea</i>	Wing length	5.368	3.993	30
	Aedeagus	5.616	3.2	30
	Tibial length	4.902	6.171	30
	Fourth abdominal segment	8.155	8.119	16
<i>Enallagma praevarum</i>	Wing length	3.678	3.724	27
	Aedeagus	6.630	2.991	27
	Tibial length	4.395	5.303	27
	Fourth abdominal segment	4.343	4.987	23

more likely to represent isometry. This is why we measured the length of tibia and fourth abdominal segment, as so far there, is no indication that these traits are under sexual or natural selection in the family of our subject species (although for at least one species of the Chlorocyphidae family, tibial length seems to be sexually selected; Jennions 1998; Steven et al. 1996). To estimate consistency during measurement, measurement error, and coefficient of variation, each trait was measured by the same person (AN-B) four times.

Using the average of the four measurements, we estimated the allometric slopes for length of aedeagus, tibia, and fourth abdominal segment separately for each body size indicator. Data were log-transformed to linearize the relationship between genital traits and body size indicators. The logarithm or multiplicative scale allows for sensible interpretation as growth is a multiplicative process (Warton et al. 2006). We employed model II regressions over a traditional ordinal least square regression approach because all morphometric variables are random and not controlled by the researcher (Warton et al. 2006). There are different versions of model II regressions, such as major axis (MA), standardized major axis (SMA), and ranged major (RMA) regressions (Legendre and Legendre 1998; Warton et al. 2006). SMA regressions are preferred over MA for allometric studies when the

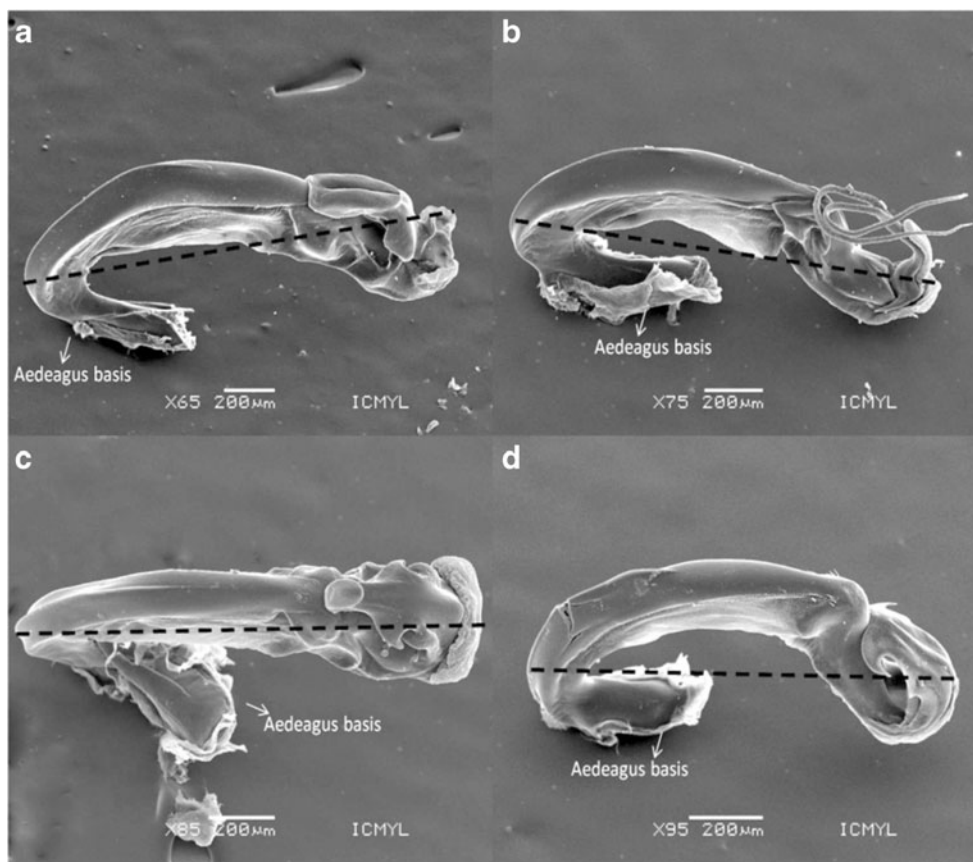
morphometric traits are in different order of magnitude or differ in their measurement units (Warton et al. 2006). The disadvantage of SMA is that there is no direct way to assess whether allometric coefficients are significantly different from zero (that is, whether there is a relationship between the genital structure and size; Quinn and Keough 2002). However, a correlation test between morphometric variables has been suggested as a proxy (Legendre and Legendre 1998; Quinn and Keough 2002). RMA analysis can be used when the variables are not in the same units, with the advantage of assessing whether the allometric slope differs from zero but is sensitive to extreme values (Legendre and Legendre 1998). To solve all these controversies, we present both SMA and RMA allometric coefficients. Also, we used Pearson's correlation for testing correlations with the SMA approach. When structures were related to body size indicators (i.e., a non-zero slope, which was tested using the RMA approach), we determined whether there was support for isometry ( $b=1$ ), negative allometry ( $b<1$ ), or positive allometry ( $b>1$ ) by looking at the confidence intervals of the allometric slope using the SMA method. For comparing the slope of aedeagus against that of tibia and fourth abdominal segment, we used their confidence intervals but after checking that the correlation between these traits and their body size indicators was significant. We used R software (R Development Core Team 2009) for statistical analysis.

## Results

Using the captured mating pairs, the aedeagus was found to be fully inserted in either the bursa copulatrix and/or spermatheca in all cases for the four species. As for morphometric measurements, measurement error for all traits indicated relatively repeatable measurements (Table 1). Wing length and head width were significantly related to aedeagus length for the four species (as shown by Pearson's correlations; Table 2). Aedeagal length was isometric both in relation to the slopes produced by the SMA and RMA methods and their significant difference from one (Table 2). Tibial length was positively allometric in *A. anceps* and *A. tezpi*, but was not significantly related to wing length in *A. extranea* and *E. praevarum*. Fourth abdominal segment length was isometric in *A. anceps* when wing was used as body size indicator, but showed positive allometry when head width was used as body size indicator. Fourth abdominal segment length was positively allometric in *A. tezpi* for both wing and head, but there was no significant relations in *A. extranea* and *E. praevarum*.

Using confidence intervals, aedeagal length showed lower slopes than tibial length in *A. anceps* for wing length but there was no difference for head width (Table 2). Also, in this species, aedeagal slope did not differ from the fourth abdominal

**Fig. 1** Aedeagus photographs of *Argia anceps* (a), *A. tezpi* (b), *A. extranea* (c), and *E. praevarum* (d). The dotted line indicates how aedeagal length was measured



segment slope for both body size indicators. In *A. tezpi*, slopes of aedeagal length were lower than that for tibial and fourth abdominal segment for both body size indicators (Table 2). The

rest of potential comparisons cannot be carried out as the relationship between tibia and fourth abdominal segment with body size indicators, was non-significant.

**Table 2** Allometry of aedeagal (referred as Aed), tibial and 4th abdominal segment (referred as Abd) length in relation to two body size indicators (wing length and head width) in four damselfly species

Species	Traits	<i>r</i> ( <i>p</i> )	RMA slope	<i>p</i> Value	SMA slope	SMA slope CI	Number
<i>A. anceps</i>	Aed/wing	0.402 (0.011)	0.849	0.013	0.729	0.522–1.019	32
	Aed/head	0.459 (0.003)	1.002	0.005	1.139	0.827–1.569	33
	Tibia/wing	0.332 (0.034)	4.596	0.029	1.617	1.139–2.197	31
	Tibia/head	0.517 (0.001)	4.295	0.001	2.429	1.174–3.325	32
	Abd/wing	0.517 (0.002)	1.957	0.003	1.178	0.856–1.622	31
	Abd/head	0.556 (0.001)	2.506	0.003	1.808	1.332–2.453	32
<i>A. tezpi</i>	Aed/wing	0.523 (<0.001)	1.058	0.002	0.911	0.670–1.238	33
	Aed/head	0.607 (<0.001)	1.076	0.001	1.081	0.811–1.440	33
	Tibia/wing	0.435 (0.005)	2.369	0.004	1.961	1.425–2.696	34
	Tibia/head	0.509 (0.0001)	2.367	0.003	2.508	1.765–3.251	33
	Abd/wing	0.699 (0.0001)	1.728	0.001	1.747	1.354–2.254	34
	Abd/head	0.546 (0.003)	1.763	0.001	2.148	1.571–2.938	34
<i>A. extranea</i>	Aed/wing	0.389 (0.016)	1.095	0.016	0.795	0.560–1.127	30
	Tibia/wing	0.097 (0.342)	-2.457	0.34	-1.568	0.562–1.122	20
	Abd/wing	0.282 (0.079)	1.554	0.132	1.997	1.204–2.871	16
<i>E. praevarum</i>	Aed/wing	0.469 (0.006)	0.728	0.009	0.763	0.534–1.089	27
	Tibia/wing	0.075 (0.365)	-0.316	0.396	-2.408	2.602–1.747	24
	Abd/wing	0.269 (0.107)	1.32	0.102	2.179	1.425–3.330	23

The table shows Pearson correlation coefficients (*r*) and their *p* value, ranged major regressions allometric coefficients (RMA slope), the *p* value obtained from permutations to test if the allometric coefficients differed from zero, standardized major axis allometric coefficients (SMA slope), and their confidence intervals (SMA slope CI), and sample sizes

## Discussion

Our measurement error was relatively high in some cases (e.g., *A. tezpi* wing length: 9.843; Table 1). Future studies should not only aim to have lower errors but also elucidate whether this has an effect on allometric estimates. Despite this, isometry (the proportional increase in size of a particular trait with respect to body size), was detected for aedeagal length. This was corroborated after comparing the slope of aedeagus against that of tibia and fourth abdominal segment, implying that aedeagal allometry is relatively negative. This difference in male slopes of genital (low slopes) versus non-sexually selected, non-genital (high slopes) traits has been already detected in arthropods (Eberhard 2009). The reasons for this difference are mainly based on how genital traits may be selected. For example, male genital traits are supposed to function on the basis of tactile stimulation (Eberhard et al. 1998; 2009). Furthermore, the fact that male genitalia are small and hidden, may make them “cheap” to develop and/or maintain and thus less likely to be used as indicators of male survival ability compared to non-genital traits (Eberhard 2009). Although the stimulatory function apply to a few odonates (reviewed by Córdoba-Aguilar and Cordero-Rivera 2008; Cordero-Rivera and Córdoba-Aguilar 2010), further studies should look at whether these reasons apply in particular to our study subjects.

While our results suggest an isometric pattern for genitalia, studies in other damselfly species do not fully support this (Córdoba-Aguilar et al. 2010; Nava-Bolaños et al. 2012). For example, Nava-Bolaños et al. (2012) found positive, negative, and non-scaling patterns for male and female genitalia in two other damselfly (one protoneurid and one coenagrionid) species. Furthermore, Outomuro and Cordero-Rivera (2012) found isometric and negative allometry for non-intromittent and intromittent male genitalia, respectively, in a calopterygid. However, these authors also found non-significant associations between both types of genitalic traits. Our interpretation of the aedeagal allometric pattern in our study species and damselflies in general, goes in terms of the nature and evolution of copulation in these animals. Damselfly males use their aedeagus to penetrate the females’ sperm storage organs and displace rival sperm (Córdoba-Aguilar et al. 2003). There are different sexual selection mechanisms and scenarios to explain why the aedeagus would evolve an elongated morphology but would also imply why the scaling nature of aedeagi may vary. One is trapping rival sperm to take it to the outside and the other is to push such rival sperm to deeper areas of the sperm storage organs, away from the fertilization site (Córdoba-Aguilar et al. 2003). These mechanisms would select for large aedeagus. Furthermore, the female “environment” (sperm storage organs) may also select for male aedeagal elongation evolution. Two particular aspects of this environment is the size of each sperm storage

organs and how deep these organs are located in the vagina. Although it is known that odonate sperm storage organs are highly diverse in terms of morphology (Córdoba-Aguilar et al. 2003), there are no records of whether this is also the case for how depth sperm storage organs are located. In case varying sperm storage organ morphology is evolving for reasons related to make sperm displacement harder, this may also favor large aedeagi. On a general level, these male- and female-driven mechanisms go along well with the idea that both sexes in damselflies are coevolving as their interests differ: males have evolved to displace stored sperm while females have evolved ways to prevent such displacement (Córdoba-Aguilar and Cordero-Rivera 2008; 2010). According to theory, sexual conflict will evolve when both sexes do not share the same reproductive interests (Parker 1979). In odonates, these interests are clear and the storage of sperm by females may be the center of the conflict as females may benefit from keeping it unreachable for later use while males would lose paternity from this female strategy (Córdoba-Aguilar and Cordero-Rivera 2008). Although there is no clear evidence of benefits of storing sperm for females (partly because this has not been investigated in detail) several sources suggest such benefits: (a) that sperm storage is a widespread trait in Odonata; (b) long-term sperm maintenance in the female’s sperm storage organs for future use in monandrous species (e.g., Grieve 1937; Cordero-Rivera 1990); and (c) a reduced female ability to keep stored sperm viable in species with frequent sperm displacement (Hayashi and Tsuchiya 2005). However, this sexual conflict scenario implies a coevolutionary process that may be driving aedeagal length producing varying scaling effects for this trait. For example, there may be times when this positive allometry is selected (for example, with the evolution of deeper sperm storage organs), but times when this is not (when sperm storage organs are not as deep as a strategy to still make sperm displacement harder). This coevolutionary scenario may equally apply to the female vaginal length given its varying scaling patterns in other odonate species (Nava-Bolaños et al. 2012). These possibilities deserve further investigation.

One final mechanism that may explain varying scaling patterns for aedeagal length is related to natural selection. A particular aedeagal length may be favored if this allows a close matching with the vagina and sperm storage organs when there is a risk of interspecific matings (Cordero-Rivera and Córdoba-Aguilar 2010). In a case like this, a fine tune may be selected to reduce the costs of such non-adaptive matings (Cordero-Rivera and Córdoba-Aguilar 2010). The fact that interspecific matings are highly common in damselflies (e.g., Leong and Hafernik 1992; Sánchez-Guillén et al. 2011) provides indirect support to this mechanism of selection. Different species-specific, scaling patterns for aedeagal length for species with high risk of interspecific matings would be expected. Again, this can be further investigated.

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