

Let's wait for the evening: nocturnal copulation in a tropical damselfly *Phylolestes ethelae* (Odonata, Synlestidae)

A. Cordero-Rivera¹, J. C. Núñez², C. Surriel³

Author affiliations:

¹ Universidade de Vigo, ECOEVO, Spain

² Jardín Botánico de Santiago Profesor Eugenio de Jesús Marcano Fondeur, República Dominicana

³ Programa doctoral en Ciencias Ambientales, INTEC, República Dominicana

Corresponding author:

A Cordero-Rivera
adolfo.cordero@uvigo.gal

Handling Editor:

Rhainer Guillermo Ferreira

Received: 23/10/2023

Cond. acceptance: 09/01/2024

Final acceptance: 15/01/2024

Published: 30/01/2024

Cite:

Cordero-Rivera A, Núñez JC, Surriel C, 2024. Let's wait for the evening: nocturnal copulation in a tropical damselfly *Phylolestes ethelae* (Odonata, Synlestidae). *Animal Biodiversity and Conservation* 47.1, 19-32. DOI: <https://doi.org/10.32800/abc.2024.47.0019>

© [2024] Copyright belongs to the authors, who license the journal *Animal Biodiversity and Conservation* to publish the paper under a Creative Commons Attribution 4.0 License.

ISSN: 1578-665 X
eISSN: 2014-928 X

Abstract

Let's wait for the evening: nocturnal copulation in a tropical damselfly *Phylolestes ethelae* (Odonata, Synlestidae). Sexual selection is one of the main causes of the diversity of reproductive behaviours observed intra- and interspecifically. Here we study the reproductive behaviour of a unique species, *Phylolestes ethelae*, the only member of the family Synlestidae (Odonata) found in America. Our hypothesis was that the phylogenetic uniqueness of this species and the fact that it lives on an island would produce novel behaviours worth protecting as they are a part of the biodiversity. Between 2017 and 2019 we studied a population of *P. ethelae* in the National Park Armando Bermúdez in the Dominican Republic by means of mark-recapture techniques and focal observations. The survival rate was estimated as 0.953 ± 0.012 (SE), which translates into an expected lifespan of 20.6 days. We found that some males showed high site fidelity but very little activity, and territorial disputes were almost inexistent. Females, on the other hand, were rarely observed on the river, and were found mainly on the nearby paths and forest. We recorded 28 tandems and observed a total of 17 copulations, lasting an average of 18.6 min. Examination of sperm volumes in females interrupted during copulation indicated that males removed most of the sperm from the female bursa copulatrix during stage I of copula, but they could not remove sperm from the paired spermathecae. The duration of precopulatory tandem was highly variable (from 3 min to 7.8 h). In some cases, copulation was observed shortly (3 min) after tandem formation, mainly during the morning. However, some pairs formed during the morning, remained in tandem for several hours, and mated late, after dusk. Females laid eggs not accompanied by the male. Mating after the main peak of activity might be a strategy which protects sperm investment because females laying eggs after dusk are unlikely to be mated again that day. Further studies are needed to decipher phenotypic correlates of the males that mate after dusk.

Key words: Territoriality, Sexual selection, Precopulatory guarding, Nocturnal behaviour, Ethodiversity, Damselfly

Resumen

Esperemos la noche: cópula nocturna en una damisela tropical *Phylolestes ethelae* (Odonata, Synlestidae). La selección sexual es una de las causas principales de la diversidad de conductas reproductivas observadas dentro de una misma especie y entre especies. En este trabajo estudiamos el comportamiento reproductivo de una especie singular, *Phylolestes ethelae*, el único miembro de la familia Synlestidae (Odonata) que se encuentra en América. Nuestra hipótesis fue que la singularidad filogenética de esta especie y el hecho de que vive en una isla producirían comportamientos nuevos, que vale la pena proteger, ya que forman parte de la biodiversidad. Estudiamos una población de *P. ethelae* que habita en el Parque Nacional Armando Bermúdez, en la República Dominicana, mediante técnicas de marcaje y recaptura y observaciones focales, entre 2017 y 2019. La tasa de supervivencia se estimó en $0,953 \pm 0,012$ (DE), lo que se traduce en una esperanza de vida de 20,6 días. Encontramos que algunos machos mostraban una alta fidelidad al sitio, pero muy poca actividad y que las disputas territoriales eran casi inexistentes. Las hembras, por otro lado, rara vez se observaban en el

río, sino que se encontraban principalmente en los senderos y bosques cercanos. Registramos 28 tandems y observamos un total de 17 cópulas, que duraron 18,6 min de promedio. El examen del volumen de esperma en las hembras a las que se interrumpió durante la cópula indica que los machos extraen la mayor parte del esperma de la bursa copulatrix de la hembra durante la etapa I de la cópula, pero no pueden extraerlo de las espermatecas. La duración del tándem precopulatorio fue muy variable (de 3 min a 7,8 h). En algunos casos, la cópula se observó poco tiempo después (3 min) de la formación del tándem, principalmente durante la mañana. Sin embargo, algunas parejas se formaron durante la mañana, permanecieron en tándem durante varias horas y se aparearon muy tarde, después del anochecer. Las hembras depositaron los huevos en ausencia del macho. El apareamiento después del máximo de actividad podría ser una estrategia que protege la inversión en esperma, ya que es poco probable que las hembras que ponen huevos después del anochecer vuelvan a aparearse ese día. Se necesitan más estudios para descifrar las características fenotípicas de los machos que se aparean después del anochecer.

Palabras clave: Territorialidad, Selección sexual, Guardia precopulatoria, Comportamiento nocturno, Etodiversidad, Damisela

Introduction

The reproductive behaviour of sexual animals is extremely diverse, with some species being strongly territorial (e.g., Suhonen et al 2008). Intense sexual selection has been a selective force for the evolution of sexual dimorphism in many animals, such as birds of paradise, for example, which are not only diverse in colouration but also in behaviour (Laman and Scholes 2012). At the intraspecific level, sexual selection is the engine that originates and maintains alternative reproductive tactics in both sexes (Gross 1996, Neff and Svensson 2013). At the individual level, the expression of condition-dependent tactics allows individuals to behave in an adaptive way when environmental circumstances change, such as the switch from territoriality to forced copulation when population density is extreme (e.g., Cordero Rivera and Andrés 2002). These three levels of variability constitute an example of behavioural diversity or ethodiversity that merits consideration in conservation biology (Cordero-Rivera 2017a).

The Odonata (dragonflies and damselflies) are well-known for their diversity of mating behaviours (Corbet 1999). Their attachment to water bodies, required by most species for the development of larvae, stimulates males to concentrate around lakes, ponds, streams and rivers, and their large size has prompted their use as model organisms in behavioural studies (Córdoba-Aguilar 2008, Córdoba-Aguilar et al 2022). However, most such studies have been performed on a few widely distributed families, such as the Calopterygidae (reviewed by Córdoba-Aguilar and Cordero-Rivera 2005), the Coenagrionidae (e.g., Sánchez-Guillén et al 2020) and the Libellulidae (e.g., Miller 1995). In this context, it is especially relevant to study the behaviour of unique or relict taxa so as to be able to incorporate this information in future studies of the phylogeny of the group because behavioural traits can provide

relevant information about the evolutionary history and the ecological pressures behind the various alternatives (Rivas-Torres et al 2019). For instance, the behaviour of the oldest extant damselfly, *Hemiphlebia mirabilis*, from Southern Australia, includes several unique features, particularly regarding precopulatory courtship (Cordero-Rivera 2016a), and illustrates that particular traits related to sperm competition are early adaptations of the odonates (Cordero-Rivera 2016b). The exceptional behaviour of *Pseudolestes mirabilis*, the only representative of its family (Pseudolestidae), is another good example of behavioural diversity related to phylogenetic uniqueness (Cordero-Rivera and Zhang 2018).

The family Synlestidae includes a small group of 33 species distributed in forest rivers of Africa, Australia, China and the island of Hispaniola (Simaika et al 2020). Their behaviour is almost unknown, even in Africa, where they are most speciose (Martens 2003). *Phylolestes ethelae* is the only synlestid found in the New World (Christiansen 1947), and it is restricted to mountain rivers in Haiti and the Dominican Republic (Westfall 1976). This species is considered endangered (EN) in the IUCN Red List of Threatened Species because it is known from only a few localities in a single highly restricted area of Hispaniola (von Ellenrieder and Paulson 2006). No information about its behaviour has been published to date. The aim of this paper was therefore to describe the general and reproductive behaviour of *P. ethelae*, which was sometimes unexpectedly observed mating after dusk.

Methods

General methods and study site

We studied a population of *P. ethelae* inhabiting the river Yaque del Norte at La Ciénaga de Manabao in the National Park Armando Bermúdez. We used mark-re-



Fig. 1. A view of the River Yaque del Norte where the observations were made. The large rocks and some trees were used as landmarks to spatially record the presence of territorial males. (Picture by A Cordero-Rivera).

Fig. 1. Vista del río Yaque del Norte, donde se realizaron las observaciones. Las grandes rocas y algunos árboles se utilizaron como puntos de referencia para registrar espacialmente la presencia de machos territoriales. (Fotografía de A Cordero-Rivera).

capture techniques and focal observations to estimate site-fidelity and survival. A first survey was completed between 7 and 11 June 2017, with a total of 24.6 h of observations, starting at around 9:00 and ending at 14:00 h. Males and females were observed perching on the riverbank vegetation, in the treetops above the river, and in the surrounding forest and paths. These preliminary observations indicated that the activity of *P. ethelae* adults was low and that they remained motionless for long periods (even for over an hour), and only one copulation was observed at noon.

In 2018, between 14 and 18 August, for a total of 47.1 h, we conducted a period of mark-recapture to test the viability of the observation method. Fieldwork was concentrated in a section of the river of about 70 m in length, with sunny open areas where adults had been observed the previous year. The area was 6-10 m wide and water depth ranged from 30 cm to 2 m (fig. 1; latitude: 19.068633, longitude: -70.876475, altitude: 1,095 m). The water velocity in this section of the river was fast (0.5-0.8 m/s, estimated with a floating dry leaf). pH was 7.5-7.8, temperature was 16.4-16.5°C in August (18.2°C in June 2017) and conductivity was 0.09-0.1 mS/cm. The studied section was divided into sectors of around 5 m, using natural marks such as large rocks and trees (fig. 1), and every observation was referred to the nearest sector, to study site attachment by males and females.

Each study day we captured all adults observed in the river and nearby forest. They were marked by writing a

number on the right hindwing, measuring them to the nearest 0.1 mm and releasing them at the site of capture. Age at marking was estimated as teneral (= newly emerged), young (flexible wings and no pruinescence) or mature (inflexible wings, full coloration, and a variable amount of whitish pruinescence on the ventral parts of the abdomen and thorax). We also tested the use of a portable mosquito net as a field insectary, by introducing 2-3 males and females. One mating was obtained with this method, but individuals generally showed little interaction and the method was abandoned.

In 2019, from August 13 to August 27 (except for days 23 and 24), we completed an intensive mark-recapture and behavioural study for a total of 73 h of observation. Given that in 2018 we detected activity in the late afternoon, the 2019 fieldwork was usually divided in two periods, from 9:00 to 13:00 h and from 14:30 to 17:30-19 h, although heavy rains on some afternoons shortened fieldwork. We recorded behaviour by direct observation, and with the help of a digital camera (Fuji FinePix S1), which allowed to read the identification numbers at a distance (e.g., fig. 2B). Due to the lack of light in the later hours, we used a red-light torch to follow tandem pairs and record their activity (fig. 3C).

Mark-recapture analyses

Males that were observed for at least two consecutive days in the same river section (usually on or very close to the same perch) were considered territorial



Fig. 2. *Phylolestes ethelae* general behaviour: males perched immobile for several hours (A) on the riverbank, with almost no agonistic interactions, but they showed high site fidelity. In the early morning and late afternoon, most males perched at the treetops for feeding (B). Females were found inside the forest (C), where they searched for sheltered perches, and when near the water, they perched at the lower end of lianas and branches (D). (Pictures by A Cordero-Rivera).

Fig. 2. Comportamiento general de *Phylolestes ethelae*: los machos permanecieron inmóviles durante varias horas (A) en la orilla del río, casi sin interacciones agonísticas y con una alta fidelidad al sitio. A primera hora de la mañana y al final de la tarde, la mayoría de los machos se posaba en las copas de los árboles para alimentarse (B). Las hembras se encontraron dentro del bosque (C), donde buscaban perchas protegidas, y cuando estaban cerca del agua, se posaban en el extremo inferior de las lianas y las ramas (D). (Fotografías de A Cordero-Rivera).

residents. We were unable to assess territorial status for males that were never recaptured, making the definition of territorial status partially circular, but most of them would have been recaptured had they remained on the riverbank (see recapture rates below). However, in the 2018 dataset, we also considered as territorial two males that were never recaptured but were observed for several hours in the place of first capture, defending the territory. Only three young males were marked in 2018, and none of them were resighted. For the 2019 dataset, only one out of 19 males marked as young was subsequently recaptured, behaving as territorial.

The recapture histories were analysed using Cormack-Jolly-Seber models by means of MARK 9.0

software (White and Burnham 1999). In a first step, we ran predefined models with groups (g) defined by sex (and age for the individuals marked in 2019) and time (t) to estimate survival and recapture probabilities. The goodness of fit of the saturated model [$\text{Phi}(g^*t) p(g^*t)$] was tested by means of the program RELEASE from within MARK. In the 2018 dataset, heterogeneity was high in males (Test 2 + Test 3, $\chi^2 = 25.6$, $df = 7$, $p < 0.001$). This was solved by subdividing males into territorial and non-territorial individuals (overall Test 2 + Test 3, $\chi^2 = 1.4$, $df = 12$, $p = 1$). The 2019 dataset also showed heterogeneity in the group of mature males (Test 2 + Test 3, $\chi^2 = 55.9$, $df = 35$, $p = 0.014$). Grouping males according to their place of first capture (river versus forest) did not improve the fit of the



Fig. 3. The precopulatory tandem in *Phyllolestes ethelae* occurs at any moment of the day, usually in sheltered places (A). Long-lasting tandems go to the treetops as the day progresses (B) and remain there until dusk (C) (a red light used to observe the tandem). Before copulation, the male translocates sperm from the genital opening, situated at the end of abdomen, to the seminal vesicle under segment 2 (D). (Pictures by A Cordero-Rivera).

Fig. 3. El tándem precopulatorio en *Phyllolestes ethelae* ocurre en cualquier momento del día, generalmente en lugares resguardados (A). Los tándems de muy larga duración van a las copas de los árboles a medida que avanza el día (B) donde permanecen hasta el anochecer (C) (Se utilizó luz roja para observar el tándem). Antes de la cópula, el macho transfiere el esperma desde la abertura genital situada al final del abdomen hasta la vesícula seminal bajo el segmento 2 (D). (Fotografías de A Cordero-Rivera).

model for males marked on the river (Test 2 + Test 3, $\chi^2 = 53.3$, $df = 33$, $p = 0.014$). However, grouping them according to their territoriality status allowed a good fit for all groups (territorial males, Test 2 + Test 3, $\chi^2 = 20.12$, $df = 20$, $p = 0.451$; overall: Test 2 + Test 3, $\chi^2 = 26.0$, $df = 50$, $p = 0.998$). Therefore, in 2019 we estimated survival and recapture rates using a model that included four groups, namely, territorial and non-territorial males, young females (including teneral) and mature females. Standard errors and confidence intervals were corrected using the overdispersion parameter, $c\text{-hat}$, which was estimated as 1.1163 in 2018 and 1.1510 in 2019, by dividing the deviance of the saturated model by the mean deviance

of 100 bootstrap runs, using the option implemented in MARK. Models were ranked by QAIC corrected for overdispersion (Burnham and Anderson 1998).

Sperm competition behaviour

To test whether males can remove rivals' stored sperm from the female genitalia, we collected females early in the morning, before mating activity (pre-copula; $N = 8$), females after copulation (post-copula, $N = 6$) and females whose copulation was interrupted at the end of stage I, but before insemination (interrupted, $N = 5$). These females were stored in 80% ethanol and genitalia was dissected, cleaned to remove fat and muscle tissue,

Table 1. Estimates of survival (Φ) and recapture (p) rates for adult *Phylloestes ethelae* in 2018 and 2019. In both years, the best supported model was $\{\Phi(.), p(g)\}$, indicating a single value of survival for all groups, but different recapture rates. The estimate of survival for 2018 is unreliable, given the value of the SE.

Tabla 1. Estimaciones de las tasas de supervivencia (Φ) y recaptura (p) de adultos de *Phylloestes ethelae* en 2018 y 2019. En ambos años, el modelo más apoyado fue $\{\Phi(.), p(g)\}$, que indica un único valor de supervivencia para todos los grupos, pero diferentes tasas de recaptura. La estimación de supervivencia de 2018 no es fiable, dado el valor de la DE.

Year and group	Parameter	Estimate	SE
2018	Φ	1.0000	0.0000
Territorial males	p	0.8056	0.0493
Non-territorial males	p	0.1037	0.0277
Females	p	0.0822	0.0340
2019	Φ	0.9526	0.0122
Territorial males	p	0.6815	0.0347
Non-territorial males	p	0.1553	0.0225
Young females	p	0.0541	0.0339
Mature females	p	0.0446	0.0172

and observed by microscope. Final sample sizes were smaller because some samples were damaged during dissection. We estimated the area of the sperm mass from calibrated microscope images using the software ImageJ. The volume of sperm was calculated from the area multiplied by a thickness of 0.2 mm, using insect minutia between the slide and the cover-slide. The volume of sperm inside the seminal vesicle of males was measured using the same method for the five males whose mating was interrupted at the end of stage I, one male that performed sperm translocation but never mated, and two males collected after copulation. The morphology of the genital ligula was observed through Scanning Electron Microscope (SEM) at CACTI facilities (University of Vigo).

If males remove sperm, the volume of sperm in interrupted females should be smaller than the volume of post-copula females, while pre-copula females should store a similar volume as post-copula females if the amount of sperm received during copulation is not consumed during oviposition. Statistical tests were computed with xIStat 2021 (xlstat.com).

Results

Survival

In 2018 we marked 80 males and 28 females and recaptured 34 and 5, respectively. In 2019 we marked 108 males and 44 females and recaptured 70 and 7 respectively. Territorial males showed high site fidelity. For example, for the 2019 dataset, one male was resighted on 11 days, three males on 10 days, and six on 9 days. Females, on the contrary, were rarely seen, being recorded on 1-3 different days. The 2018 dataset was too sparse to allow precise estimation of survival. In this dataset, the best supported model

(table 1s in supplementary material) was $\{\Phi(.), p(g)\}$, suggesting similar survival for territorial males, non-territorial males, and females, but the estimated value was 1.0, with a SE=0, indicating that the parameter could not be correctly estimated. There were, however, clear differences in recapture rates, with a high value for territorial males (0.806) and a very low value for non-territorial males (0.104) and females (0.082) (table 1). This might be explained in part by the impossibility to define some never-recaptured males as territorial. However, the longer period of fieldwork in 2019 gave more precise estimates of these parameters for all groups. The best supported model was again $\{\Phi(.), p(g)\}$ (table 1s in supplementary material). The survival rate was estimated as 0.953 ± 0.012 (table 1), which gives an expected lifespan of 20.6 days using the formula $-1/\text{LN}(\Phi)$ (Cook et al 1967). As expected, recapture rates were higher for territorial males (0.682) than for non-territorial males (0.155) and young (0.054) and mature females (0.045). In both years, the second model had lower support than the first model (table 1s in supplementary material). Parameters were therefore estimated from the first model only.

General activity

Territorial males started to arrive at the riverbank at about 9:30, when the temperature was around 23 °C. They perched on the riverbank at a height of 1-2 m, with a distance between them of 2-5 m, and they remained motionless in the same spot for long periods (fig. 2A). Their inactivity was so extreme that they were easily approached and could be collected by hand in most cases. Some males remained in the area for the whole day, leaving only after dusk. For example, male 167 was observed from 9:32 to 17:10 h in his territory on 21 August 2019, being recorded

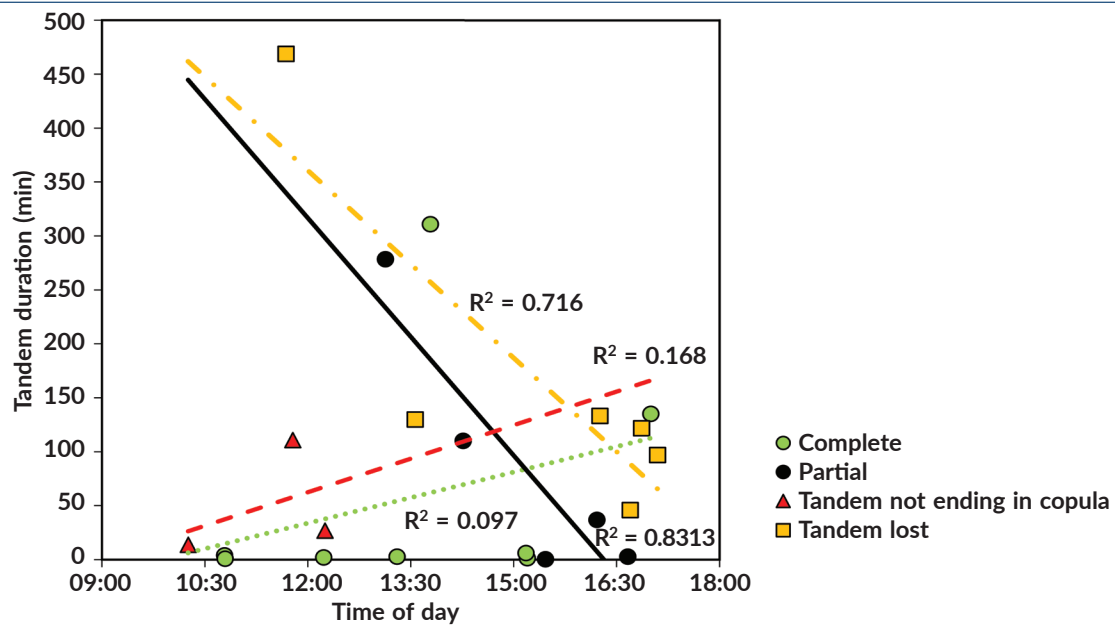


Fig. 4. The relationship between the duration of the precopulatory tandem and time of day in *Phyllostes ethelae*. Circles represent tandems that ended in copulation, which were observed from their start (complete) or not (partial). Triangles indicate three pairs which ended by the male releasing the female without copulation. Tandems lost (squares) refers to tandems whose end could not be recorded, and it is therefore unknown whether they mated or not.

Fig. 4. Relación entre la duración del tándem precopulatorio y la hora del día en *Phyllostes ethelae*. Los círculos representan los tándems que terminaron en cópula y que se observaron desde el inicio (completos) o no (parciales). Los triángulos indican tres parejas en las que el macho liberó a la hembra sin cópula. Los cuadrados ("Tandem lost") se refieren a los casos en que no se pudo registrar el final del tándem y, por lo tanto, se desconoce si hubo apareamiento o no.

eight times that day. Agonistic encounters between territorial males were almost inexistent. Only two fights were recorded in 2018 and three in 2019, all lasting only a few seconds. After these encounters, the males returned to their previous perches or disappeared. At the end of the day males were observed perching on treetops (fig. 2B). Females were mostly seen on the path inside the forest (fig. 2C), feeding on treetops or perched over the river (fig. 2D).

Reproductive behaviour

We recorded 28 tandems (22 of which were timed) and observed a total of 17 copulations. Males grasped females in tandem without previous courtship (fig. 3A), at any moment of the day, the earliest time noted being 10:15 and the latest at 17:06 h. From eight male-female encounters we found that females may try to escape by dropping to the ground ($N = 1$) while others confronted the male ($N = 3$), opening their wings in a clear refusal display ($N = 1$), or they successfully flew away when males approached ($N = 3$). Pairs in tandem remained perched on the shoreline vegetation, and the male showed occasional movements, moving the abdomen in an attempt to make contact with female genitalia (= copulation invitation). From time to time, the pairs in tandem made short flights, going higher up the trees each time (fig. 3B; sometimes after being disturbed by

other males), and therefore, some were lost before copulation due to low light conditions hampering observation (fig. 3C). The duration of the precopulatory tandem was highly variable (fig. 4). Mating followed shortly (on average in 3 min) in 6 out of 8 tandems whose start was observed, but in two cases the males remained in tandem for 135 and 311 min before copulation. Five tandems whose start was not observed ended in copulation and lasted 85.9 ± 52.1 min (range: 0.6 to 279 min; fig. 4). Six pairs that were lost from sight remained in tandem for 166.2 ± 62.0 min (range: 46 to 469 min). Whether or not they copulated is unknown, but at 19:30 h we had to abandon observation of the pair that remained in tandem for 469 min as it was impossible to continue in total darkness. At 21:56 h, the site was checked by torchlight and the male was still on the same perch, suggesting the couple mated in complete darkness. In three cases males released the female –without reaching copulation– after being in tandem for 50.6 ± 30.4 min (range: 14 to 111 min).

Males in tandem performed the intra-male sperm translocation by flexing their abdomen to contact the seminal vesicle on segment 2 with the primary genitalia (fig. 3D). This behaviour lasted 5.7 ± 0.4 seconds ($N = 11$) and took place 77.02 ± 35.6 min (range: 0.2 to 311 min) after the first observation of the tandem. We timed 9 copulations (fig. 5; all but one from the onset), with a duration of 18.61 ± 4.09 min



Fig. 5. Copulation behaviour and oviposition in *Phyllolestes ethelae*. The first minutes of copulation or stage I are characterised by pumping movements of the male abdomen (A), during which the male removes stored sperm from the female *bursa copulatrix*. In the last minute, the typical movements of stage II are observed, when the abdomen of the male is curved downwards, and the sperm is transferred to the female (B). After copulation, the male might remain for a short time perched with its mate (C), which a few minutes later is observed laying eggs on branches above the river (D). (Pictures by A Cordero-Rivera).

Fig. 5. Comportamiento de cópula y oviposición en *Phyllolestes ethelae*. Los primeros minutos de la cópula o etapa I se caracterizan por los movimientos de bombeo del abdomen masculino (A), durante los cuales el macho extrae el esperma almacenado de la bursa copulatrix femenina. En el último minuto, se observan los movimientos típicos de la etapa II, cuando el abdomen del macho se curva hacia abajo y se transfiere el esperma a la hembra (B). Después de la cópula, el macho puede permanecer por un corto tiempo posado con su pareja (C), que unos minutos más tarde se observa poniendo huevos en las ramas sobre el río (D). (Fotografías de A Cordero-Rivera).

(range: 5.8 to 46.6 min), and a negative relation with time of day ($r = -0.73$, $p = 0.027$; fig. 6). The movements of male genitalia during the first part of copulation were similar to those described as stage I by Miller and Miller (1981), a phase that lasted 12.55 ± 1.82 min (range: 5.2 to 25.2; $N = 12$; fig. 5A). This phase was followed by the typical movements of stage II, with a duration of 0.50 ± 0.07 min (range 0.37 to 0.75, $N = 8$; fig. 5B). After copulation, the post-copulatory tandem was very brief, lasting 1.07 ± 0.25 min ($N = 8$), and both individuals left or remained perched (fig. 5C).

Nine females were observed laying eggs, alone in all cases. Three of them were observed in oviposition immediately after copulation, but the remaining

females found laying eggs had not been seen mating previously. Oviposition took place on lianas and branches hanging over the river (fig. 5D), the earliest at 9:39 and the latest at 19:14 h, in almost complete darkness, only 6 min after the end of one mating.

Sperm competition mechanism

The genitalia of female *P. ethelae* show a large spherical *bursa copulatrix*, and very long and thin paired spermathecae, united to the bursa by a common duct (fig. 7A-7B). Male genital ligula is spoon-like, with a smooth surface and a median tubercle in the distal part (fig. 7C-7D). The ligula has no horns or filaments that could be used to remove sperm from the spermathecae.

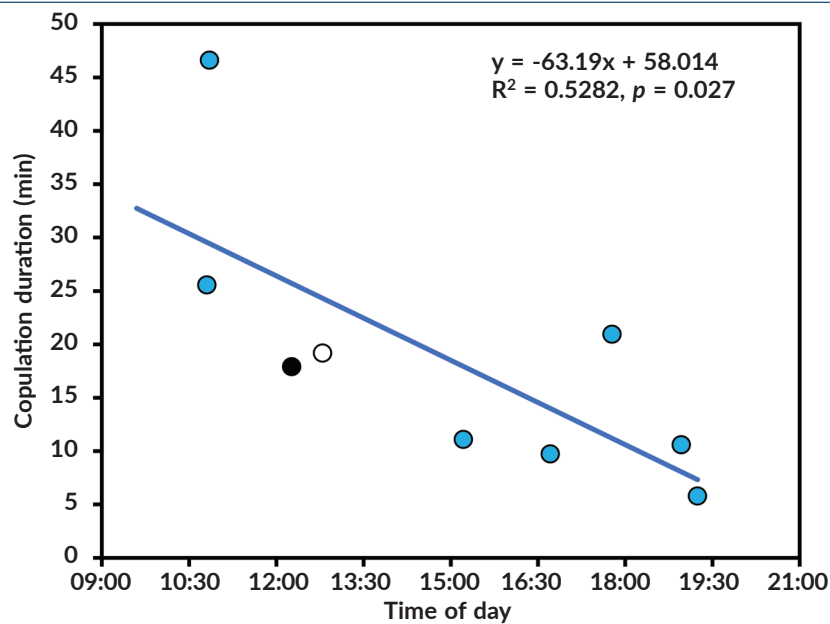


Fig. 6. The relationship between duration of copulation and time of day in *Phylolestes ethelae*. The black dot indicates a mating observed inside an insectary, and the blank circle indicates a mating whose start was not observed.

Fig. 6. Relación entre la duración de la cópula y la hora del día en *Phylolestes ethelae*. El punto negro indica un apareamiento observado dentro de un insectario y el círculo blanco un apareamiento cuyo inicio no se observó.

Three of the dissected females had no sperm at all and were therefore assumed to be virgin (one interrupted and two pre-copula females). These individuals were excluded from further analyses. The volume of sperm in the bursa copulatrix differed significantly between treatments, with a lower value in interrupted females (ANOVA, $F_{2,11} = 5.81$, $p = 0.019$), whose bursa stored only 33.7% of sperm compared to post-copula females (fig. 8). In contrast, there were no significant differences in the volume of the spermathecae between treatments ($F_{2,11} = 0.43$, $p = 0.662$).

Post-copula males showed an empty seminal vesicle ($N = 2$), as expected after insemination. The volume of sperm in the seminal vesicle of males interrupted during stage I was $0.019 \pm 0.003 \text{ mm}^3$, whereas the total volume of sperm in post-copula females was $0.062 \pm 0.011 \text{ mm}^3$. The male collected after sperm translocation had 0.023 mm^3 of sperm in his seminal vesicle.

Discussion

In agreement with our initial hypothesis, our results indicate that *Phylolestes ethelae* has several unusual features, likely related to its isolation from the geographic and phylogenetic perspectives. This large damselfly shows very limited activity. Our mark-recapture study indicates that most male behaviour is territorial, showing high site fidelity, but agonistic interactions were almost never observed. The most unique behaviour is the fact that some males delayed

copulation until dusk, a finding previously unknown in odonates. From the point of view of postcopulatory sexual selection, this species is able to remove sperm from the bursa copulatrix of the female, but not from the spermathecae, an organ which is the main source of sexual conflict for fertilization in odonates (Cordero-Rivera and Córdoba-Aguilar 2016). We will discuss these findings in the context of behavioural evolution.

Males of *P. ethelae* perch in prominent places on the riverine vegetation where they are highly conspicuous due to their size (fig. 2). Most individuals return to the same spot day after day, and could therefore be described as territorial (Baker 1983). However, they rarely engage in aggressive encounters with other males, and when they do, these last for a only a few seconds. From this point of view and the fact that they do not defend oviposition sites or other resources needed by females, their behaviour can hardly be considered territorial. Their passivity is extreme, so much so that they remain perched even if the observer approaches and grasps them by hand. They seem to have no specialised predators, and in our experience, it was rare to see birds around the river. Both sexes were observed on treetops early in the morning, taking the first sunshine. Temperature could perhaps be a limiting factor for this large species because it inhabits areas at an altitude of around 1,000 m. In agreement with this observation, small species of odonates have been found to be more tolerant to higher temperatures than larger species (Bota-Sierra et al 2022), and in our experience, *P. ethelae* individuals commonly perched in shaded places in the central hours of the day. As a

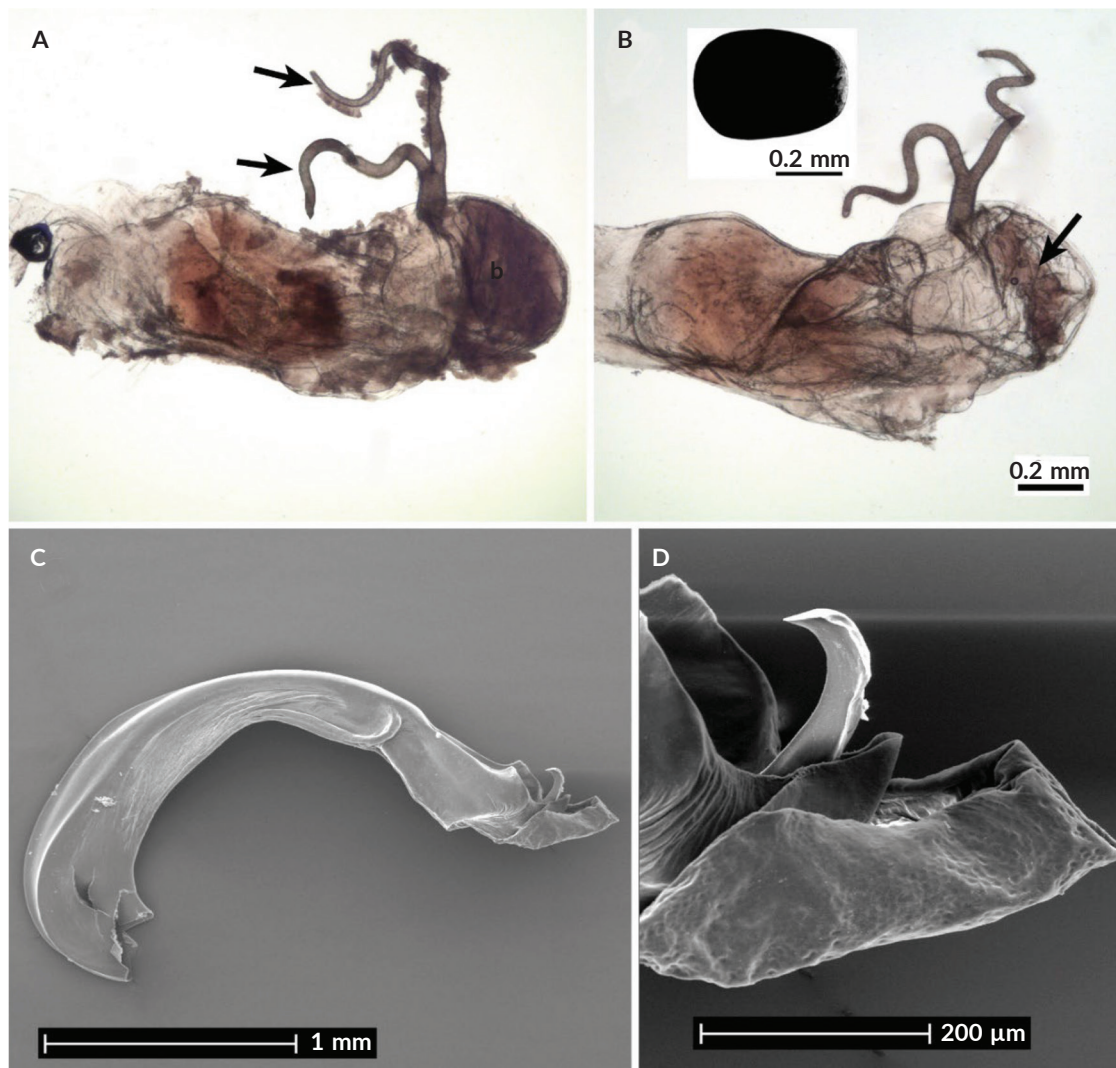


Fig. 7. Female and male genitalia of *P. ethelae*: A, vaginal plates, bursa copulatrix (b) and spermathecae (arrows) of a female captured early in the morning (pre-copula), showing bursa with a large amount of sperm (dark mass); B, genitalia of a female interrupted at the end of stage I, with the bursa almost empty (the arrow indicates the sperm mass); the insert shows the sperm mass dissected from the seminal vesicle of a male, at the same scale. Male genital ligula observed at SEM (C), with a detail of the distal part (D). Images were edited to remove dust and particles, and in (A) the tip of the lower spermathecae was digitally pasted in its place because it was broken during dissection. (Pictures by A Cordero-Rivera).

Fig. 7. Genitales femeninos y masculinos de *P. ethelae*: A, placas vaginales, bursa copulatrix (b) y espermatecas (flechas) de una hembra capturada a primera hora de la mañana (antes de la cópula), donde se observa la bursa con una gran cantidad de esperma (masa oscura); B, genitales de una hembra a la que se interrumpió al final de la etapa I, con la bursa casi vacía (la flecha indica la masa espermática); en la imagen superpuesta se muestra la masa espermática extraída de la vesícula seminal de un macho, a la misma escala. Lígula genital masculina observada con un microscopio electrónico de barrido (C), donde se muestra un detalle de la parte distal (D). Las imágenes se editaron para eliminar el polvo y las partículas y en (A), la punta de las espermatecas inferiores se pegó digitalmente en su lugar porque se había roto durante la disección. (Fotografías de A Cordero-Rivera).

tropical forest species, the narrow range and habitat specialization of this species is an important argument in support of forest conservation (Bota-Sierra et al 2021). Furthermore, as an endangered riverine species (von Ellenrieder and Paulson 2006), this taxon could be used as a flag species for the conservation of mountain tropical streams.

The high site fidelity of males allowed us to mark and recapture most individuals, and their recapture histories indicate that survival was high (0.953 daily

survival rate; table 1) and similar between sexes. However, the recapture rate was much higher for males, particularly the 'territorial' males, than for females. In a recent review of mark-recapture studies in odonates, daily survival rates for males were on average 0.895 (Sanmartín-Villar and Cordero-Rivera 2022), and few species have been found to have daily survival values above 0.95 (Cordero 1988, Cordero-Rivera et al 2019, Gabela-Flores et al 2019, Munguía-Steyer et al 2010). Therefore, we can describe *P. ethelae* as a long-lived

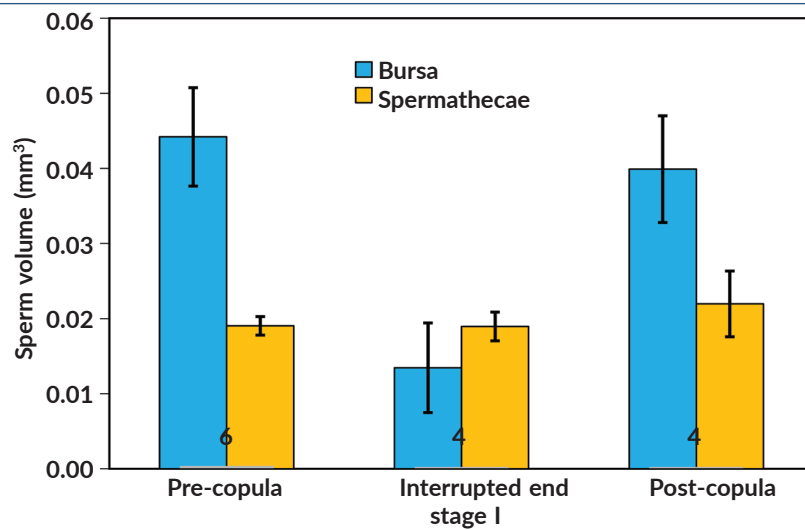


Fig. 8. The volume of sperm (mean \pm SE) in the *bursa copulatrix* and spermathecae of *Phyllostes ethelae* females collected before copulation (pre-copula), interrupted at the end of stage I of copulation, and after copulation (post-copula). The volume of sperm in the *bursa* was significantly lower for interrupted females, but there were no significant differences in the sperm volume of the spermathecae. Numbers on bars indicate sample size.

Fig. 8. Volumen de esperma (media \pm EE) en la bursa copulatrix y las espermatecas de las hembras de *Phyllostes ethelae* recogidas antes de la cópula (precópula), al final de la etapa I de la cópula y después de la cópula (postcópula). El volumen de esperma en la bursa fue significativamente menor en las hembras a las que se interrumpió, pero no hubo diferencias significativas en el volumen de esperma de las espermatecas. Los números en las barras indican el tamaño de la muestra.

species, a strategy that might be common in tropical damselflies (Cordero-Rivera et al 2019). The sex ratio was heavily biased to males, clearly because females visited the river only to mate and lay eggs, producing a large difference in recapture rates between sexes and ages (reviewed by Sanmartín-Villar and Cordero-Rivera 2022).

Copulatory behaviour of *P. ethelae* was similar to the general pattern described for zygopterans. First, males translocate sperm to their seminal vesicle before copulation, but after having grasped the female in tandem (for a review of this behaviour see Rivas-Torres et al 2019). A brief copulation then follows, the duration of which is negatively related to the time of day (fig. 6), a common pattern in zygopterans (e.g., Rivas-Torres et al 2023). The movements of the male abdomen and genitalia during copulation are similar to those described for other species, which allowed Miller and Miller (1981) to define stage I, associated to rivals' sperm removal and stage II, where insemination takes place. From this point of view, this species shows a typical zygopteran behaviour. *Synlestes weyersii*, an Australian species of the same family, has been observed mating for 6 min (only two copulations recorded; Coy 2014), a much shorter time than that for most of our observations. More research on this small family is needed to better understand the evolution of copulation behaviour in the order Odonata, and to put our findings into context, because as far as we know, no other information is available on any species of the Synlestidae, and therefore we do not know whether nocturnal copulation is found in other species of this family.

The estimate of sperm volumes in females gave unambiguous results, indicating that sperm is removed by males from the bursa using their genital ligula, even if they apparently have no spines to remove sperm, unlike most odonates (Córdoba-Aguilar et al 2003). The long, thin morphology of the spermathecae suggests that males cannot remove sperm from this organ as they lack specialised structures in the penis head, and sperm volumes did not differ between treatments. This contrasts with other species of damselflies, whose males can remove sperm from the spermathecae using genital flagella (Cordero-Rivera 2017b). Therefore, we predict last-male sperm precedence in this species in the short term, but sperm mixing in the long term if females lay eggs without further mating. Males of *P. ethelae* did not guard females after copulation, unlike *Synlestes weyersii*, whose males remained close to the females during oviposition (Coy 2014). Unguarded females could mate again, but given the low frequency of copulations observed during fieldwork, this is unlikely. Possibly, some matings were undetected, particularly if they occurred at dusk, but low mating frequencies have also been reported in some other tropical odonates (Sanmartín-Villar and Cordero-Rivera 2016), making the strategy of unguarded oviposition successful for males. The volume of sperm estimated in the seminal vesicle of males whose mating was interrupted before insemination (0.019 mm³) was clearly smaller than the volume estimated in post-copula females (0.062 mm³). This can be explained by the clearly higher density of sperm in males, whose sperm mass was completely opaque to light (fig. 7B), in contrast to the sperm mass inside females.

The most surprising result was the delay of copulation until late afternoon, even until dusk, by some males (fig. 3). Nocturnal copulation in mainly diurnal insects has been reported in some tropical regions, for instance in stalk-eyed flies (Lorch et al 1993) and butterflies (Molleman et al 2020), but appears to be a rare behaviour. Some temperate fruit flies show high variability in copulation duration, sometimes including the night (Rull et al 2017). In contrast, crepuscular and nocturnal copulation is the typical behaviour of *Panorpa* scorpionflies (Thornhill 1980). Some odonates, particularly Aeshnidae, are crepuscular and even nocturnal (e.g., Borkenstein et al 2016), and a few species show nocturnal roosting aggregations (Paulson et al 2020). There are even reports of ovipositing tandem pairs of *Indolestes peregrinus* that remained attached throughout the night and resumed oviposition the next day when kept in net cages in the field (Naraoka 2013). However, as far as we know, no other odonate is known to show mating behaviour by night. If *P. ethelae* males were very susceptible to high temperatures, this could explain the observation that that some matings occurred late. Nevertheless, some males mated at midday, in full solar exposure, and for this reason we consider this explanation unlikely. Another explanation could be related to the fact that males seem unable to remove sperm from the spermathecae (fig. 8). In some species of damselflies, there is evidence of biased fertilization using bursal or spermathecal sperm, apparently under the control of females (Siva-Jothy and Hooper 1996). In *Ceriagrion tenellum*, males are also unable to remove sperm from the spermatheca, and by prolonging copulation achieve higher reproductive success (Andrés and Cordero-Rivera 2000). These ideas point to a sexual conflict over fertilization, with males engaged in long-lasting tandems as a way to induce females to give priority to their sperm during oviposition, i.e., cryptic female choice (Córdoba-Aguilar and Cordero-Rivera 2008). Further experiments would be needed to test this hypothesis, for instance by estimating male fertilization success after long and short precopulatory tandems, as the natural variation in this behaviour is huge (fig. 4).

In conclusion, *Phyllostes ethelae* shows unique behaviours, is a conspicuous and dominant species in the high-altitude rivers of Hispaniola and is completely dependent on the shaded environments that the tropical forest provides. Given its phylogenetic uniqueness, its rarity, and its reduced distribution, this species is a valuable sentinel for the conservation of tropical forest ecosystems and mountain rivers in the Antilles.

References

- Andrés JA, Cordero-Rivera A, 2000. Copulation duration and fertilization success in a damselfly: An example of cryptic female choice? *Animal Behaviour* 59(4), 695-703. DOI: [10.1006/anbe.1999.1372](https://doi.org/10.1006/anbe.1999.1372)
- Baker RR, 1983. Insect territoriality. *Annual Review of Entomology* 28, 65-89. DOI: [10.1146/annurev.en.28.010183.000433](https://doi.org/10.1146/annurev.en.28.010183.000433)
- Borkenstein A, Schröter A, Jödicke R, 2016. Aeshna viridis is an early bird - matutinal matings in a crepuscular species (Odonata: Aeshnidae). *Odonatologica* 45(1/2), 37-56. DOI: [10.5281/zenodo.50848](https://doi.org/10.5281/zenodo.50848)
- Bota-Sierra CA, Flórez-V C, Escobar F, Sandoval-H J, Novelo-Gutiérrez R, Londoño GA, Cordero-Rivera A, 2021. The importance of tropical mountain forests for the conservation of dragonfly biodiversity: A case from the Colombian Western Andes. *International Journal of Odonatology* 24, 233-247. DOI: [10.23797/2159-6719_24_18](https://doi.org/10.23797/2159-6719_24_18)
- Bota-Sierra CA, García-Robledo C, Escobar F, Novelo-Gutiérrez R, Londoño GA, 2022. Environment, taxonomy and morphology constrain insect thermal physiology along tropical mountains. *Functional Ecology* 36(8), 1924-1935. DOI: [10.1111/1365-2435.14083](https://doi.org/10.1111/1365-2435.14083)
- Burnham KP, Anderson DR, 1998. *Model Selection and Inference. A Practical Information-Theoretic Approach*. Springer, NY. DOI: [10.1007/978-1-4757-2917-7](https://doi.org/10.1007/978-1-4757-2917-7)
- Christiansen KA, 1947. A new genus and species of damselfly from Southern Haiti (Odonata). *Psyche* 54(4), 256-262. DOI: [10.5281/zenodo.3241358](https://doi.org/10.5281/zenodo.3241358)
- Cook LM, Brower PP, Crozer HJ, 1967. The accuracy of a population estimation from multiple recapture data. *Journal of Animal Ecology* 36, 57-60. DOI: [10.2307/3014](https://doi.org/10.2307/3014)
- Corbet PS, 1999. *Dragonflies. Behaviour and Ecology of Odonata*. Harley Books, Colchester, UK.
- Cordero A, 1988. Estudio ecológico de una población de *Lestes viridis* Vander Linden, 1825 (Zygoptera: Lestidae). *Limnética* 4, 1-8. <https://www.limnetica.com/documentos/limnetica/limnetica-4-1-p-1.pdf>
- Cordero-Rivera A, 2016a. Demographics and adult activity of *Hemiphysalia mirabilis*: A short-lived species with a huge population size (Odonata: Hemiphysaliidae). *Insect Conservation and Diversity* 9, 108-117. DOI: [10.1111/icad.12147](https://doi.org/10.1111/icad.12147)
- Cordero-Rivera A, 2016b. Sperm removal during copulation confirmed in the oldest extant damselfly, *Hemiphysalia mirabilis*. *PeerJ* 4, e2077. DOI: [10.7717/peerj.2077](https://doi.org/10.7717/peerj.2077)
- Cordero-Rivera A, 2017a. Behavioral diversity (ethodiversity): A neglected level in the study of biodiversity. *Frontiers in Ecology and Evolution* 5, 1-7. DOI: [10.3389/fevo.2017.00007](https://doi.org/10.3389/fevo.2017.00007)
- Cordero-Rivera A, 2017b. Sexual conflict and the evolution of genitalia: Male damselflies remove more sperm when mating with a heterospecific female. *Scientific Reports* 7(1), 7844. DOI: [10.1038/s41598-017-08390-3](https://doi.org/10.1038/s41598-017-08390-3)
- Cordero Rivera A, Andrés JA, 2002. Male coercion and convenience polyandry in a Calopterygid damselfly (Odonata). *Journal of Insect Science* 2, 14. DOI: [10.1093/jis/2.1.14](https://doi.org/10.1093/jis/2.1.14)
- Cordero-Rivera A, Córdoba-Aguilar A, 2016. Selección postcópula: Competencia espermática y elección femenina críptica. In: *Etología adaptativa: El comportamiento como producto de la selección natural*: 471-497 (J. Carranza (Ed). UCopress y Publicaciones de la Universidad de Extremadura. <http://hdl.handle.net/10261/138196>
- Cordero-Rivera A, Sanmartín-Villar I, Sánchez Herrera M, Rivas-Torres A, Encalada A, 2019. Survival and longevity in neotropical damselflies (Odonata, Polythoridae). *Animal Biodiversity and Conservation* 42(2), 293-300. DOI: [10.32800/abc.2019.42.0293](https://doi.org/10.32800/abc.2019.42.0293)
- Cordero-Rivera A, Zhang H, 2018. Ethological uniqueness of a damselfly with no near relatives: The relevance of behaviour as part of biodiversity. *Animal Biodiversity and Conservation* 41(1), 161-174. DOI: [10.32800/abc.2018.41.0161](https://doi.org/10.32800/abc.2018.41.0161)
- Córdoba-Aguilar A, 2008. *Dragonflies and Damselflies. Model Organisms for Ecological and Evolutionary Research*. Oxford University Press, DOI: [10.1093/acprof:oso/9780199230693.001.0001](https://doi.org/10.1093/acprof:oso/9780199230693.001.0001)
- Córdoba-Aguilar A, Beatty CD, Bried JT (Eds), 2022. *Dragonflies and Damselflies. Model Organisms for Ecological and Evolutionary Research* (2nd ed.). Oxford University Press. DOI: [10.1093/acprof:oso/9780192898623.001.0001](https://doi.org/10.1093/acprof:oso/9780192898623.001.0001)
- Córdoba-Aguilar A, Cordero-Rivera A, 2005. Evolution and ecology of Calopterygidae (Zygoptera: Odonata): Status of knowledge and research perspectives. *Neotropical Entomology* 34(6), 861-879. DOI: [10.1590/S1519-566X2005000600001](https://doi.org/10.1590/S1519-566X2005000600001)
- Córdoba-Aguilar A, Cordero-Rivera A, 2008. Cryptic female choice and sexual conflict. In: *Dragonflies and damselflies. Model organisms for ecological and evolutionary research*: 189-202 (A Córdoba-Aguilar, Ed). Oxford University Press. DOI: [10.1093/acprof:oso/9780199230693.001.0001](https://doi.org/10.1093/acprof:oso/9780199230693.001.0001)
- Córdoba-Aguilar A, Uhía E, Cordero-Rivera A, 2003. Sperm competition in Odonata (Insecta): The evolution of female sperm storage and rivals' sperm displacement. *Journal of Zoology* 261(4), 381-398. DOI: [10.1017/S0952836903004357](https://doi.org/10.1017/S0952836903004357)
- Coy R, 2014. *Synlestes weyersii*: Observations of mating and oviposition. *Victorian Entomologist* 44(4), 74-79.
- Gabela-Flores MV, Sanmartín-Villar I, Rivas-Torres A, Encalada AC, Cordero-Rivera A, 2019. Demography and territorial behaviour of three species of the genus *Hetaerina* (Odonata: Calopterygidae) along three tropical stream ecosystems. *Odonatologica* 48(1/2), 79-100. DOI: [10.5281/zenodo.2677691](https://doi.org/10.5281/zenodo.2677691)
- Gross MR, 1996. Alternative reproductive strategies and tactics: Diversity within sexes. *Trends in Ecology and Evolution* 11(2), 92-98. DOI: [10.1016/0169-5347\(96\)81050-0](https://doi.org/10.1016/0169-5347(96)81050-0)

- Laman T, Scholes E, 2012. *Birds of Paradise: Revealing the World's Most Extraordinary Birds*. National Geographic Society, USA.
- Lorch PD, Wilkinson GS, Reillo PR, 1993. Copulation duration and sperm precedence in the stalk-eyed fly *Cyrtodiposis whitei* (Diptera: Diopsidae). *Behavioral Ecology and Sociobiology* 32, 303-311. DOI: [10.1007/BF00183785](https://doi.org/10.1007/BF00183785)
- Martens A, 2003. Reproductive behaviour of African Odonata – A review. *Cimbebasia* 18, 225-241.
- Miller PL, 1995. Sperm competition and penis structure in some libellulid dragonflies (Anisoptera). *Odonatologica* 24, 63-72.
- Miller PL, Miller CA, 1981. Field observations on copulatory behaviour in Zygoptera, with an examination of the structure and activity of male genitalia. *Odonatologica* 10(3), 201-218.
- Molleman F, Halali S, Kodandaramaiah U, 2020. Brief mating behavior at dawn and dusk and long nocturnal matings in the butterfly *Melanitis leda*. *Journal of Insect Behavior* 33(2-4), 138-147. DOI: [10.1007/s10905-020-09753-x](https://doi.org/10.1007/s10905-020-09753-x)
- Munguía-Steyer R, Córdoba-Aguilar A, Romo-Beltrán A, 2010. Do individuals in better condition survive for longer? Field survival estimates according to male alternative reproductive tactics and sex. *Journal of Evolutionary Biology* 23(1), 175-184. DOI: [10.1111/j.1420-9101.2009.01894.x](https://doi.org/10.1111/j.1420-9101.2009.01894.x)
- Naraoka H, 2013. Diurnal activity and reproductive behavior of *Indolestes peregrinus* (Ris. 1916) (Lestidae: Odonata). *Tombo* 55, 91-98.
- Neff BD, Svensson EI, 2013. Polyandry and alternative mating tactics. *Philosophical Transactions of the Royal Society of London, Series B Biological Sciences* 368, 20120045. DOI: [10.1098/rstb.2012.0045](https://doi.org/10.1098/rstb.2012.0045)
- Paulson DR, Landeira-Dabarcá A, Haave-Audet E, 2020. First nocturnal roosting aggregations of dragonflies reported from the New World tropics. *Notulae Odonatologicae* 9(6), 263-268. DOI: [10.60024/zenodo.4268593](https://doi.org/10.60024/zenodo.4268593)
- Rivas-Torres A, Di Pietro V, Cordero-Rivera A, 2023. Sex wars: A female genital spine forces male damselflies to shorten copulation duration. *Evolution* 77(7), 1659-1666. DOI: [10.1093/evolut/qpad073](https://doi.org/10.1093/evolut/qpad073)
- Rivas-Torres A, Outomuro D, Lorenzo-Carballa MO, Cordero-Rivera A, 2019. The evolution and diversity of intra-male sperm translocation in Odonata: A unique behaviour in animals. *Behavioral Ecology and Sociobiology* 73, 54. DOI: [10.1007/s00265-019-2660-5](https://doi.org/10.1007/s00265-019-2660-5)
- Rull J, Abraham S, Schlisermann P, Ordano M, Ovruski S, 2017. Mating behavior and basic biology of *Haywardina cuculi* (Diptera: Tephritidae), a poorly known species exhibiting high variability in copulation duration. *Journal of Insect Behavior* 30(4), 439-453. DOI: [10.1007/s10905-017-9629-x](https://doi.org/10.1007/s10905-017-9629-x)
- Sánchez-Guillén RA, Ceccarelli S, Villalobos F, Neupane S, Rivas-Torres A, Sanmartín-Villar I, Wellenreuther M, Bybee SM, Velásquez-Vélez MI, Realpe E, Chávez-Ríos JR, Dumont HJ, Cordero-Rivera A, 2020. The evolutionary history of colour polymorphism in *Ischnura* damselflies (Odonata: Coenagrionidae). *Odonatologica* 49(3-4), 333-370. DOI: [10.5281/zenodo.4268559](https://doi.org/10.5281/zenodo.4268559)
- Sanmartín-Villar I, Cordero-Rivera A, 2016. Female colour polymorphism and unique reproductive behaviour in *Polythore* damselflies (Zygoptera: Polythoridae). *Neotropical Entomology* 45, 658-664. DOI: [10.1007/s13744-016-0417-7](https://doi.org/10.1007/s13744-016-0417-7)
- Sanmartín-Villar I, Cordero-Rivera A, 2022. Odonata survival: Insights from mark-recapture experiments. In: *Dragonflies and Damselflies: Model Organisms for Ecological and Evolutionary Research*: 129-140 (A Córdoba-Aguilar, CD Beatty, JT Bried, Eds). Oxford University Press. DOI: [10.1093/oso/9780192898623.001.0001](https://doi.org/10.1093/oso/9780192898623.001.0001)
- Simaika JP, Ware JL, Garrison RW, Samways MJ, 2020. Phylogeny of the Synlestidae (Odonata: Zygoptera), with an emphasis on *Chlorolestes* Selys and *Ecchlorolestes* Barnard. *Scientific Reports* 10, 15088. DOI: [10.1038/s41598-020-72001-x](https://doi.org/10.1038/s41598-020-72001-x)
- Siva-Jothy MT, Hooper RE, 1996. Differential use of stored sperm during oviposition in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). *Behavioral Ecology and Sociobiology* 39, 389-393. DOI: [10.1007/s002650050305](https://doi.org/10.1007/s002650050305)
- Suhonen J, Rantala MJ, Honkavaara J, 2008. Territoriality in odonates. In: *Dragonflies and Damselflies. Model Organisms for Ecological and Evolutionary Research*: 203-218 (A Córdoba-Aguilar, Ed). Oxford University Press. DOI: [10.1093/acprof:oso/978019230693.001.0001](https://doi.org/10.1093/acprof:oso/978019230693.001.0001)
- Thornhill R, 1980. Rape in *Panorpa* scorpionflies and a general rape hypothesis. *Animal Behaviour* 28(1), 52-59. DOI: [10.1016/S0003-3472\(80\)80007-8](https://doi.org/10.1016/S0003-3472(80)80007-8)
- Von Ellenrieder N, Paulson D, 2006. *Phylolestes ethelae*. *The IUCN Red List of Threatened Species*, e.T17233A6869479. DOI: [10.2305/IUCN.UK.2006.RLTS.T17233A6869479.en](https://doi.org/10.2305/IUCN.UK.2006.RLTS.T17233A6869479.en)
- Westfal MJ, 1976. Taxonomic relationships of *Diceratobasis macrogaster* (Selys) and *Phylolestes ethelae* Christiansen of the West Indies as revealed by their larvae (Zygoptera: Coenagrionidae, Synlestidae). *Odonatologica* 5(1), 65-76.
- White GC, Burnham KP, 1999. Program mark: Survival estimation from populations of marked animals. *Bird Study* 46, S120-S139. DOI: [10.1080/00063659909477239](https://doi.org/10.1080/00063659909477239)

Acknowledgements

We thank Olalla Lorenzo and two anonymous referees for their comments on a previous version of this paper. This work was possible thanks to the logistics provided by the Museo Nacional de Historia Natural 'Prof. Eugenio de Jesús Marcano' of Santo Domingo. We warmly thank all the staff at the National Park Armando Bermúdez, at La Ciénaga de Manabao, and particularly Germán de Jesús Adames, for always having a coffee ready!

Author contributions

A Cordero-Rivera: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing the original draft, writing-review and editing.

C Suriel and JC Núñez: data curation, investigation, methodology, validation, writing-review and editing.

Conflicts of interest

No conflicts of interest declared.

Funding

A Cordero-Rivera was further funded by grants CGL2014-53140-P and PGC2018-096656-B-I00 from MCIN/AEI/10.13039/501100011033 and from 'ERDF A way of making Europe', by the 'European Union'. Permits to study and capture specimens of odonates were issued by the Ministry of Environment and Natural Resources of the Dominican Republic to ACR (number APB-07023).

Complete affiliations

Adolfo Cordero-Rivera, Universidade de Vigo, ECOEVO Lab, Escola de Enxeñaría Forestal, Campus A Xunqueira, 36005 Pontevedra, Spain.

Juan Carlos Núñez, Jardín Botánico de Santiago Profesor Eugenio de Jesús Marcano Fondecu, Avenida del Botánico Dr. José de Jesús Jiménez Almonte 1, Santiago de los Caballeros, República Dominicana.

Carlos Suriel, Programa doctoral en Ciencias Ambientales, Instituto Tecnológico de Santo Domingo (INTEC), República Dominicana.

Supplementary material

Table 1s. Results of model selection for *Phylolestes ethelae* in 2018 and 2019. Models are ordered by increasing QAIC.Tabla 1s. Resultados de la selección de modelos para *Phylolestes ethelae* en 2018 y 2019. Los modelos se ordenan en orden creciente del QAIC.

Model	QAICc	Delta QAICc	AICc Weights	Model Likelihood	Num. Par	QDeviance
2018: dispersion parameter, c-hat = 1.1163						
{Phi(.) p(g)}	189.544	0	0.623	1	4	43.320
{Phi(t) p(g)}	191.781	2.238	0.204	0.327	7	39.075
{Phi(g) p(g)}	193.082	3.539	0.106	0.170	6	42.566
{Phi(.) p(g*t)}	194.402	4.859	0.055	0.088	13	27.928
{Phi(g) p(g*t)}	198.572	9.028	0.007	0.011	15	27.251
{Phi(t) p(g*t)}	199.249	9.705	0.005	0.008	15	27.928
{Phi(g*t) p(g)}	203.855	14.311	0.000	0.001	15	32.534
{Phi(g*t) p(g*t)}	212.875	23.331	0.000	0	21	26.166
{Phi(g) p(.)}	225.618	36.074	0	0	4	79.394
{Phi(g) p(t)}	228.731	39.187	0	0	7	76.025
{Phi(g*t) p(.)}	235.589	46.045	0	0	13	69.114
{Phi(g*t) p(t)}	238.375	48.832	0	0	15	67.054
{Phi(.) p(.)}	278.507	88.963	0	0	2	136.465
{Phi(.) p(t)}	280.973	91.430	0	0	5	132.617
{Phi(t) p(.)}	282.196	92.652	0	0	5	133.839
{Phi(t) p(t)}	284.866	95.323	0	0	7	132.160
2019: dispersion parameter, c-hat = 1.1510						
{Phi(.) p(g)}	770.692	0	0.922	1	5	464.914
{Phi(g) p(g)}	775.735	5.044	0.074	0.080	8	463.718
{Phi(t) p(g)}	781.351	10.660	0.004	0.005	16	452.165
{Phi(.) p(g*t)}	804.863	34.171	0	0	49	395.548
{Phi(g) p(g*t)}	811.616	40.925	0	0	52	394.169
{Phi(t) p(g*t)}	817.279	46.587	0	0	59	380.226
{Phi(g*t) p(g)}	852.311	81.620	0	0	50	440.303
{Phi(g*t) p(g*t)}	887.629	116.937	0	0	83	375.861
{Phi(g) p(t)}	889.679	118.988	0	0	16	560.493
{Phi(g) p(.)}	914.881	144.190	0	0	5	609.104
{Phi(.) p(t)}	921.591	150.899	0	0	13	598.935
{Phi(t) p(t)}	937.580	166.888	0	0	23	592.704
{Phi(.) p(.)}	945.935	175.243	0	0	2	646.292
{Phi(t) p(.)}	954.496	183.804	0	0	13	631.841
{Phi(g*t) p(t)}	960.211	189.520	0	0	57	528.852
{Phi(g*t) p(.)}	978.463	207.772	0	0	47	574.485