# The status of *Rhionaeschna galapagoensis* (Currie, 1901) with notes on its biology and a description of its ultimate instar larva (Odonata, Aeshnidae)

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## Abstract

*The status of* Rhionaeschna galapagoensis *(Currie, 1901) with notes on its biology and a description of its ultimate instar larva (Odonata, Aeshnidae).*— A morphological, molecular, and behavioural characterization of *Rhionaeschna galapagoensis* is presented, based on a series of specimens and observations from San Cristóbal Island, Galápagos, including both adults and larvae. Several of the characters proposed earlier to distinguish between the adults of this species and its closest relative, *R. elsia*, are found to be variable, but the presence of a black band over the fronto–clypeal suture is confirmed as a good diagnostic character. The ultimate instar larvae of *R. galapagoensis* is described for the first time, and diagnosed from its closest relatives by a combination of characters, including the acute angle between the prothoracic apophyses, absence of lateral spines on abdominal segment 6, and length of cerci relative to paraprocts. Molecular analysis confirmed that *R. galapagoensis* and *R. elsia* are sister species, and showed that their genetic distance is the closest among the analyzed species, which is to be expected given the young age of the Galápagos Islands. The larvae of *R. galapagoensis* were very common and widespread in the mountain streams and a pond in the southwest of San Cristóbal. Swarms of tens of individuals formed at sunrise in the coastal vegetation, together with adults of *Tramea* cf. *cophysa*, feeding on small flying insects. Males showed patrolling behaviour on small sections of the streams and at a pond. Only one copulation was observed, lasting 10 minutes. Females oviposited alone on floating vegetation in running and standing waters. Our observations corroborate that *R. galapagoensis* and *R. elsia* are parapatric species, that are morphologically and genetically close. In San Cristóbal, *R. galapagoensis* had large populations, apparently not threatened.

Key words: Odonata, Aeshnidae, Island species, Sibling species, Endemism, *Rhionaeschna*

## Resumen

*El estado de* Rhionaeschna galapagoensis *(Currie, 1901) con notas sobre su biología y una descripción de su último estadío larvario (Odonata, Aeshnidae).*— Se presenta una caracterización morfológica, molecular y comportamental de *Rhionaeschna galapagoensis*, basada en una serie de especímenes, tanto adultos como larvas, y observaciones realizadas en la isla de San Cristóbal, en las Galápagos. Se ha observado que varios de los caracteres propuestos anteriormente para distinguir entre los adultos de esta especie y los de su pariente más próximo, *R. elsia*, son variables; sin embargo, se ha confirmado que la presencia de una banda negra en la sutura frontoclipeal es un buen carácter diagnóstico. Se describe por primera vez el último estadio larvario de *R. galapagoensis* y se distingue de sus parientes más cercanos mediante una combinación de caracteres que incluye el ángulo agudo entre las apófisis protorácicas, la ausencia de espinas laterales en el sexto segmento abdominal y la longitud de los cercos en relación con los paraproctos. El análisis molecular confirmó que *R. galapagoensis* y *R. elsia* son especies hermanas, y mostró que la distancia genética entre ellas es la menor entre las especies analizadas, lo cual es previsible dada la edad reciente de las islas Galápagos. Las larvas de *R. galapagoensis* eran muy comunes y estaban ampliamente distribuidas en los arroyos de montaña y en un estanque en el suroeste de San Cristóbal. Se observó la formación de enjambres de decenas de individuos en la vegetación costera al amanecer que, junto con adultos de *Tramea* cf. *cophysa*, se alimentaban de pequeños insectos. Los machos patrullaban pequeñas secciones de los arroyos y en un estanque. Solo

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se observó una cópula, que duró unos 10 minutos. Las hembras ovipositaron solas en la vegetación flotante de los arroyos y el estanque. Nuestras observaciones corroboran que *R. galapagoensis* y *R. elsia* son dos especies parapátricas, morfológica y genéticamente cercanas. Las poblaciones de *R. galapagoensis* en San Cristóbal son grandes y aparentemente no están amenazadas.

Palabras clave: Odonata, Aeshnidae, Especies insulares, Especies gemelas, Endemismo, *Rhionaeschna*

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## Introduction

Islands are evolutionary laboratories where speciation occurs at a high rate due to isolation (MacArthur & Wilson, 1967; Whittaker, 1998). The Galápagos are especially significant in this context in view of their effect on Charles Darwin's theory of evolution by natural selection (Darwin, 1901). Flying animals, with their high dispersal ability, are less prone to island endemism, but archipelagos situated far from the mainland are sufficiently isolated to promote speciation and evolution of novel traits. This is the case of the Azores Islands, whose poor odonata fauna (only four resident species) harbors the only case of parthenogenesis known in the entire order of Odonata (Cordero Rivera et al., 2005).

The Odonata of the Galápagos, with only nine species currently known for the archipelago (Peck, 1992; Muddeman, 2007), are a clear example of the effect of isolation on colonization events. Only one *Rhionaeschna* species, *R. galapagoensis*, has been described from the Galápagos Islands, and it is currently considered the only endemic species of the order in the Galápagos archipelago. *Rhionaeschna galapagoensis* was described by Currie (1901) as *Aeshna galapagoensis* from a male and a female collected in the Galápagos Island of San Cristóbal. It was later also described from the islands of Santa Cruz and Isabela (Calvert, 1956; Turner Jr., 1967). The male caudal appendages were depicted in Martin (1908), and the species was listed by Campos (1922) from Ecuador. Calvert (1956) included it in the subgenus *Neureclipa* Navás and provided a redescription and illustrations of the caudal appendages of males and females. Turner (1967) provided a new island record, and Belle (1991) published a few observations about its behaviour. *Aeshna galapagoensis* was transferred by von Ellenrieder (2003) to the genus *Rhionaeschna*, accompanied by a characterization, diagnosis, and illustrations of various morphological characters based on examination of the type specimens. The preliminary phylogenetic analysis of the genus based on morphological characters of the adults (von Ellenrieder, 2003) placed *R. galapagoensis* as the sister species of *R. elsia* (Calvert) within a clade including the other species previously included in the subgenus *Neureclipa* by Calvert (1956). These species share the combination of supratriangles usually free, two rows of cells between RP1 and RP2 in Hw beginning at the distal end of the pterostigma or further distally, and male cercus with dorso–distal crest as high or higher than the width of cercus at base, a prominent sub–basal tooth, and external margin concave (von Ellenrieder, 2003). Both species can be distinguished from all remaining species of *Rhionaeschna* by the combination of rounded clypeal lobes and ventral tubercle of S1 bearing only a few denticles (10 or less) restricted to its apex (von Ellenrieder, 2003). Calvert (1952) described *R. elsia* without providing any diagnosis from *R. galapagoensis*. In his monograph of the group (Calvert, 1956), he used the thoracic and membranule color pattern and shape of male caudal appendages in dorsal view to separate them. Von Ellenrieder (2000) redescribed *R. elsia*, and later (von Ellenrieder, 2003) showed that thoracic color pattern and dorsal shape of male cerci were variable in *R. elsia* and unreliable as diagnostic characters, proposing the presence or absence of a black band over the fronto–clypeal suture, and the shape of the anterior hamule anterior tip, female cercus tip, and male cercus dorso–distal crest in lateral view to distinguish between the two species.

Needham (1904) provided a brief larval description of *R. galapagoensis* based on an early instar larva. The ultimate instar larvae of slightly over half of the known species of *Rhionaeschna* were described by Calvert (1956), Walker (1958); Musser (1962), Santos (1966), Rodrigues Capítulo (1980), De Marmels (1982, 1990, 2001), Limongi (1983), Novelo–Gutiérrez & González–Soriano (1991), von Ellenrieder (1999, 2001), von Ellenrieder & Costa (2002), von Ellenrieder & Muzón (2003), Müller & Schiel (2012) and Rodriguez & Molineri (2014).

Given the scarcity of specimens of *R. galapagoensis* available for study to date, further data were needed to assess the status of *R. galapagoensis* as a different taxon from *R. elsia*. To this end, we: (i) examined morphological characters of adult and ultimate instar larvae, (ii) provided some observations on general and reproductive behaviour, and (iii) used nuclear and mitochondrial DNA sequences to contrast the specific status for these sister taxa.

## Material and methods

All observations were performed at San Cristóbal Island (Galápagos Archipelago) between 20 II and 6 III 2014. Most of the island has no road access so we were limited to the populated areas in the southwest of the island. We sampled permanent streams and ponds around the 'Hacienda El Cafetal'. Further observations were carried out at Punta Carola, where young and mature adults were found in swarms feeding on shrubs of *Hippomane mancinella* Linnaeus. Specimens collected were preserved in 80% ethanol for further study.

Acronyms for collections are as follows: ACR. Adolfo Cordero Rivera, Pontevedra, Spain; CSCA. California State Collection of Arthropods, Sacramento, California, USA; RWG. Rosser W. Garrison, Sacramento, California, USA; USFQ. Museo de Entomología Acuática, Universidad San Francisco de Quito, Ecuador.

In the laboratory, the variability of characters of adult specimens of *R. galapagoensis* was studied, documented and compared with those of a series of adult *R. elsia* in order to re–evaluate which diagnostic characters reliably identified the two species. Illustrations were made with the aid of a camera lucida coupled to a Nikon SMZ1500 stereomicroscope. Exuviae were photographed using a Canon Eos 7D mark II camera, and images were combined by means of a procedure of photo–stacking using Adobe Photoshop CS6 software (www.adobe.com).

Ultimate instar larvae were photographed and measured with an AxionCam ICc3 coupled to a ZEISS Discovery V12 and the software Axion Vision version 4.8. Mandibular formula follows Watson (1956). All measurements are given in millimeters; average dimensions are given as average ± standard deviation; hind wing measurement excludes basal sclerites; total length includes caudal appendages; larval wing cases, lateral spines on abdomen, cercus, and paraproct were measured along their inner margin. Abbreviations used throughout the text are as follows: Dept.: Department; Prov.: Province; Fw: forewing; Hw: hindwing; pnx: postnodal crossveins; S1–10: abdominal segments 1 to 10.

Material studied is detailed in appendix 1.

### Molecular analysis and phylogenetic reconstruction

#### DNA extraction and sequencing

DNA was extracted from one leg of each adult dragonfly using a GeneJet Genomic DNA Purification kit (Thermo Scientific, Waltham, USA). Three genes were amplified: mitochondrial cytochrome oxidase I (COI); hypervariable D7 region of the large–subunit 28S (rDNA) (28S); and nuclear Histone 3 (H3), using PCR according to Kohli's et al. (2014) protocol. We selected two mitochondrial and one nuclear gene based on their diverse evolutionary rates, to allow us to reconstruct both internal and external branches, respectively (Fritz et al., 1994). Successfully amplified samples were sent to Macrogen (www.macrogen.com) for bidirectional sequencing.

#### Genetic distances and phylogenetic reconstruction

Forward and reverse sequences were edited in BioEdit version 7.5.0.3 (Hall, 1999) and consensus sequences aligned with SeqMan DNAStar version 5.03 (www.dnastar.com). Variable positions were revised by eye, and only high quality sequences were considered for further analyses.

Genetic distances among the seven *Rhionaeschna* species sequenced were estimated by using Kimura 2–parameter genetic distances (Kimura, 1980) of the three genes separately; COI (20 sequences, 367 bp), nuclear H3 (21 sequences, 251 bp), and 28S (20 sequences, 454 bp). All samples of each species clustered in the same species–group, and genetic distances were estimated between groups. The gamma distribution (shape parameter = 1) was used to modulate the rate of variation among sites with MEGA 6 (Tamura et al., 2013).

A Neighbor–Joining tree (based on Maximum composite Likelihood method; Tamura et al., 2004) and a Maximum Likelihood tree (based on Tamura–Nei model; Tamura & Nei, 1993) were generated for the three concatenated genes: COI (19 sequences, 367 bp), H3 (19 sequences, 251 bp), and 28S (19 sequences, 454 bp) using MEGA 6 (Tamura et al., 2013). After deleting all positions containing gaps and missing data, we performed both phylogenetic reconstructions based on 1,065 positions.

Phylogenetic relationships among haplotypes were also estimated using Bayesian inference with the program MrBayes version 3.0 (Huelsenbeck & Ronquist, 2001). To this end, we investigated models of nucleotide substitution of the three genes and ranked them by Akaike information criterion implemented in the program jModelTest (Posada, 2008). The model Generalized Time–Reversible plus Gamma (GTR)+G (Tavaré, 1986) was inferred as the most appropriate model to estimate nucleotide substitutions for two genes (COI and H3) because it allows for a different rate of transitions and transversions as well as unequal frequencies of the four nucleotides (base frequencies). However, the model of nucleotide substitution inferred for the nDNA gene 28S was the Hasegawa–Kishino–Yano model (HKY)+G (Hasegawa et al., 1985), a model that also allows for a different rate of transitions and transversions as well as unequal frequencies of the four nucleotides (base frequencies). Thus, because the nDNA gene 28S has a different model of nucleotide substitution and a low percentage of informative positions to be analyzed alone (see details in Results section) it was not included in the Bayesian analyses. Therefore, only two genes, COI and H3, were concatenated and analyzed together. We conducted two independent runs that consisted of four Markov chains (one cold and three heated chains) each. We ran 100,000 generations, sampling every 10 generations and discarding the first 2,500 (25%) generations (burn–in time). The resulting phylogenetic tree was rooted with *Anax amazili* and drawn with FigTree version 1.3.1 (http://tree.bio.ed.ac.uk/).

## Results

### Morphological characterization of the adults

Clypeal lobes rounded; clypeus and frons light blue to pale brown, brown spots lateral to yellow area surrounding T–spot stem; T–stem widening posteriorly, with convex sides; vertex yellow or light blue with latero–posterior margins black, with a black stripe on fronto–clypeal suture (fig. 1A); wide black stripe on fronto–ocular groove. Pale mesanepisternal stripes present at basal 25% in teneral specimens to absent in older specimens; mesepimeral and metepimeral stripes whitish, wide, and complete in teneral specimens to light blue, narrow, and faint or incomplete to absent in older specimens. Membranule dark except basal 15% (fig. 2A) to 30% white (fig. 2B). Abdomen dark brown with light blue spots; female S2 with a narrow medio–longitudinal dorsal yellow stripe usually spanning along anterior 0.75 of segment length, rarely along entire length or limited to anterior 0.50 of segment. Abdominal ventral terga narrow (length/width of S5 at basal 25% higher than 4), maximum width of S5–6 at distal 66%, basal 30% of inner and outer lateral carinae of S4 concave. Ventral tubercle of S1 bearing few denticles (10 or less) restricted to its apex; dorsal margin of anterior lamina spine concave;

Fig. 1. A. A pair of *Rhionaeschna galapagoensis* in copula photographed by Adolfo Cordero–Rivera on 24 II 2014 in Ecuador, San Cristóbal Island, Camarones stream at Hacienda El Cafetal (arrow in insert points at black band over fronto–clypeal suture); B. Male of *Rhionaeschna elsia* photographed by Dennis Paulson on 26 IV 2014 in Peru, Lima Department, Chorrillos, Pantanos de Villa near Lima (arrow in insert points at fronto–clypeal suture, devoid of a black band).

*Fig. 1. A. Pareja de* Rhionaeschna galapagoensis *en cópula fotografiada por Adolfo Cordero–Rivera el 24 II 2014 en Ecuador, en la isla de San Cristóbal, arroyo Camarones, dentro de la Hacienda El Cafetal (la flecha en el detalle indica la banda negra de la sutura frontoclipeal); B. Un macho de* Rhionaeschna elsia *fotografiado por Dennis Paulson el 26 IV 2014 en Perú, departamento de Lima, Chorrillos, Pantanos de Villa, cerca de Lima (la flecha en el detalle indica la sutura frontoclipeal, sin banda negra).*

tip of hamular anterior process rounded (fig. 3A) to pointed in ventro–lateral view (fig. 3B); auricles with two teeth. Male cercus black, lacking pale basal spot in outer surface; dorso–distal crest rising gradually, as high as base of cercus in lateral view, extending along distal 0.30–0.35 of cercus length (figs. 4A, 4B). Tip of female cercus rounded to pointed (figs. 5A, 5B).

Dimensions: head width: 8–8.9 male, 8.5–8.7 female; Hw length: 37.6–40.8 male, 38.5–40.5 female; Hw width: 12–13.2 male, 13–13.9 female; Hw pterostigma length: 2.8–3.2 male, 2.9–3.4 female; cerci length: 4.5–5.3 male, 3.9–4.5 female; female cerci maximum width: 0.65–0.75; total length: 57–61.8 male, 55.7–59.7 female.

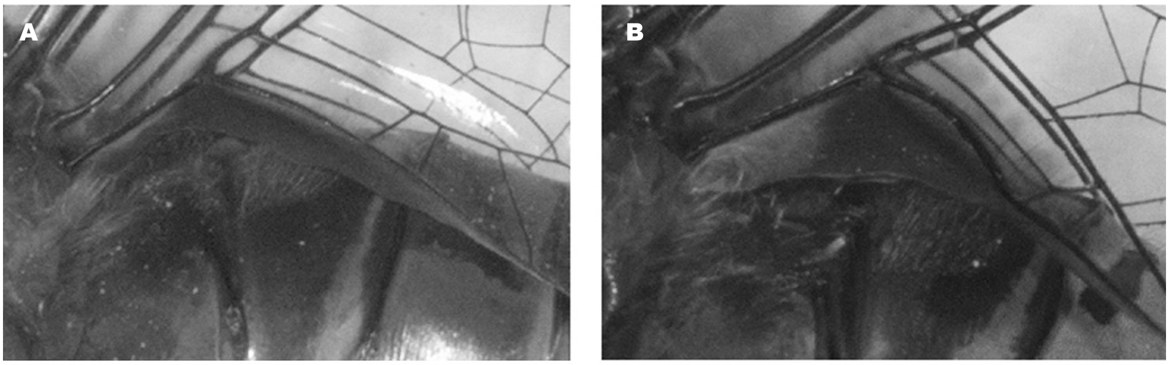

Fig. 2. Variability in extension of white coloring in hind wing membranule in *Rhionaeschna galapagoensis*: A. Female from pond, Hacienda El Cafetal (#883); B. Male from Camarones stream, Hacienda El Cafetal (#869).

*Fig. 2. Variabilidad en la extensión del color blanco en la membránula del ala posterior de* Rhionaeschna galapagoensis*: A. Hembra del estanque, Hacienda El Cafetal (#883); B. Macho del arroyo Camarones, Hacienda El Cafetal (#869).*

## Morphological description of ultimate instar larva

### Head (figs. 6A, 6B)

Approximately 1.34–1.42 times as wide as long. Occipital margin slightly concave with 7–8 pilose patches on each side; posterolateral portion of occipital lobes rounded. Antennae 7–segmented, the third antennomere the longest. Prementum reaching caudad base to midlevel between second coxae; prementum (figs. 6C, 6D) as wide as 0.91–1 of its length, border of medial lobe of ligula with fringe of setae and with two small tubercles, one on each side of median cleft, both shorter than setae. Labial palp (fig. 6D) with a small infra–apical tooth, inner margin with 25–30 denticles; movable hook 1.2–1.3 times as long as inner margin of palp. Mandibles (fig. 7) with no accessory tooth k below the molar crest, only a swollen area on the equivalent position, and no accessory tooth y between the incisive and the molar crest on left mandible. Molar crest with two small accessory denticles on the right mandible, none on the left mandible. Mandibular formula: L 1234 0 a b/ R 1234 y a($m^{1,2}$)b.

### Thorax

Prothoracic supracoxal apophyses with apices blunt, posterior longer and broader at base than anterior, cleft between them forming an acute angle (fig. 6B); wing pads nearly parallel, the external pad reaching caudad base of S4; femora and tibiae with four diffuse dark rings (fig. 6A).

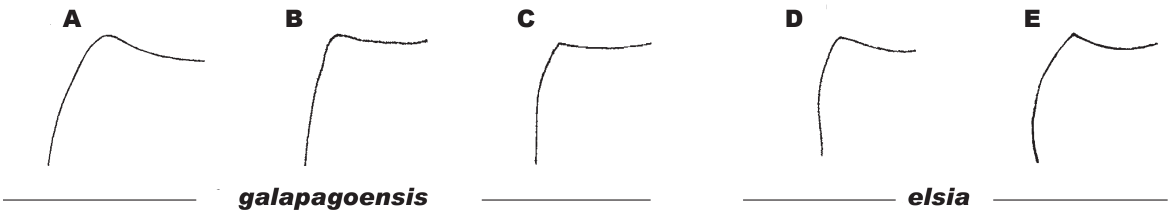

Fig. 3. Variability in shape of anterior hamule anterior process in *Rhionaeschna galapagoensis* (A–C) and *R. elsia* (D–E): A. Santa Cruz Island (redrawn from fig. 293b in von Ellenrieder, 2003); B. Camarones stream, Hacienda El Cafetal (#851); C. Camarones stream, Hacienda El Cafetal (#852); D. Peru, humedales de Ite (RWG); E. Chile, Arica (redrawn from fig. 292b in von Ellenrieder, 2003).

*Fig. 3. Variabilidad en la forma del proceso anterior del hámulo anterior de* Rhionaeschna galapagoensis *(A–C) y* R. elsia *(D–E): A. Isla de Santa Cruz (redibujado de la fig. 293b en von Ellenrieder, 2003); B. Arroyo Camarones, Hacienda El Cafetal (#851); C. Arroyo Camarones, Hacienda El Cafetal (#852); D. Perú, humedales de Ite (RWG); E. Chile, Arica (redibujado de la fig. 292b en von Ellenrieder, 2003).*

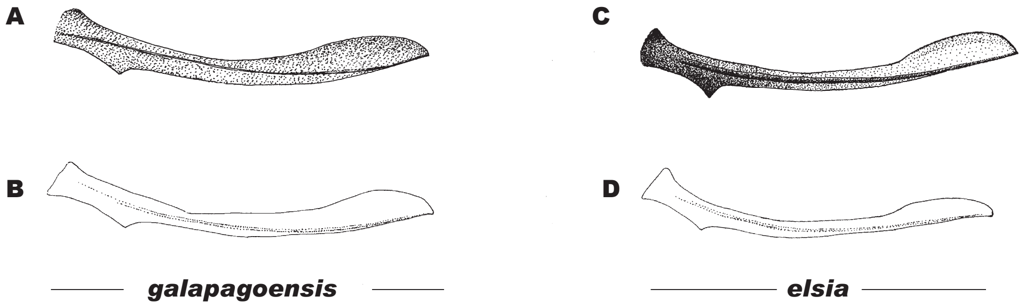

Fig. 4. Variability in shape of male cercus dorso–distal crest in *Rhionaeschna galapagoensis* (A–B) and *R. elsia* (C–D): A. Lectotype, San Cristóbal Island (redrawn from fig. 374b in von Ellenrieder, 2003); B. Camarones stream, Hacienda El Cafetal (#851); C. Peru, humedales de Ite (RWG); D. Paratype, Peru near Villa (redrawn from fig. 373b in von Ellenrieder, 2003).

*Fig. 4. Variabilidad en la forma de la cresta dorsodistal del cerco del macho de R*hionaeschna galapagoensis *(A–B) y* R. elsia *(C–D): A. Lectotipo, isla de San Cristóbal (redibujado de la fig. 374b en von Ellenrieder, 2003); B. Arroyo Camarones, Hacienda El Cafetal (#851); C. Perú, humedales de Ite (RWG); D. Paratipo, Perú, cerca de Villa (redibujado de la fig. 373b en von Ellenrieder, 2003).*

Abdomen
Widest on S6–7. Dorsal color pattern as in figure 6A. Lateral spines present on S7–9, those on S8 the longest. Female gonapophyses (fig. 8A) not reaching posterior margin of S9. Cerci (fig. 8B) shorter than epiproct, epiproct with middorsal ridge and two apical short spines; male basal lamina with blunt tip, as long as 0.42–0.45 of epiproct.

Measurements are presented in table 1.

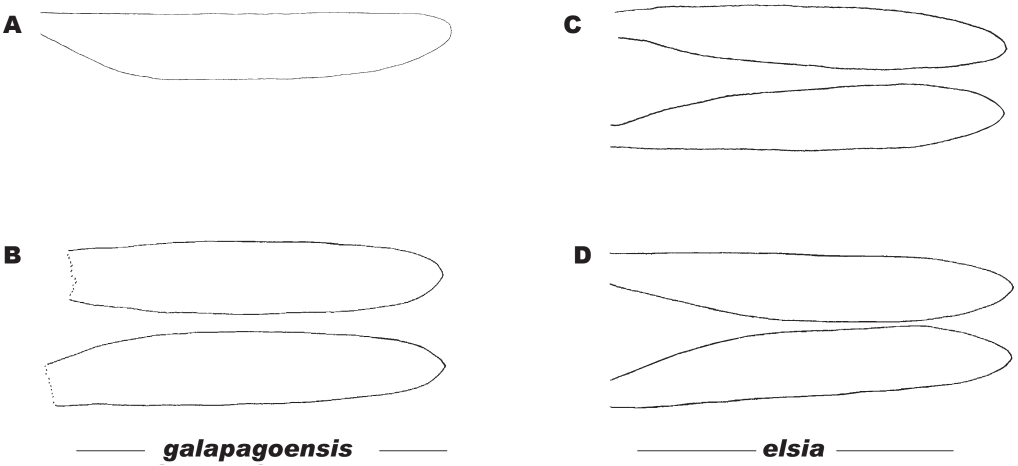

Fig. 5. Variability in shape of female cercus tip in *Rhionaeschna galapagoensis* (A–B) and *R. elsia* (C–D): A. Paralectotype, San Cristóbal Island (redrawn from fig. 374c in von Ellenrieder, 2003); B. Puerto Baquerizo Moreno, Punta Carola Beach (#879); C. Peru, humedales de Ite (CSCA); D. Peru, Lima (redrawn from fig. 373c in von Ellenrieder, 2003).

*Fig. 5. Variabilidad en la forma del extremo del cerco de la hembra de* Rhionaeschna galapagoensis *(A–B) y* R. elsia *(C–D): A. Paralectotipo, isla de San Cristóbal (redibujado de la fig. 374c en von Ellenrieder, 2003); B. Puerto Baquerizo Moreno, playa de Punta Carola (#879); C. Perú, humedales de Ite (CSCA); D. Perú, Lima (redibujado de la fig. 373c en von Ellenrieder, 2003).*

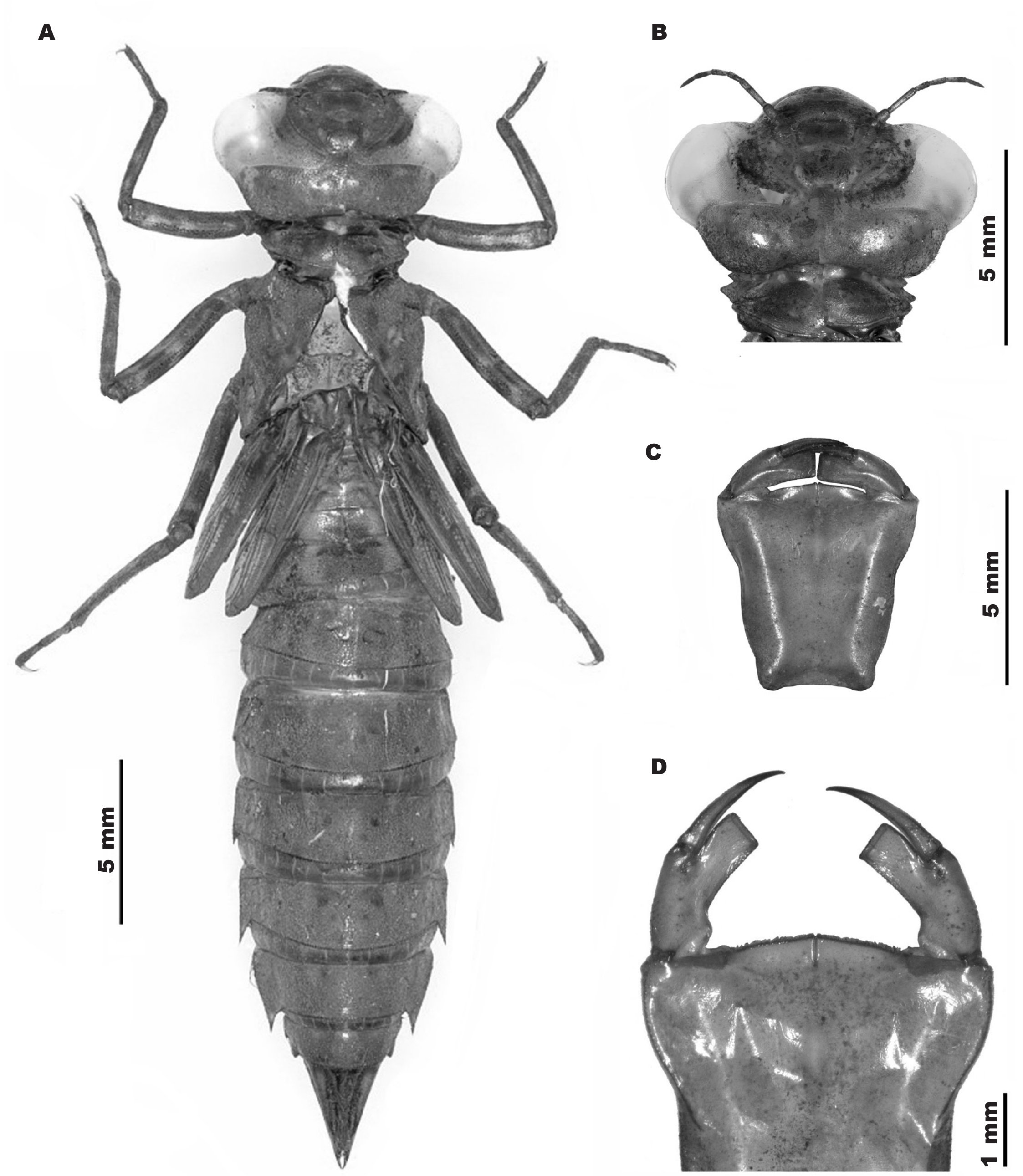

Fig. 6. Ultimate larval instar of *Rhionaeschna galapagoensis:* male exuvia from Ecuador, San Cristóbal Island, Hacienda El Cafetal, Camarones stream, 20 II 2014 (ACR) (A, C–D); male exuviae from same locality but pond, 27 II 2014 (ACR) (B): A. General view of body, dorsal view; B. Head and pronotum, dorsal view; C. Prementum, ectal view; D. Detail of labial palps and ligula, ental view.

*Fig. 6. Último estadio larvario de* Rhionaeschna galapagoensis*: exuvia del macho de Ecuador, isla de San Cristóbal, Hacienda El Cafetal, arroyo Camarones, 20 II 2014 (ACR) (A, C–D); exuvia del macho de la misma localidad pero del estanque, 27 II 2014 (ACR) (B): A. Vista general del cuerpo en vista dorsal; B. Cabeza y pronoto en vista dorsal; C. Prementón en vista ectal; D. Detalle de los palpos labiales y la lígula en vista ental.*

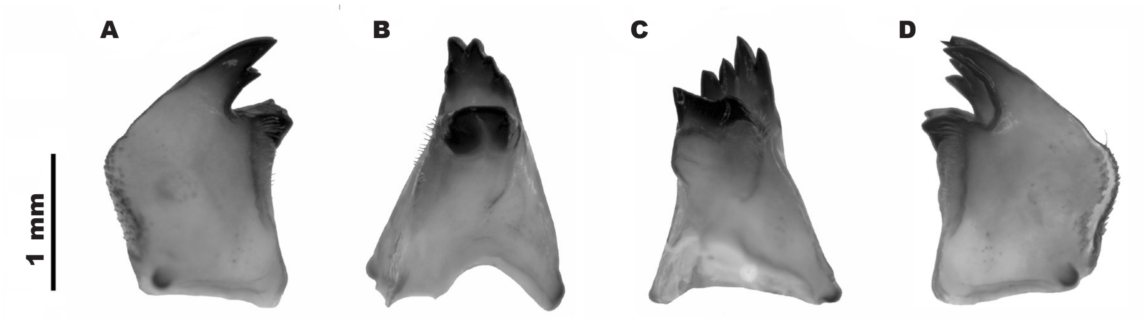

Fig. 7. Ultimate larval instar of *Rhionaeschna galapagoensis*, female larva from Ecuador, San Cristóbal Island, pond at El Cafetal, 22 II 2014 (USFQ): A. Right mandible, lateral view; B. Right mandible, medial view; C. Left mandible, medial view; D. Left mandible, lateral view.

*Fig. 7. Último estadio larvario de* Rhionaeschna galapagoensis*, larva hembra de Ecuador, isla de San Cristóbal, estanque en El Cafetal, 22 II 2014 (USFQ): A. Mandíbula derecha en vista lateral; B. Mandíbula derecha en vista medial; C. Mandíbula izquierda en vista medial; D. Mandíbula izquierda en vista lateral.*

Larval habitat

Larvae were found commonly at streams (fig. 9A) but also at a pond (fig. 9B) near the Hacienda El Cafetal. The streams were generally small (width from 90 to 130 cm), most with pool and riffle sections and substrate composed mostly of pebble and cobble, but some streams also had sand and lime substrate. Water transparency varied from clear to milky (1.97 to 10.49 NTU), average conductivity was 99.6 ± 2.3 μS/cm, water temperature varied from 20 to 23ºC and discharge values ranged from 1 to 3 L/s (Ochoa, pers. comm.). Vegetation around streams was composed mostly of ferns (*e.g., Diplazium subobtusum*) and planted trees of *Coffea arabica*

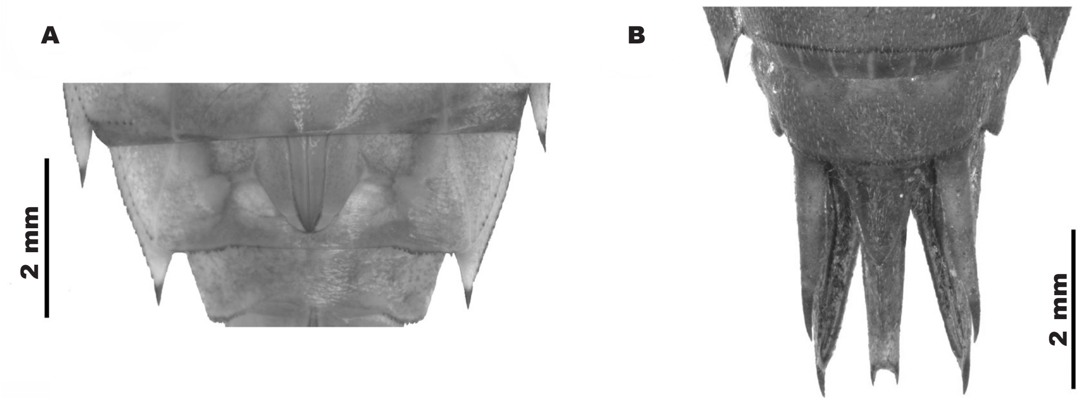

Fig. 8. Ultimate larval instar of *Rhionaeschna galapagoensis*: A. Female gonapophyses, ventral view, larva from Ecuador, San Cristóbal Island, pond at El Cafetal, 22 II 2014 (USFQ); B. Male S10 and caudal appendages, dorsal view, exuvia from Ecuador, San Cristóbal Island, Hacienda El Cafetal, Camarones stream, 20 II 2014 (ACR).

*Fig. 8. Último estadio larvario de* Rhionaeschna galapagoensis*: A. Gonapófisis femeninos en vista ventral, larva de Ecuador, isla de San Cristóbal, estanque en El Cafetal, 22 II 2014 (USFQ); B. S10 y apéndices caudales del macho en vista dorsal, exuvia de Ecuador, isla de San Cristóbal, Hacienda El Cafetal, arroyo Camarones, 20 II 2014 (ACR).*

Table 1. Measurements of ultimate instar larvae of *Rhionaeschna galapagoensis*. Measurements are given in mm, as average ± standard deviation followed by range in square brackets.

*Tabla 1. Medidas del último estadio larvario de* Rhionaeschna galapagoensis*. Las medidas se dan en mm, como media ± desviación estándar, seguidas del intervalo entre corchetes.*

| | ♂ (N = 3) | ♀ (N = 2) |
|---|---|---|
| Total length | 33.5 ± 0.71 [34–33] | 32.8 ± 0.28 [32.6–33] |
| Maximum head width | 7.81 ± 0.18 [7.64–8] | 7.6 ± 0.05 [7.56–7.63] |
| Maximum head length | 5.72 ± 0.08 [5.65–5.8] | 5.67 ± 0.06 [5.63–5.71] |
| Maximum prementum width | 4.8 ± 0 [4.8] | 4.65 ± 0.07 [4.6–4.7] |
| Maximum prementum length | 5.1 ± 0.28 [4.9–5.3] | 4.69 ± 0.02 [4.67–4.7] |
| Labial palp movable hook | 1.70 ± 0.05 [1.65–175] | 1.66 ± 0 [1.66] |
| Antennomere I | 0.30 ± 0 [0.27–0.33] | 0.30 ± 0.04 [0.27–0.33] |
| Antennomere II | 0.37 ± 0.03 [0.35–0.40] | 0.33 ± 0.04 [0.30–0.35] |
| Antennomere III | 0.92 ± 0.08 [0.85–1] | 0.83 ± 0.11 [0.75–0.90] |
| Antennomere IV | 0.38 ± 0.03 [0.36–0.41] | 0.32 ± 0.02 [0.30–0.33] |
| Antennomere V | 0.42 ± 0.03 [0.39–0.45] | 0.38 ± 0.01 [0.37–0.38] |
| Antennomere VI | 0.48 ± 0.02 [0.47–0.50] | 0.44 ± 0.01 [0.43–0.45] |
| Antennomere VII | 0.50 ± 0.01 [0.49–0.51] | 0.47 ± 0.04 [0.44–0.49] |
| Femur I length | 3.63 ± 0.15 [3.5–3.8] | 3.65 ± 0.07 [3.6–3.7] |
| Femur II length | 4.8 ± 0.2 [4.6–5] | 4.6 ± 0 [4.6] |
| Femur III length | 5.93 ± 0.15 [5.8–6.1] | 5.75 ± 0.07 [5.7–5.8] |
| Tibia I length | 4.73 ± 0.06 [4.7–4.8] | 4.75 ± 0.07 [4.7–4.8] |
| Tibia II length | 5.07 ± 0.06 [5–5.1] | 5.1 ± 0.14 [5–5.2] |
| Tibia III length | 6.18 ± 0.1 [6.1–6.3] | 6.05 ± 0.35 [5.8–6.3] |
| Internal wing pad length (inner margin) | 7.4 ± 0.1 [7.3–7.5] | 7.2 ± 0 [7.2] |
| External wing pad length (inner margin) | 6.77 ± 0.12 [6.7–6.9] | 6.6 ± 0.14 [6.5–6.7] |
| Maximum length of S5 | 2.4 ± 0.1 [2.3–2.5] | 1.88 ± 0.18 [1.75–2] |
| Maximum length of S6 | 2.57 ± 0.15 [2.4–2.7] | 2.67 ± 0.15 [2.5–2.8] |
| Maximum length of S7 | 2.67 ± 0.15 [2.5–2.8] | 2.22 ± 0.31 [2–2.44] |
| Maximum length of S8 | 2.43 ± 0.21 [2.2–2.6] | 1.93 ± 0.11 [1.85–2] |
| Maximum length of S9 | 2.13 ± 0.12 [2–2.22] | 1.9 ± 0.07 [1.85–1.95] |
| Maximum length of S10 | 1.38 ± 0.03 [1.35–1.4] | 1.03 ± 0.04 [1–1.05] |
| Lateral spines (inner margin) on S7 | 0.56 ± 0.04 [0.52–0.6] | 0.58 ± 0.04 [0.55–0.6] |
| Lateral spines (inner margin) on S8 | 0.9 ± 0 [0.9] | 0.96 ± 0.01 [0.95–0.96] |
| Lateral spines (inner margin) on S9 | 0.85 ± 0 [0.85] | 0.9 ± 0 [0.9] |
| Epiproct length | 3.03 ± 0.06 [3–3.1] | 3.26 (one malformed) |
| Cercus length (inner margin) | 2.7 ± 0.17 [2.6–2.9] | 2.68 ± 0.04 [2.65–2.7] |
| Paraproct length (inner margin) | 3.63 ± 0.06 [3.6–3.7] | 3.88 ± 0.11 [3.8–3.96] |
| Inner gonapophyses (inner margin) | | 1.93 ± 0.25 [1.75–2.1] |

and some introduced trees (*i.e., Cedrella odorata).* The only other odonate larva sharing the habitat in the streams was *Ischnura hastata* Say and several aquatic invertebrate species including *Chironomus* sp., Orthocladiinae unind. (Diptera, Chironomidae), *Simulium ochraceum* Walker (Diptera, Simuliidae), *Geranomyia tibialis* (Loew) (Diptera, Limoniidae*)*, *Haliplus gravidus* Aubé (Coleoptera, Haliplidae), *Gyrinus galapagoensis* Van Dyke (Coleoptera, Gyrinidae), *Typhlatya galapagoensis* Monod & Cals (Decapoda, Atyidae) and *Macrobrachium hancocki* Holthuis (Decapoda, Palaemonidae).

Table 2. Localities where *Rhionaeschna galapagoensis* was found at San Cristóbal Island, Galápagos, II 2014: Long. Longitude; Lat. Latitude; Alt. Altitude (in m). (Coordinates in the WGS84 datum.)

*Tabla 2. Localidades donde se encontró* Rhionaeschna galapagoensis *en la isla de San Cristóbal, Galápagos, II 2014: Long. Longitud; Lat. Latitud; Alt. Altitud (en m). (Datum de coordenadas WGS84.)*

| Life stage | Habitat | Locality | Long. | Lat. | Alt. |
|---|---|---|---|---|---|
| Adults | Waterfall in stream | El Chino | –89.458717 | –0.911821 | 210 |
| Adults, larvae, exuviae | Pond | Hacienda el Cafetal | –89.538821 | –0.924274 | 191 |
| Adults, larvae, exuviae | Camarones stream | Hacienda el Cafetal | –89.539510 | –0.925664 | 282 |
| Adults, larvae | Camarones stream | Hacienda el Cafetal | –89.538821 | –0.924274 | 191 |
| Adults, larvae | Stream | Nariz del Diablo | –89.522702 | –0.918473 | 348 |
| Adults | Punta Carola beach | Puerto Baquerizo Moreno | –89.611999 | –0.890603 | 3 |
| Adults | Tijeretas beach | Puerto Baquerizo Moreno | –89.603032 | –0.883341 | 2 |
| Adults | Stream | Unnamed | –89.491606 | –0.908980 | 431 |

The pond had a surface of approximately 10 m$^2$, turbid water. It was surrounded by native junco (*Junco pallescens*), other shrubs and herbaceous plants (*i.e., Ludwigia erecta*), and introduced vegetation (*Psidium guajava*, *Rubus niveus, Cedrella odorata*). Other odonate sharing the habitat were *Ischnura hastata*, *Anax amazili*, *Brachymesia herbida*, and *Tramea* cf. *cophysa*. Several other aquatic invertebrate species were present, including *Tanyponus* sp. (Diptera, Chironomidae), *Trichocorixa reticulata* (Guerin–Meneville) (Heteroptera, Corixidae), *Copelatus galapagoensis* Waterhouse and *Rhantus galapagoensis* Balke & Peck (Coleoptera, Dytiscidae).

### Diagnosis

Adults of *R. galapagoensis* and *R. elsia* can be distinguished from all other species of *Rhionaeschna* by the combination of clypeal lobes rounded and ventral tubercle of S1 bearing only a few denticles (10 or less) restricted to its apex (von Ellenrieder, 2003). Adults of *R. galapagoensis* differ from those of *R. elsia* by the presence of a wide black band over the fronto–clypeal suture (fig. 1A). In *R. elsia,* there is no dark color over the fronto–clypeal suture at all or only a faint narrow brown line (fig. 1B).

Among the known ultimate instar larvae of *Rhionaeschna*, still only about 57% of the species in the genus, *R. galapagoensis* shares only with *R. brasiliensis* (von Ellenrieder & Costa), *R. elsia*, and *R. marchali* the absence of the lateral spines on S6 (Limongi, 1983; Müller & Schiel, 2012; von Ellenrieder & Costa, 2002). Ultimate instar larvae of *R. galapagoensis* can be recognized from those of *R. brasiliensis* and *R. elsia* by the acute angle between the prothoracic supracoxal apophyses (orthogonal to obtuse in *R. brasiliensis* and *R. elsia*), and from *R. marchali* by the well developed prothoracic supracoxal apophyses (absent in *R. marchali*). Ultimate instar larvae of *R. galapagoensis* differ further from those of *R. elsia* by the longer lateral spines on S7–9 and shorter cerci in relation to paraproct length (values of *R. galapagoensis* first): length of lateral spine on S7: 0.52–0.6 *vs*. 0.4; length of lateral spine on S8: 0.9–0.96 *vs*. 0.63; length of lateral spine on S9: 0.85–0.9 *vs*. 0.63; length cerci/ length paraprocts: 0.68–0.78 *vs*. 0.64.

Both adults and ultimate instar larvae of *R. galapagoensis* are overall larger than those of *R. elsia*, although some of their ranges overlap partly: total length of adults: 57–61.8 in males, 55.7–59.7 in females (*vs*. 54.3–58.2 in males, 54.3–56.4 in females in *R. elsia*); Hw length 37.6–40.8 in males, 38.5–40.5 in females (*vs*. 35.8–38.7 in males, 35.9–39 in females in *R. elsia*); adult male cercus length of 4.5–5.3 (*vs*. 4.3–4.6 in *R. elsia*); total length of ultimate instar larvae: 33–34 in males, 32.6–33 in females (*vs*. 27.5–28.7 in males, 26.9–31 in females in *R. elsia*); head width of ultimate instar larvae: 7.64–8 in males, 7.56–7.63 in females (*vs*. 6.67–6.99 in males, 6.65–6.97 in females in *R. elsia*); length cercus of ultimate instar larvae: 2.6–2.9 in males, 2.65–2.7 in females (*vs*. 1.75–2.21 in males, 1.93–2.43 in females in *R. elsia*); length paraproct of ultimate instar larvae: 3.6–3.7 in males, 3.8–3.96 in females (*vs*. 3.09–3.39 in males, 3.11–3.33 in females in *R. elsia*).

## Distribution and biological observations

*Rhionaeschna galapagoensis* was found in all streams visited, both as adults and as larvae of various instars (table 2). We recorded abundant specimens at the Camarones Stream inside the 'Hacienda el Cafetal' and in a nearby pond (fig. 9B). Teneral and mature specimens were found near roads and in the village of Puerto Baquerizo but were particularly common along the coast at Punta Carola and nearby places.

Larvae and exuviae were found in the Camarones Stream but also in the pond, indicating

Fig. 9. Habitat of *Rhionaeschna galapagoensis* in San Cristóbal Island, Ecuador: A. Waterfall at El Chino stream; B. Pond at Hacienda El Cafetal. Photos by Adolfo Cordero–Rivera.

*Fig. 9. Hábitat de* Rhionaeschna galapagoensis *en la isla de San Cristóbal, Ecuador: A. Cascada en el arroyo El Chino; B. Estanque en la Hacienda El Cafetal. Fotos de Adolfo Cordero–Rivera.*

that the species is able to complete development in both running and still water. Analyses of gut content of 20 larvae suggested a highly diverse diet, including several Chironomidae, Gyrinidae, Dytiscidae, and also specimens of *Ischnura hastata*. In agreement with larval habitat preference, males were seen patrolling sections of streams and around the pond, and females were seen laying eggs in both types of habitat. Nevertheless, individuals were more commonly observed at the streams than at the pond. Mate–searching males patrolled sections of the stream of a few meters, flying at about 30–50 cm above the surface of the water. They remained at the stream for short periods (a few minutes) and were observed in the morning and afternoon. Females approached the stream and oviposited on floating vegetation. One female was observed laying eggs at 16:00 h. Another female approached the stream at mid–day (12:20 h), was captured in tandem by a patrolling male, and the pair mated perched in a nearby tree for about 10 min (fig. 1A).

Swarms of tens of individuals (fig. 10A, video 1) were observed feeding just after sunrise around the beaches and shrubland at Punta Carola. The total number of individuals swarming in the area was clearly enormous, although not easily quantifiable. These swarms appeared at about 6:00 h, immediately after sunrise, and dispersed at about 7:00 h, when the sun became stronger. Some specimens of *Tramea* cf. *cophysa* were found in the same swarms. These swarms attracted bird predators, and one successful predation event (on a teneral *R. galapagoensis*) was observed (fig. 10B).

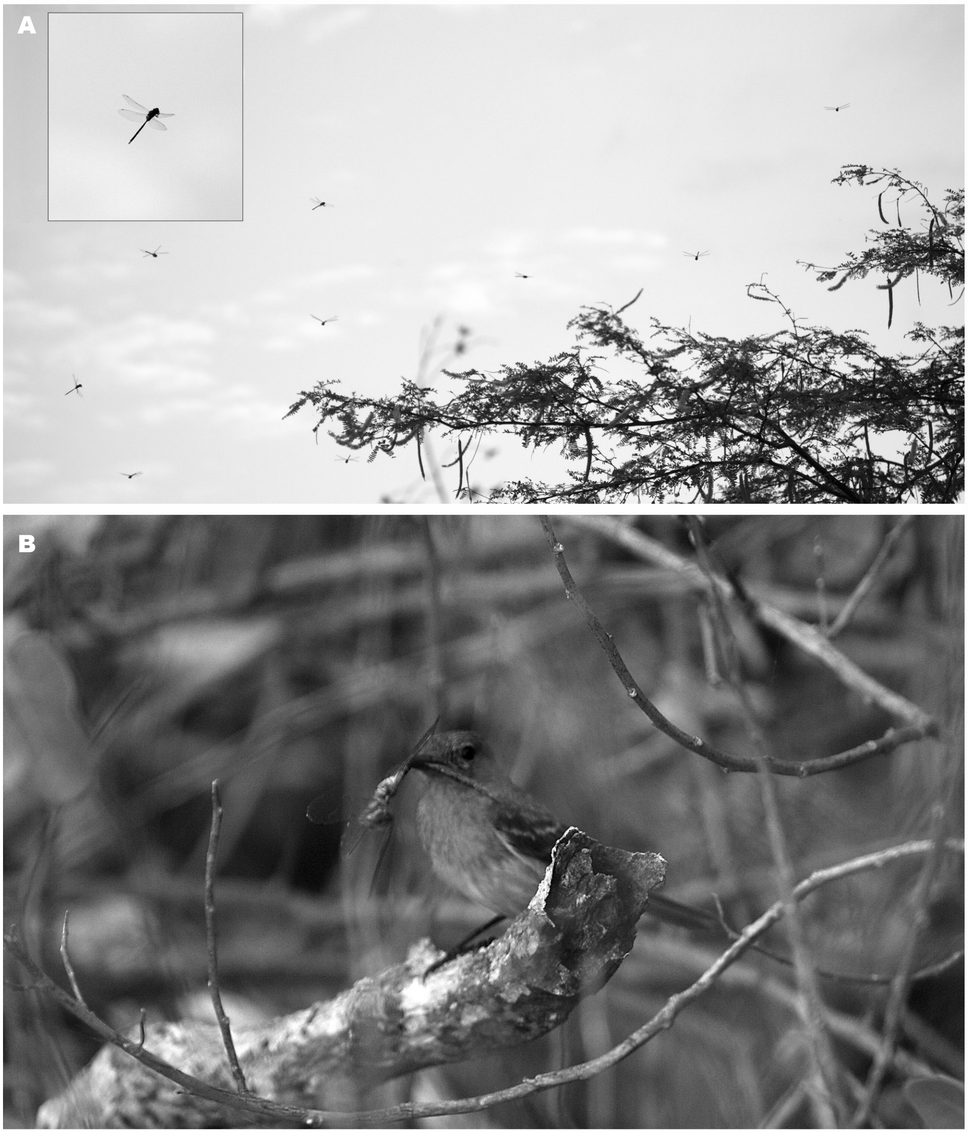

Fig. 10. Swarm of adults of *Rhionaeschna galapagoensis*: A. Feeding just after sunrise around the beaches and shrubland at Punta Carola; B. Documented predation event of birds (a Galapagos flycatcher, *Myiarchus magnirostris*) on a teneral *R. galapagoensis*. Photos by Adolfo Cordero–Rivera.

*Fig. 10. Enjambre de adultos de* Rhionaeschna galapagoensis*: A. Alimentándose al amanecer alrededor de la playa y en los arbustos en Punta Carola; B. Caso documentado de depredación por aves (un atrapamoscas de Galápagos,* Myiarchus magnirostris*) sobre un individuo recién emergido de* R. galapagoensis*. Fotos de Adolfo Cordero–Rivera.*

## Genetic characterization and phylogenetic reconstruction

Alignments of mtDNA COI, H3, and nDNA 28S fragments included 367, 251, and 454 bp positions respectively. Sequences can be accessed at GenBank under accession numbers provided in table 3. The mtDNA COI fragment showed 56 parsimony–informative positions, while there were only 23 and 17 in nDNA H3 and nDNA 28S. Pairwise genetic distances between the seven *Rhionaeschna* species ranged from 1.7 to 8.8% for mtDNA A, 1.2 to 4.0% mtDNA C and 0.01 to 0.04% for mtDNA B (table 4). Similar topologies were obtained from Bayesian and maximum likelihood phylogenetic reconstructions. The Bayesian posterior probability approach produced a tree with a topology (based on COI and H3) largely resolved, but the two main clades were not well–supported

Table 3. GenBank accession numbers for COI (367 bp), 28S (454 bp), and H3 (259 bp) sequences.

*Tabla 3. Números de accesión de la genoteca GenBank para las secuencias COI (367 bp), 28S (454 bp) y H3 (259 bp).*

| | | GenBank accession numbers | | |
|---|---|---|---|---|
| Species | Code | COI | 28S gene | Histone 3 |
| *Anax amazili* | 871 | KR110051 | KP793444 | KR864847 |
| *Rhionaeschna absoluta* | Ra1 | – | KP895577 | KR864853 |
| *Rhionaeschna absoluta* | Ra2 | KR189020 | KP992515 | – |
| *Rhionaeschna bonariensis* | Rb1 | KR110055 | KP793448 | KR864843 |
| *Rhionaeschna bonariensis* | Rb2 | KR189017 | KP992512 | KR864854 |
| *Rhionaeschna bonariensis* | Rb3 | KR189021 | KP992516 | KR864857 |
| *Rhionaeschna bonariensis* | Rb4 | KR110054 | KP793447 | KR864844 |
| *Rhionaeschna cornigera* | 234 | KR139935 | KP793439 | KR864851 |
| *Rhionaeschna cornigera* | 240 | KR011722 | KP793440 | KR864860 |
| *Rhionaeschna cornigera* | 241 | KR011723 | KP793441 | KR864850 |
| *Rhionaeschna diffinis* | Rd1 | KR110052 | KP793449 | KR864842 |
| *Rhionaeschna diffinis* | Rd2 | KR189018 | KP992513 | KR864855 |
| *Rhionaeschna diffinis* | Rd3 | KR189022 | KP992517 | KR864858 |
| *Rhionaeschna elsia* | Re1 | KR189016 | KP895576 | KR864852 |
| *Rhionaeschna elsia* | Re2 | KR189019 | KP992514 | KR864856 |
| *Rhionaeschna elsia* | Re3 | KR189023 | KP992518 | KR864859 |
| *Rhionaeschna galapagoensis* | 851 | KR011724 | KP793442 | KR864849 |
| *Rhionaeschna galapagoensis* | 852 | KR066402 | KP793443 | KR864848 |
| *Rhionaeschna galapagoensis* | 877 | KR110050 | KP793445 | KR864846 |
| *Rhionaeschna galapagoensis* | 878 | KR110053 | KP793446 | KR864845 |
| *Rhionaeschna marchali* | 222 | – | KP723677 | KR259168 |
| *Rhionaeschna marchali* | 223 | KP866411 | KP749923 | KR259169 |

(see fig. 11A): (i) the '*marchali*–clade' that included four species (*R. elsia*, *R. galapagoensis*, *R. cornigera* and *R. marchali*); and (ii) the '*absoluta*–clade' that included the remaining three species (*R. bonariensis, R. diffinis*, and *R. absoluta*). Although both clades presented well–supported species–clades, in the '*marchali*–clade' *R. elsia* and *R. galapagoensis* positions were relatively unresolved, likely due to their recent speciation. However, their close position respect to the remaining two species in the clade (*R. cornigera* and *R. marchali*) was well–supported. Similar topologies were obtained from neighbor–joining and maximum likelihood phylogenetic reconstructions. The neighbor–joining tree based on the three genes (COI, H3, and 28S) (see fig. 11B) identified three clades: (i) the '*marchali*–clade' (*R. elsia*, *R. galapagoensis*, and *R. marchali*); the '*cornigera*–clade' (*R. cornigera* and *R. bonariensis*); and (iii) the '*absoluta*–clade' (*R. diffinis* and *R. absoluta*). Moreover, the neighbor–joining tree confirmed the close position of both species *R. elsia* and *R. galapagoensis*, which were placed in the same cluster but in different branches. However, in the maximum likelihood tree, *R. elsia* and *R. galapagoensis* positions were relatively unresolved.

## Discussion

Several diagnostic characters to differentiate between *R. galapagoensis* and *R. elsia*, based on examination of type specimens, have been proposed (von Ellenrieder, 2003). These include the color of membranule (fig. 2), the shape of anterior hamule anterior tip fig. 3), dorso–distal crest of male cercus (fig. 4) and female cercus tip (figs. 5). However, we found that these characters are variable in the larger

Table 4. Average genetic distances (%) (Kimura 2–parameter) between the seven *Rhionaeschna* species sampled for COI (367 bp, 21 sequences), H3 (259 bp, 20 sequences), and 28S (454 bp, 20 sequences).

*Tabla 4. Distancias genéticas medias (%) (parámetro Kimura–2) entre las siete especies de* Rhionaeschna *muestreadas para COI (367 bp, 21 secuencias), H3 (259 bp, 20 secuencias) y 28S (454 bp, 20 secuencias).*

| COI | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1. *R. absoluta* | | | | | | | |
| 2. *R. bonariensis* | 0.031 | | | | | | |
| 3. *R. cornigera* | 0.086 | 0.063 | | | | | |
| 4. *R. diffinis* | 0.025 | 0.022 | 0.082 | | | | |
| 5. *R. elsia* | 0.056 | 0.051 | 0.088 | 0.053 | | | |
| 6. *R. galapagoensis* | 0.052 | 0.043 | 0.086 | 0.049 | 0.017 | | |
| 7. *R. marchali* | 0.054 | 0.039 | 0.082 | 0.045 | 0.055 | 0.043 | |

| H3 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1. *R. absoluta* | | | | | | | |
| 2. *R. bonariensis* | 0.020 | | | | | | |
| 3. *R. cornigera* | 0.018 | 0.022 | | | | | |
| 4. *R. diffinis* | 0.005 | 0.026 | 0.023 | | | | |
| 5. *R. elsia* | 0.033 | 0.028 | 0.034 | 0.035 | | | |
| 6. *R. galapagoensis* | 0.020 | 0.016 | 0.022 | 0.026 | 0.012 | | |
| 7. *R. marchali* | 0.024 | 0.020 | 0.026 | 0.028 | 0.040 | 0.028 | |

| 28S | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1. *R. absoluta* | | | | | | | |
| 2. *R. bonariensis* | 0.002 | | | | | | |
| 3. *R. cornigera* | 0.004 | 0.003 | | | | | |
| 4. *R. diffinis* | 0.002 | 0.001 | 0.004 | | | | |
| 5. *R. elsia* | 0.002 | 0.001 | 0.003 | 0.001 | | | |
| 6. *R. galapagoensis* | 0.002 | 0.001 | 0.002 | 0.001 | 0.001 | | |
| 7. *R. marchali* | 0.002 | 0.002 | 0.003 | 0.003 | 0.002 | 0.001 | |

series of specimens of both species available in this study, and cannot be used as diagnostic. The only seemingly reliable character to distinguish between adults of the two species was the black band over the fronto–clypeal suture. The presence or absence of a black band over the fronto–clypeal suture has been found to be a stable character in other species of this genus, always either present or absent in all specimens of a particular species (von Ellenrieder, 2003). The ultimate instar larvae of both species can be recognized from all other *Rhionaeschna* larvae so far described except for *R. brasiliensis* and *R. marchali* by the obsolete to absent lateral spines on S6. These two species differ in the shape of the angle formed between the prothoracic supracoxal apophyses, the length of the lateral spines on S7–9, and of the length of cerci relative to paraprocts, as detailed above. As these color and morphological differences between the two species were consistent in all adult and larval specimens available to us in this study, we consider that *R. galapagoensis* and *R. elsia* can be maintained as separate species, although they are very close due to their recent speciation.

*Rhionaeschna galapagoensis* was commonly found in streams, far from the coast, perhaps because freshwater ponds are scarce in San Cristóbal. In contrast, the typical habitat of *R. elsia* is brackish waters in coastal deserts (Müller & Schiel, 2012). Therefore, our results also suggest that the two taxa have different ecological preferences. Further field studies in other islands and other areas of San Cristóbal are needed to confirm our findings.

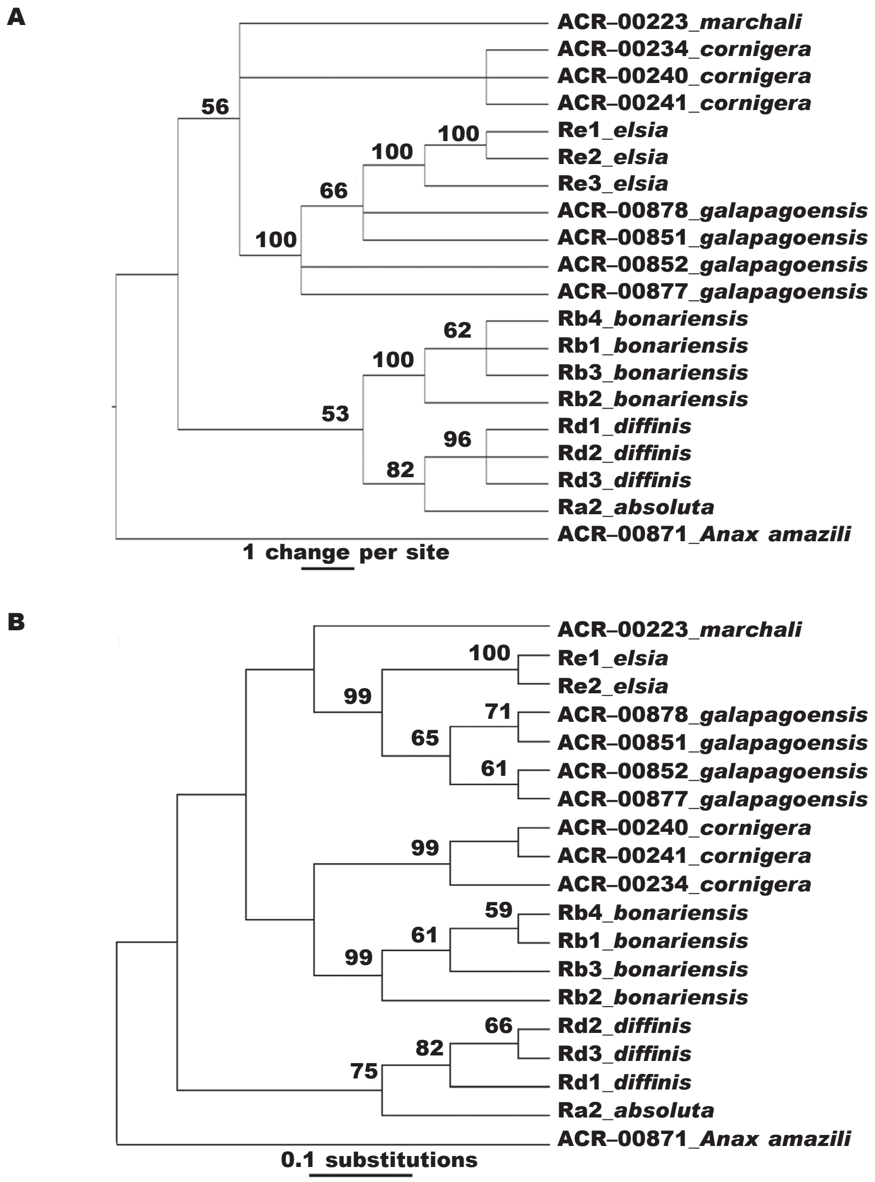

Fig. 11. Phylogenetic relationships within *Rhionaeschna* derived by: A. Bayesian inference based on mtDNA (COI) and nDNA (H3) concatenated sequences from 19 samples under a GTR+G model of evolution; B. Maximum likelihood tree based on mtDNA (COI), nDNA (H3) and nDNA (28S) concatenated sequences from 18 samples. (Numbers at nodes indicate posterior probabilities higher than 50%.)

*Fig. 11. Relaciones filogenéticas dentro de* Rhionaeschna *obtenidas mediante inferencia Bayesiana: A. Basada en secuencias concatenadas de ADNmt (COI) y ADNn (H3) de 19 muestras con el modelo evolutivo GTR+G; B. Árbol de máxima verosimilitud basado en secuencias concatenadas de ADNmt (COI) ADNn (H3) y ADNn (28S) obtenido a partir de 18 muestras. (Los números en los nodos indican probabilidades* a posteriori *mayores del 50%.)*

Although the genetic distances between *R. galapagoensis* and *R. elsia* are less than the most common 2% divergence between congeneric pairs of animal species (Hebert et al., 2003), other odonate species considered to be good species show similar levels of interspecific divergence. This is the case of the *Ischnura elegans*–group of species (*I. elegans*, *I. genei*, *I. graellsii*, and *I. saharensis*) which show less than 1% divergence (Sánchez–Guillén et al., 2014). Consistently with their low genetic divergence, which is to be expected given the young age of the Galápagos Islands, phylogenetic reconstruction confirms the monophyletic origin of *R. elsia* and *R. galapagoensis* as it had been suggested in the preliminary phylogeny of the genus based on morphology (von Ellenrieder, 2003), with both species clustering together in the three reconstructed trees (Bayesian, Maximum likelihood, and Neighbor–Joining). Even though there was no absolute agreement on the position of all species, all three phylogenetic reconstructions also hint at the paraphyletic nature of the Neureclipa–group, with *R. galapagoensis* and *R. elsia* clustering with *R. marchali* or with *R. cornigera* and *R. marchali*, rather than with the remaining species of the Neureclipa–group.

In conclusion, morphological, ecological and genetic evidence indicate that *R. galapagoensis* and *R. elsia* can be maintained as closely related but separate species.

## Acknowledgements

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## References

Belle, J., 1991. A visit to the Galapagos islands. *Selysia,* 20: 2.

Calvert, P. P., 1952. New taxonomic entities in Neotropical Aeshnas (Odonata: Aeshnidae). *Entomological News*, 63: 253–264.

– 1956. The Neotropical species of the 'subgenus *Aeschna'* sensu Selysii 1883 (Odonata). *Memoirs of the American Entomological Society*, 15: 1–251.

Campos, F. R., 1922. Catálogo sistemático y sinonímico de los Odonatos del Ecuador. *Revista del Colegio Nacional Vicente Rocafuerte*, 8–9: 1–75.

Cordero Rivera, A., Lorenzo Carballa, M. O., Utzeri, C. & Vieira, V., 2005. Parthenogenetic *Ischnura hastata* (Say, 1839), widespread in the Azores Islands (Zygoptera: Coenagrionidae). *Odonatologica,* 34: 1–9.

Currie, R. P., 1901. Papers from the Hopkins Satndford Galapagos expedition, 1898–1899. III. Entomological results (3): Odonata. *Proceedings Washington Academy Sciences*, 3: 381–389.

Darwin, C., 1901. *The origin of species*. John Murray, London.

De Marmels, J., 1982. Dos náyades nuevas de la familia Aeshnidae (Odonata: Anisoptera). *Boletín de Entomología de Venezuela, Nueva Serie*, 2(12): 102–106.

– 1990. Nine new Anisoptera larvae from Venezuela (Gomphidae, Aeshnidae, Corduliidae, Libelulidae). *Odonatologica,* 19(1): 1–15.

– 2001. *Aeshna* (*Hesperaeschna*) *condor* sp. nov. from the Venezuelan Andes, with a redescription of *A* (*H.*) *joannisi*, comments on other species and descriptions of larvae (Odonata, Aeshnidae). *International Journal of Odonatology*, 4(2): 119–134.

Fritz, G. N., Conn, J., Cockburn, A. & Seawright, J., 1994. Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of *Anopheles nuneztovari* (Diptera: Culicidae). *Molecular Biology and Evolution,* 11: 406–416.

Hall, T. A., 1999. BioEdit: a user–friendly biological sequence alignment editor and analysis program for windows 95/98/T. *Nucleic Acids Symposium Series*, 41: 95–98.

Hasegawa, M., Kishino, K. & Yano, T., 1985. Dating the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22: 160–174.

Hebert, P. D. N., Ratnasingham, S. & de Waard, J. R., 2003. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B (Suppl.)*, 270: 96–99.

Huelsenbeck, J. P. & Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics,* 17: 754–755.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16: 111–120.

Kohli, M. K., Schneider, T., Müller, O. & Ware, J. L., 2014. Counting the spots: a molecular and morphological phylogeny of the spotted darner *Boyeria* (Odonata: Anisoptera: Aeshnidae) with an emphasis on European taxa. *Systematic Entomology*, 39: 190–195.

Limongi, J., 1983 [1985]. Estudio morfo–taxonómico de nayades en algunas especies de Odonata (Insecta) en Venezuela. *Memorias de la Sociedad de ciencias naturales 'La Salle'*, 43(119): 95–117.

MacArthur, R. H. & Wilson, E. O., 1967. *The theory of island biogeography*. Princeton University Press, Princeton.

Martin, R., 1908. Aeschnines. C*ollections zoologiques du Baron Edmund de Sélys–Longchamps, Catalogue Systématique et Descriptif*, 18: 1–84.

Muddeman, J., 2007. A new species for the Galapagos Islands: Great Pondhawk (*Erythemis vesiculosa*). *Argia*, 19: 17–18.

Müller, O. & Schiel, F.–J., 2012. Description of the final instar larva of *Rhionaeschna elsia* (Calvert, 1952) (Odonata: Aeshnidae). *Libellula Supplement*, 12: 133–142.

Musser, R. J., 1962. Dragonfly nymphs of Utah (Odonata: Anisoptera). *University of Utah Biological Series,* 12(6): vii + 74 pp.

Needham, J. G., 1904. New dragonfly nymphs in the United States National Museum. *Proceedings of The United States National Museum,* 27: 685–720.

Novelo–Gutiérrez, R. & González–Soriano, E., 1991. Odonata de la Reserva de la Biósfera la Michilia, Durango, Mexico. Parte II. Náyades. *Folia Entomológica Mexicana,* 81: 107–164.

Peck, S. B., 1992. The dragonflies and damselflies of the Galapagos Islands, Ecuador (Insecta: Odonata). *Psyche,* 99: 309–321.

Posada, D., 2008. jModel test: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, 25: 1253–1256.

Rodrigues Capítulo, A., 1980. Contribución al conocimiento de los Anisoptera de la republica Argentina. I. Descripción de los estadios preimaginales de *Aeshna bonariensis* Rambur (Insecta Odonata). *Limnobios,* 2(1): 1–21.

Rodríguez, J. S. & Molineri, C., 2014. Description of the final instar larva of *Rhionaeschna vigintipunctata* (Ris, 1918) (Odonata: Aeshnidae). *Zootaxa,* 3884(3): 267–274.

Sánchez–Guillén, R. A., Córdoba Aguilar, A., Cordero Rivera, A. & Wellenreuther, M., 2014. Genetic divergence predicts reproductive isolation in damselflies. *Journal of Evolutionary Biology*, 27: 76–87.

Santos, N. D., 1966. Notas sobre *Aeshna* (*Hesperaeschna*) *punctata* Martin, 1908 e sua ninfa (Odonata, Aeshnidae). *Atas da Sociedade de Biologia do Rio de Janeiro*, 10(4): 97–100.

Tamura, K. & Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10: 512–526.

Tamura, K., Nei, M. & Kumar, S., 2004 Prospects for inferring very large phylogenies by using the neighbour–joining method. *Proceedings of the National Academy of Sciences*, 101:11030–11035.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution,* 30: 2725–2729.

Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences*, 17: 57–86.

Turner, P. E., Jr., 1967. Odonata of the Galápagos islands. *Pan–Pacific Entomologist*, 43: 285–291.

von Ellenrieder, N., 1999. Description of the last larval instar of *Aeshna* (*Hesperaeschna*) *cornigera planaltica* Calvert, 1952 (Odonata: Aeshnidae). *Revista de la Sociedad Entomológica Argentina,* 58(3–4): 151–156.

– 2000. *Aeshna tinti* sp. nov. from Chile and redescription of *A. elsia* Calvert (Anisoptera: Aeshnidae). *Odonatologica,* 29: 347–358.

– 2001. The larvae of the Patagonian species of the genus *Aeshna* Fabricius (Anisoptera: Aeshnidae). *Odonatologica,* 30: 423–434.

– 2003. A synopsis of the Neotropical species of '*Aeshna'* Fabricius: the genus *Rhionaeschna* Förster (Odonata: Aeshnidae). *Tijdschrift voor Entomologie,* 146: 67–207.

von Ellenrieder, N. & Costa, J. M., 2002. A new species of *Aeshna*, *A. brasiliensis* (Odonata, Aeshnidae) from South and Southeastern Brazil, with a redescription of its larva. *Neotropical Entomology,* 31(3): 369–376.

von Ellenrieder, N. & Muzón, J., 2003. Description of the last larval instar of *Aeshna* (*Marmaraeschna*) *pallipes* Fraser, 1947 (Anisoptera: Aeshnidae). *Odonatologica,* 32(1): 95–98.

Walker, E. M., 1958. *The Odonata of Canada and Alaska*, vol. 2. Part III: *The Anisoptera, four families*. University of Toronto Press, Toronto.

Watson, M. C., 1956. The utilization of mandibular armature in taxonomic studies of anisopterous nymphs. *Transactions of the American Entomological Society,* 81: 155–205.

Whittaker, R. J., 1998. *Island biogeography. Ecology, evolution and conservation*. Oxford University Press, Oxford.

Appendix 1. Material studied: * Specimens for which DNA was sequenced.

*Apéndice 1. Material estudiado: * Especímenes para los que se secuenció el ADN.*

*Anax amazili* (Burmeister). 1♀ (#871)*, Ecuador, Colón Prov., San Cristóbal Island, unnamed stream (0º 54' 32.69" S, 89º 29' 29.78" W, 431 m), 26 II 2012, A. Cordero Rivera leg. [ACR].

*Rhionaeschna absoluta* (Calvert). 1♂ (#Ra1)*, Argentina, Salta Prov., pond at Finca Los Sauces (24° 28' 24" S, 65° 22',45" W, 1,568 m), 2 XII 2013, R. W. Garrison & N. von Ellenrieder leg. [RWG]; 1♂ (#Ra1)*, Argentina, Salta Prov., Chicoana, Quebrada de Tilián (25° 7' 51" S, 65°32' 24" W, 1,350 m), 26 I 2012, R. W. Garrison & N. von Ellenrieder leg. [RWG].

*Rhionaeschna bonariensis* (Rambur). 1♂ (#Rb4)*, Argentina, Salta Prov., pond at Finca Los Sauces (24° 28' 24" S, 65° 22' 45" W, 1,568 m), 2 XII 2013, R. W. Garrison & N. von Ellenrieder leg. [CSCA]; 1♀ (#Rb1)*, Uruguay, Paysandu Dep., arroyo Soto, ruta 26, km 52 (32° 3' 10" S, 57° 40' 23" W, 43 m), 12 IX 2008, D. Emmerich leg. [CSCA]; 1♀ (#Rb2)*, Argentina, Salta Prov., Chicoana, Quebrada de Tilián (25° 7' 51" S, 65° 32' 24" W, 1,350 m), 26 I 2012, R.W. Garrison & N. von Ellenrieder leg. [RWG]; 1♀ (#Rb3)*, Argentina, Salta Prov., Dique El Tunal, pond below dam (25° 13' 18" S, 64° 28' 31" W, 460 m), 27 I 2012, N. von Ellenrieder & R. W. Garrison leg. [RWG].

*Rhionaeschna cornigera* (Brauer). 1♂ (#234)*, Ecuador, Pichincha prov., river at Mindo (0° 04' 27" S, 78° 45' 49" W, 1,517 m), 15 V 2011, A. Cordero Rivera leg. [ACR]; 1♂ (#240)*, same data but (0° 04' 33" S, 78° 34' 39" W, 1,528 m) [ACR]; 1♂ (#241)*, Pichincha prov., Santa Rosa River, Maquipucuna (#241, 0º 07' 15" N, 78º 37' 58" W, 1,313 m), 7 V 2011, A. Cordero Rivera leg. [ACR].

*Rhionaeschna diffinis* (Rambur). 1♀ (Rd1)*, Chile, De Los Lagos Region, Valdivia Prov., río by road Coñaripe–Carrigüe, 5 II 1999, N. von Ellenrieder leg. [CSCA]; 1♀ (Rd2)*, Chile, De Los Lagos Region, Valdivia Prov., road San José de Mariquina–Valdivia, 30 km N Valdivia, 7 II 1999, N. von Ellenrieder leg. [CSCA]; 1♂ (Rd3)*, Chile, De Los Lagos Region, Osorno Prov., road Hueyusca–Bahía de San Pedro, 9 II 1999, N. von Ellenrieder leg. [CSCA].

*Rhionaeschna elsia* (Calvert). 7♂, 4♀: Peru, Arequipa Dept.: 1♂, Majes Canyon at Puerta Colorada (16° 16' 29" S, 72° 27' 22" W, 628 m), 22 I 1981, D. A. L. Davies leg. [RWG]; Lima Dept.: 1♂ paratype, vicinity of Villa (12° 12' S, 77° 1' W, 120 m), 15 III 1936, F. Woytkowski leg. [RWG]; Huanuco Dept.: 1♀, vicinity of Huanuco (9° 55' S, 76° 14' W, 1,793 m), 2 IX 1937, F. Woytkowski leg. [RWG]; 2♂ (Re1)*, 1♀ (Re2)*, Tacna Dept., Ite Wetlands (17° 55' 32" S, 70° 56' 11" W, 68 m), 2005, N. Flores leg. [CSCA]; 1♂ (Re3)*, same but [RWG]. Chile: Tarapacá Region, Arica Prov.: 1♀, Pampa de Chaca (18° 34' S, 70° 10' W), 5–8 XI 1955, L.E. Peña leg. [RWG]; 1♂, Azapa (18° 34' S, 70° 0' W), 8–10 XI 1955, L. E. Peña leg. [RWG]; 1♂ 1♀, Camarones, fertile valley in the middle of the desert (19° 0' S, 69° 47' W), 27–30 XI 1952, L. E. Peña leg. [CSCA].

*Rhionaeschna galapagoensis* (Currie). 6♂, 6♀, 3♂ ultimate instar exuviae, 2♀ ultimate instar larvae: Ecuador, Colón Prov., San Cristóbal Island: 2♂ (#851–#852)*, 1♀ (#853), 1♂ ultimate instar exuviae (#854), Hacienda El Cafetal, Camarones Stream (0° 55' 32" S, 89° 32' 22" W, 282 m), 20 II 2014, A. Cordero Rivera leg. [ACR]; 1♀ (#870), same data but 25 II 2014 [USFQ]; 1♂ (#869), same data but (0° 55' 27" S, 89° 32' 20" W, 191 m), J. Málaga leg. [USFQ]; 1♀ (#883), 2♂ ultimate instar exuviae (#860), same data but pond, 28 II 2014, A. Cordero Rivera leg. [ACR]; 1♂ (#884), same data but 3 III 2014 [RWG]; 2♀ ultimate instar larvae, same data but 22–23 II 2014, A. C. Encalada leg. [USFQ]. 1♂ (#866), Puerto Baquerizo Moreno, Playa Tijeretas (0° 53' 0" S, 89° 36' 11" W, 2 m), 24 II 2014, A. Cordero Rivera leg. [USFQ]; 1♀ (#878)*, Puerto Baquerizo Moreno, Playa Punta Carola (0° 53' 26" S, 89° 36' 43" W, 3 m), 27 II 2014, A. C. Encalada leg. [USFQ]; 1♂ (#880), 1♀ (#879), same data but [CSCA]; 1♀ (#877)*, same data but [RWG].

*Rhionaeschna marchali* (Rambur). 1♂, 1♀ (#222)*: Ecuador, El Ángel, Carchi prov.: 1♂ (0º 42' 59" N, 78º 00' 54" W, 3,669 m), 4 V 2011, A. Cordero Rivera leg. [ACR]; 1♀ (#223)*, Pichincha prov.: Paluguillo (0º 18' 11.2" N, 78º 13' 41" W, 3,966 m), 4 V 2011, A. Cordero Rivera leg. [ACR].