

Behavior of the Amazonian damselfly *Chalcopteryx scintillans* McLachlan (Zygoptera: Polythoridae) and comments on its morphological distinction from *C. rutilans* (Rambur)

Rhainer Guillermo-Ferreira^{a,b,*}, Ulisses Gaspar Neiss^c, Neusa Hamada^d and Pitágoras C. Bispo^b

^aFaculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados/UFGD, Dourados, Mato Grosso do Sul, Brazil; ^bDepartamento de Ciências Biológicas, Faculdade de Ciências e Letras de Assis, Universidade Estadual Paulista/UNESP, Assis, São Paulo, Brazil; ^cInstituto de Natureza e Cultura - INC/BC, Universidade Federal do Amazonas/UFAM, Benjamin Constant, Amazonas, Brazil; ^dInstituto Nacional de Pesquisas da Amazônia/INPA, Coordenação de Biodiversidade/CBio, Manaus, Amazonas, Brazil

(Received 26 June 2014; accepted 28 October 2014)

Polythorid damselflies are Neotropical stream dwellers, whose behavior has rarely been recorded. Here we describe the territorial and courtship behavior of *Chalcopteryx scintillans* McLachlan, an Amazonian damselfly with shiny copper-colored hind wings. Territorial behavior consists of aerial contests, when males engage in threat displays and mutual pursuits in ascending and rocking flights. During courtship, males hold their coppery hind wings still while hovering with their forewings, showing the hind wings to females, which hover in front of the male in response. After copulation, the male exhibits the courtship flight again by hovering over the oviposition resource (i.e. fallen tree trunk) on the stream. The females oviposit on the trunk while the males guard them by perching near and hovering around them constantly. We also present behavioral notes on reproductive and oviposition behavior, and comments on the differentiation between *C. scintillans* and *C. rutilans* (Rambur).

Keywords: dragonfly; aquatic insect; Odonata; animal behavior; reproduction; territoriality

Introduction

Polythoridae comprises 57 species of Neotropical damselflies, distributed mainly in Andes foothills and Amazonian forests (Garrison, von Ellenrieder & Louton, 2010). These damselflies are stream dwellers, easily observable by their colorful and shiny wings in sunny patches in the midst of the forest (Altamiranda & Ortega, 2012; Bick & Bick, 1985, 1986; Sánchez-Herrera & Realpe, 2010).

There are only a few behavioral records, with observations on female oviposition on fallen tree trunks, male-female interactions and male territorial behavior (Burmeister & Börzsöny, 2003; Dunkle, 2001; Esquivel, 2006; Fraser & Herman, 1993; Gonzalez-Soriano & Verdugo-Garza, 1984; Pritchard, 1996; Resende & De Marco, 2010). However, there is no detailed description of courtship or fighting behavior.

*Corresponding author. Email: rhainerguillermo@gmail.com

Thus, here we provide descriptions of male fighting and courtship behavior of *Chalcopteryx scintillans* McLachlan, 1870. We also provide notes on reproductive behavior and clarify possible identification misunderstandings between this species and *C. rutilans* (Rambur, 1842).

Materials and methods

Study species

Larvae and adults of *C. scintillans* are abundant in typical sand bed Amazonian igarapés (i.e. streams) within the tropical rainforest. In these igarapés, larvae are found with relative abundance in moderately to fast flowing streams, associated with roots and litter substrates retained in the flowing water regions (Neiss, unpublished data). This species, like other *Chalcopteryx*, is usually restricted to primary forests, and hence is considered as a good bioindicator of environmental integrity. Polythorid larvae are morphologically peculiar, exhibiting a nearly unique trait in Odonata, shared only with Euphaeidae: the presence of lateral-ventral filamentous abdominal gills, on segments 2–7 (2–8 in Euphaeidae). However, lateral gills in these groups are probably not homologous (Dijkstra, Kalkman, Dow, Stokvis, & Van Tol, 2014). Moreover, caudal lamellae also exhibit distinctive characteristics: a sac-like shape, with conical or digital-shaped posterior projections, and dorsally covered by a dense coat of scale-shaped setae.

Fieldwork

We conducted our study on *C. scintillans* (Figure 1) in the Barro Branco stream in the Reserva Florestal Ducke (RFD), Manaus, Amazonas state, Brazil (2°57'07.4" S, 59°57'27.6" W; altitude 75 m) in November 2011 and 2012. We made 22 hours of behavioral observations from 10:00 (when males began to fight and court females) to 15:00 (when sexual and fighting activity declined). We marked a total of 34 males with white correction fluid on the thorax and abdomen to prevent sampling the same male multiple times. We observed and timed with a chronometer 18 individual fights. In one fight, the males involved were not marked. Fights usually consisted of 1 to 6 bouts (total $N = 33$), when the fight was interrupted for a while but was restarted by the same males.

To describe both the courtship and fighting behavior, we used the *sequence sampling* method (Altmann, 1974). Territorial behavior was observed by the presence of the following categories (descriptions from Pajunen, 1966): (i) chase, one male chased another male for a brief time with



Figure 1. A male of *Chalcopteryx scintillans* McLachlan, 1870 (Odonata, Polythoridae), left lateral view. (Photo: U. G. Neiss).

no trajectory pattern; (ii) frontal threat, the males faced each other in flight; (iii) reversed threat, with both males in flight, one male with its back turned to the other; (iv) lateral threat, the males flew side by side; (v) circling, the males flew in circles in mutual pursuit; (vi) rocking flight, the males rose and dived with side-to-side rocking movements.

Courtship behavior was observed by the presence of the following categories: (i) courtship flight (Heymer, 1973), when the male hovers in an arc in front of the female; (ii) cross display (Waage, 1973), when the male perches on the oviposition site and spread his wings forming an “X”; (iii) float display (Gibbons & Pain, 1992), when the male falls to the water and floats with the flow.

Additional observations were made during copulation and oviposition, with the help of photo and video recording (Fuji Finepix HS10, Fujifilm, Japan). Since there may be confusions between *C. scintillans* and *C. rutilans*, we also present a figure with traits that can be used to avoid identification mistakes.

Specimens examined

Chalcopteryx scintillans: Brazil, Amazonas state: Manaus: Reserva Florestal Ducke, highway AM 010-km 26 (2°57'07.4" S, 59°57'27.6" W), *igarapé* (small stream): (Cod. 066): 1♂, 1♀, 24 October 2009; BR 174-km 38 – Fazenda Experimental da UFAM – PPBio grid (02°39'29.8" S, 60°04'28" W) (Cod. 70): 1♂, 10 November 2008; BR 174-km 56, ZF-2 (02°35'57.3" S, 60°13'01.2" W), small stream (Cod. 129): 1♂, 22 May 2008. Neiss, U. G. leg. *Chalcopteryx rutilans*: Brazil, Pará state: Rurópolis, Ramal do Lagarto, *igarapé* Grovão de Pedra (04°14'39.5" S, 55°00'28.6" W): 4♂, 2♀, 14 October 2008. Hamada, N. and Silva, J.O. leg.

Results

Male fights and courtship behavior

All male–male interactions ($N = 33$ bouts) involved aerial contests (See Supplemental Video 1) composed of (ii) frontal and (iii) reversed threat displays, with some ($N = 6$ bouts) also including ascending flights and rocking flights (vi). The typical circle-fighting (v) was not observed. We did not observe any short chases (i), since males always engaged in aerial displays. Fights lasted 242.84 ± 895.68 s (range: 5 s to 86 min). Males apparently defended fallen tree trunks on the stream, which is the resource used by females for oviposition. Males engaged in long aerial contests, exhibiting the coppery surface of the wings to each other. The bobbing flight may be a consequence of flight being maintained largely by the forewings, while the hind wings are used mainly for the threat display.

Courtship behavior (Figure 2a) began when a female approached the male territory. The male engaged the female in the air and exhibited the (i) courtship flight, flying facing the female while holding the hind wings immobile ($N = 6$). The female might perch and make a warding-off display by spreading her wings, then fly away from the male ($N = 2$) or respond the male display ($N = 4$) by exhibiting a display similar to male frontal threat display (Figure 2b), following him face-to-face until the male perches on the oviposition resource (i.e. fallen tree trunk). Males did not raise or curl the abdomen up or down during contests and courtship displays.

The female then perched on the territory and the male clasped her prothorax, initiating tandem formation and quickly entering copulation. After mating, the male released the female and hovered over the oviposition site making the courtship flight again with hind wings held inert ($N = 4$), apparently guiding the female to the site. Two of these four females left the site immediately after mating, while the males tried to chase them with no success.

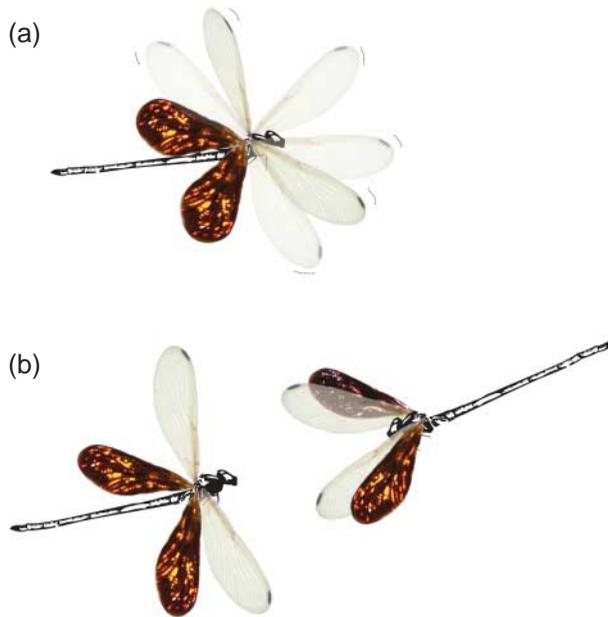


Figure 2. Positions of wings during male courtship behavior (a) and territorial contests (b) of *Chalcopteryx scintillans*. The female exhibits the same pattern in (b) during courtship.

The other four females perched on the oviposition site while the male perched nearby, periodically hovering over her. If the female tried to leave prematurely ($N = 1$), the male exhibited the courtship flight again, which led the female to return to oviposit. Copulations ($N = 5$) lasted 10.4 ± 4.9 min (range: 6.5–19 min) and ovipositions ($N = 6$) lasted 52.6 ± 42 min (range: 7–112 min). Females may aggregate to oviposit on the same trunk (Figure 4), more commonly after raining. During oviposition, male and female signal to each other with wingclaps, which show the angle-dependent metallic coloration on the dorsal surface of their wings (See Supplemental Video 2).

Female ovipositing behavior

Three females were observed in the field inserting the terminal region of their abdomens into the bark of a fallen tree trunk (Figure 3). At first, we thought they were laying eggs, since it is widely known that Polythoridae females oviposit on decomposing trunks, but we could not see eggs being laid in the videos we recorded. After the observations, we collected the bark where females were puncturing to search for the eggs in the laboratory, under a microscope, but did not find any.

The area of the trunk where females were active was located approximately 1 m above the water surface. The bark of the trunk was superficially decomposed. Females were observed mainly over the upper surface of the trunk, but one was also observed on the inferior surface. A female would land on the bark surface with its legs widely separated, bend its abdomen and insert the gonapophysis (anterior and posterior) into the bark; the lateral valves provided some support at the end of this movement.

The female repeated this behavior several times in a small patch and then moved to an adjacent patch where this action was continued. After some time, the area she punctured looks like a plowed field, as a result of small pieces of wood being overturned. During oviposition, both males and females exhibit wing-clapping behavior, showing the coppery surface of hind wings.



Figure 3. Females of *Chalcopteryx scintillans* during aggregated oviposition in a fallen tree trunk.

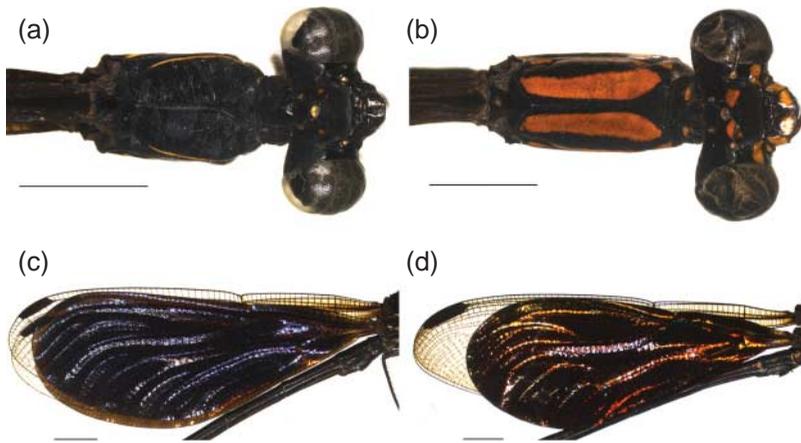


Figure 4. Dorsal thorax, head and right lateral view of wings of *C. scintillans* (a, c) and *C. rutilans* (b, d). (Scale bar: 2 mm)

Distinctions between species of Chalcopteryx

Although the species mentioned in other studies in the Ducke Reserve is *C. rutilans* (Delgado, 2002; Juen & De Marco, 2011; Resende & De Marco, 2010), Neiss (2012) found that only *C. scintillans* occurs in the Manaus region. Therefore, we also present clear distinctions between *C. scintillans* and *C. rutilans* to help in their identification (Figure 4). The male adults of both species can be distinguished mainly by the differences in the coloration of pterothorax (compare Figures 4a and 4b), and length of hind wing (colored wing) in comparison with the length of forewing (hyaline wing) (compare Figures 4c and 4d). Moreover, the species exhibit different metallic reflection coloration on superior surfaces of the hind wings, which can be easily seen during the flight: *C. scintillans* has a coppery reflection, whereas *C. rutilans* has an iridescent multi-colored reflection (mainly greenish, purplish, reddish and coppery).

Discussion

The present study provides the first description of the courtship behavior in Polythoridae. The male displays during pre and post-copulatory courtship resemble those of Calopterygidae,

especially *Neurobasis*, whose males also hover holding their colorful hind wings inert while the hyaline forewings sustain the flight (Günther, 2006; Günther, Hilfert-Rüppell, & Rüppell, 2014). However, a clear difference between *Chalcopteryx* and calopterygids is the absence of the cross display in the polythorid, which is a display calopterygids make with wings spread when perched on the oviposition site (Waage, 1973) or near the females (Guillermo-Ferreira & Bispo, 2012). In *C. scintillans*, males guided the female to the oviposition resource by moving towards the fallen tree trunk while still facing the female and presenting the hind wings. Another genus with shiny wings and complex courtship displays is Chlorocyphidae, but their behavior is quite different from polythorids. Males of many chlorocyphid species have pruinosity on their legs, which they show to females while they fly in an arc above them during the courtship flight (Orr, 1996).

The territorial behavior also seems to be similar to other polythorids (Gonzalez-Soriano & Verdugo-Garza, 1984; Pritchard, 1996), chlorocyphids (Günther, 2008; Orr, 1996) and calopterygids (Córdoba-Aguilar & Cordero-Rivera, 2005; Guillermo-Ferreira & Bispo, 2012; Heymer, 1973; Pajunen, 1966). Males engage in aerial contests, exhibiting the coppery surface of wings to each other in mutual threat displays and pursuits. It resembles the threat displays of Chlorocyphidae (Günther, 2008; Orr, 1996) and *Neurobasis* (Günther, 2006; Günther et al., 2014), when males engage in frontal displays and a series of rapid feints at one another, showing the metallic coloration of the wings. Circle-fighting, abdomen raising and lateral threat displays are traits that were not observed in *C. scintillans*, representing characters that differentiate them from calopterygids (Córdoba-Aguilar & Cordero-Rivera, 2005).

The proposed (Dumont, Vierstraete, & Vanfleteren, 2010) close phylogenetic relationship between Polythoridae and Calopterygidae may indicate that they share similar courtship and territorial displays that first evolved in a common ancestor, presumably a damselfly with colored wings. Nevertheless, other phylogenetic hypotheses suggest a different scenario, where polythorids are more closely related to clear-winged damselflies (e.g. Philogangidae, Heteragrionidae and Dicteriadidae) (Dijkstra et al., 2014) that are not known to exhibit courtship behavior. In this context, the structural coloration and complex behavioral displays could be considered traits that are not homologous but the result of convergent evolution in different groups (i.e. Calopterygidae, Polythoridae, Chlorocyphidae and Euphaeidae). Since we lack information about the behavior of some groups that are closely related to polythorids, it is also possible that further studies may result in different evolutionary scenarios.

During female ovipositing behavior, interestingly, we could see that females were not actually laying their eggs, but instead were simply puncturing the bark of the trunk. Oviposition in Odonata often involves the activity of the cutting valves, which move alternately to penetrate plant tissue in endophytic oviposition (Matushkina & Gorb, 2007; Matushkina & Lambret, 2011). The presence of mechanoreceptors and gustatory sensilla in the cutting valves of Odonata (Matushkina & Gorb, 2002; Matushkina & Lambret, 2011; Rebora, Piersanti, Dell'Otto, & Gaino, 2013) provide evidence that allow us to suggest that *C. scintillans* females test substrates repeatedly with typical oviposition behavior.

In conclusion, we provide a detailed description of the courtship and territorial behavior of *C. scintillans*. We expect that with the morphological comments provided here, it will be easier to distinguish this species from *C. rutilans*.

Acknowledgments

RGF thanks CAPES for financial support and FAPESP (13/00406-7) for a post-doc fellowship grant. UGN and NH thanks PRONEX-CNPq-FAPEAM, FAPEAM - FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DO AMAZONAS Edital 020/2013 - PAPAC and PRO-Equipamentos/CAPES/INPA for financial support. NH also thanks CNPq for a research fellowship. PCB thanks CNPq (307577/2011-2) for research fellowship and FAPESP (12/21196-8) for financial support.

Supplemental data

Supplemental data for this article can be accessed via the online version (<http://dx.doi.org/10.1080/13887890.2014.983189>)

References

- Altamiranda, M., & Ortega, O. (2012). Estructura poblacional de *Polythore gigantea* (Odonata: Polythoridae) en sistemas lóticos con diferentes estados de conservación. Antioquia, Colombia. *Revista de Biología Tropical*, 60, 1205–1216.
- Altmann, J. (1974). Observational study of behaviour: sampling methods. *Behaviour*, 49, 227–267. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0034-77442012000300020
- Bick, G. H., & Bick, J. C. (1985). A revision of the *picta* group of *Polythore*, with a description of a new species, *P. lamerceda* spec. nov., from Peru (Zygoptera: Polythoridae). *Odonatologica*, 14, 1–28.
- Bick, G. H., & Bick, J. C. (1986). The genus *Polythore* exclusive of the *picta* group (Zygoptera: Polythoridae). *Odonatologica*, 15, 245–273.
- Burmeister, E. G., & Börzsöny, L. (2003). *Polythore spaeteri* spec. nov., from the Peruvian tropical rainforest (Panguana), with remarks on its ecology (Zygoptera: Polythoridae). *Spixiana*, 26, 43–48.
- Córdoba-Aguilar, A., & Cordero-Rivera, A. (2005). Evolution and ecology of Calopterygidae (Zygoptera: Odonata): status of knowledge and research perspectives. *Neotropical Entomology*, 34, 861–879. doi:10.1590/S1519-566X200500600001
- Delgado, C. (2002). Spatial and temporal distribution patterns of Odonata larvae in the streams of a Terra Firma Forest of the Central Amazon, Brazil. *Journal of Freshwater Ecology*, 17, 555–561.
- Dijkstra, K. D. B., Kalkman, V. J., Dow, R. A., Stokvis, F. R., & Van Tol, J. (2014). Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata). *Systematic Entomology*, 39(1), 68–96. doi:10.1111/syen.12035
- Dumont, H. J., Vierstraete, A., & Vanfleteren, J. R. (2010). A molecular phylogeny of the Odonata (Insecta). *Systematic Entomology*, 35, 6–18. doi:10.1111/j.1365-3113.2009.00489.x
- Dunkle, S. (2001). Family Polythoridae (Bannerwings). In J. Silsby (Ed.), *Dragonflies of the world* (pp. 84–86). Collingwood, Australia: CSIRO Publishing.
- Esquivel, H. C. (2006). *Libélulas de Mesoamérica y el Caribe. Dragonflies and Damselflies of Middle America and the Caribbean*. Santo Domingo de Heredia, Costa Rica: Instituto Nacional de Biodiversidad (INBio).
- Fraser, F. C., & Herman T. (1993). Territorial and reproductive behaviour in a sympatric species complex of the neotropical damselfly *Cora* Selys (Zygoptera: Polythoridae). *Odonatologica*, 22, 411–429.
- Garrison, R. W., von Ellenrieder, N., & Louton, J. A. (2010). *Damselfly genera of the new world: An illustrated and annotated key to the Zygoptera*. Baltimore: The Johns Hopkins University Press.
- Gibbons, D. W., & Pain, D. (1992). The influence of river flow rate on the breeding behaviour of *Calopteryx* damselflies. *Journal of Animal Ecology*, 61, 283–289.
- Gonzalez-Soriano, E., & Verdugo-Garza, M. (1984). Estudios en Odonatos Neotropicales II: notas sobre el comportamiento reproductivo de *Cora marina* Selys (Odonata: Polythoridae). *Folia Entomologica Mexicana*, 62, 3–15.
- Guillermo-Ferreira, R., & Bispo, P. C. (2012). Male and female interactions during courtship of the Neotropical damselfly *Mnesarete pudica* (Odonata: Calopterygidae). *Acta Ethologica*, 15, 173–178. doi:10.1007/s10211-012-0122-4
- Günther, A. (2006). Reproductive behaviour of *Neurobasis kaupi* (Odonata: Calopterygidae). *International Journal of Odonatology*, 9, 151–164. doi:10.1080/13887890.2006.9748274
- Günther, A. (2008). *Vergleichende Untersuchungen zum Reproduktionsverhalten südostasiatischer Chlorocyphidae und Calopterygidae (Odonata: Zygoptera)* (PhD thesis). TU Bergakademie Freiberg, Germany. <http://nbn-resolving.de/urn:nbn:de:bsz:105-5111029>
- Günther, A., Hilfert-Rüppell, D., & Rüppell, G. (2014). Reproductive behaviour and the system of signalling in *Neurobasis chinensis* (Odonata, Calopterygidae)—a kinematic analysis. *International Journal of Odonatology*, 17, 31–52. doi:10.1080/13887890.2014.881305
- Heymer, A. (1973). Etude du comportement reproducteur et analyse des mécanismes déclencheurs innes (MDI) optiques chez les Calopterygidae (Odon. Zygoptera). *Annales de la Société entomologique de France*, 9, 219–255.
- Juen, L., & De Marco, P. (2011). Odonate biodiversity in terra-firme forest streamlets in Central Amazonia: on the relative effects of neutral and niche drivers at small geographical extents. *Insect Conservation and Diversity*, 4, 265–274. doi:10.1111/j.1752-4598.2010.00130.x
- Matushkina, N., & Gorb, S. (2002). Stylus of the odonate endophytic ovipositor: a mechanosensory organ controlling egg positioning. *Journal of Insect Physiology*, 48, 213–219. doi:10.1016/S0022-1910(01)00166-4
- Matushkina, N., & Gorb, S. (2007). Mechanical properties of the endophytic ovipositor in damselflies (Zygoptera, Odonata) and their oviposition substrates. *Zoology*, 110, 167–175.
- Matushkina, N. A., & Lambert, P. H. (2011). Ovipositor morphology and egg laying behaviour in the dragonfly *Lestes macrostigma* (Zygoptera: Lestidae). *International Journal of Odonatology*, 14, 69–82. doi:10.1080/13887890.2011.568190
- Neiss, U. G. (2012). *Taxonomia de Odonata (Insecta), com ênfase na caracterização morfológica e biologia de larvas, na Amazônia Central, Brasil* (PhD thesis). Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil. 331 pp.

- Orr, A. G. (1996). Territorial and courtship displays in Bornean Chlorocyphidae (Zygoptera). *Odonatologica*, 25, 119–141.
- Pajunen, V. (1966). Aggressive behavior and territoriality in a population of *Calopteryx virgo* L. (Odonata: Calopterygidae). *Annales Zoologici Fennici*, 3, 201–214.
- Pritchard, G. (1996). The life history of a tropical dragonfly: *Cora marina* (Odonata: Polythoridae) in Guanacaste, Costa Rica. *Journal of Tropical Ecology*, 12, 573–581. doi:10.1017/S0266467400009792
- Rebora, M., Piersanti, S., Dell’Otto, A., & Gaino, E. (2013). The gustatory sensilla on the endophytic ovipositor of Odonata. *Arthropod Structure and Development*, 42, 127–134. doi:10.1016/j.asd.2012.10.005
- Resende, D., & De-Marco, P. (2010). First description of reproductive behavior of the Amazonian damselfly *Chalopteryx rutilans* (Rambur) (Odonata, Polythoridae). *Revista Brasileira de Entomologia*, 54, 436–440. doi:10.1590/S0085-56262010000300013
- Sánchez-Herrera, M., & Realpe, E. (2010). Population structure of *Polythore procera* at a Colombian stream (Odonata: Polythoridae). *International Journal of Odonatology*, 13, 27–37.
- Waage, J. K. (1973). Reproductive behaviour and its relationship to territoriality in *Calopteryx maculata* (Beauvois) (Odonata: Calopterygidae). *Behaviour*, 47, 240–256.