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New distribution records and notes on the habitat of *Magneuptychia flavofascia* Zacca & Siewert, 2014 (Lepidoptera: Nymphalidae)

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Abstract: The recently described butterfly *Magneuptychia flavofascia* was formerly known from only two localities in the Brazilian Cerrado savanna. Here, we report new distribution records, DNA barcode sequences, and information on the habitat, adult behavior and systematic position of this species.

Key words: Chapada dos Guimarães, DNA barcoding, grassland butterflies, Paryphthimoides, Satyrinae

The Brazilian savanna vegetation named Cerrado originally covered an area of over two million square kilometers in Central Brazil (Oliveira and Marquis 2002). It is the most extensive savanna in South America, presenting high species richness and endemism (including butterflies; see Brown and Gifford 2002) and is considered a global hotspot of biodiversity (Mittermeier et al. 2005). The vegetation consists of savannic tree-shrub formations in the well-drained interfluves with gallery forests along the watercourses (Figure 1). Different vegetation types are included under Cerrado *sensu lato*, whose physiognomies vary from pure grassland, through savanna with different woody biomass, to mesophytic forests in richer soils (Oliveira-Filho and Ratter 2002).

Recently, Zacca et al. (2014) described *Magneuptychia flavofascia* Zacca & Siewert, 2014, a species of Satyrinae from the Brazilian Cerrado. This species was described based on museum specimens collected in the 1960s and 1970s. In addition to being recorded from only two localities, there is a lack of information about its habitat preference and behavior. Here, we report recent new distribution data and DNA barcode sequences, and we discuss information about habitat, adult behavior and systematic position.

The species was studied in the field at Parque Nacional da Chapada dos Guimarães (PNCG), Chapada dos Guimarães municipality, Mato Grosso state, Brazil. The park contains almost all Cerrado physiognomies, with altitudes ranging from 200 to 900 m (for details see Pinto and Oliveira-Filho 1999). The study was carried out along two different trails, 5 km long, each containing three sampling units, 2 km apart. Each sampling unit consisted of five portable bait traps (see Uehara-Prado et al. 2007). The traps were spaced linearly along trails in the Cerrado *sensu stricto* of PNCG, suspended 0.8–1.5 m above the ground, with a distance of at least 20 m between adjacent traps. A standard mixture of mashed banana and sugar cane juice, fermented for at least 48 h, was used as the attractant. The bait was placed inside the traps in plastic pots with a perforated cover. The traps were checked and the baits replaced every 24 hr over four days. Sampling was done in December 2012 and March, May and June 2013. Additionally, sampling with entomological nets was also done in different vegetation types in PNCG, including gallery forests, mesophytic forests, and open Cerrado areas, totaling a sampling effort of ~100 net-hours. Adults were collected with entomological nets and legs were removed from each individual and kept for DNA barcoding analysis. The mitochondrial gene Cytochrome C Oxidase I (*CoxI*, ca. 658 bp) was amplified by using the following primer combination: LCO + HCO (Folmer et al. 1994). Reactions were done in a 25 µL final volume using 2 µL of total DNA, 2.0 mM of $MgCl_2$, 40 µM of dNTPs, 0.5 µM of each primer, 1U of GoTaq DNA Polymerase (Promega, Madison, Wisconsin, USA), and 10% of 1X Taq buffer. The amplification program included an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, and polymerization at 72°C

Figure 1. General view of the *Magneuptychia flavofascia* habitat in Chapada dos Guimarães, state of Mato Grosso, Brazil; specimens were observed in areas of contact between gallery forests and the Cerrado savanna (arrow).

for 1 min, followed by an extension step at 72°C for 10 min (Silva-Brandão et al. 2005). PCR products were purified of primers and deoxynucleotides with ExoSAP-IT (GE Healthcare, Buckinghamshire England), and then sequenced by ABI Prism BigDye Kit protocol in a 3500xL Genetic Analyser (Applied Biosystems – Hitachi), with primers used for amplification.

Additional distribution data for *Magneuptychia flavofascia*

BRAZIL: Distrito Federal: Brasília: Parque do Gama, 2 females, 23–26.ii.1968; 3 males and 2 females, 14–17.v.1969; 2 males and 3 females, 07.ii.1970; Brown Jr. *leg*. *Mato Grosso*: Chapada dos Guimarães – Parque Nacional da Chapada dos Guimarães (15°40′S, 055°83′ W, 590–600 m above sea level), 3 males and 1 female, 01.VI.2013; 1 male, 25.XI.2013; Kaminski and Dell'Erba *leg*. Material deposited in the Museu de Zoologia da Universidade Estadual de Campinas (ZUEC), Unicamp, Campinas, São Paulo, Brazil.

Molecular data

DNA sequences of *CoxI* from four individuals were obtained (DNA vouchers YPH-0485, YPH-0488, YPH-0489 and YPH-0490); GenBank accession numbers are KP994892, KP994893, KP994894 and KP994895 respectively.

Habitat and behavior of *Magneuptychia flavofascia*

Adults of *M. flavofascia* were only observed in areas of contact between gallery forests and the Cerrado savanna (Figure 1). Extensive censuses inside the gallery forests, mesophytic forests, and cerrado areas (both with entomological nets and bait traps) in the Chapada dos Guimarães showed that *M. flavofascia* was absent in these habitats, being observed exclusively in the interface between open vegetation and forest. This habit preference reported for *M. flavofascia* is distinct to that observed for several other species of *Magneuptychia,* which are associated with forest habitats (e.g., DeVries 1987; Kaminski and Freitas 2008). For example, the three widespread species *Magneuptychia libye* (Linnaeus, 1767), *Magneuptychia pallema* (Schaus, 1902), and *Magneuptychia ocypete* (Fabricius, 1776), were only observed inside the gallery forests and the mesophytic forests in the same region (R. Dell'Erba, pers. obs.), never syntopic with *M. flavofascia*. This same pattern was observed in Diamantino (MT), where *M. flavofascia* is found only in rocky Cerrado sites near gallery forests (E. Furtado, pers. comm.). The habitat requirements that might explain its localized distribution are unknown and need to be investigated in detail.

The reported habitat preference of *M. flavofascia* is potentially important information for understanding not only its ecology and natural history, but also its systematic position. The genus *Magneuptychia* is clearly polyphyletic (Peña et al. 2010), and preliminary phylogenetic analysis (unpublished results), comparing the barcode sequences of *M. flavofascia* with available sequences of other species of Euptychiina, recovered the species close to *Paryphthimoides poltys* (Prittwitz,

1865), a species of open habitats, and not to the forest species of *Magneuptychia*. It is also interesting that the old specimens here reported (from the former collection of Keith S. Brown Jr., and now part of ZUEC), were identified by Dr. Keith Brown as an "undescribed species of *Erichthodes* Forster, 1964", and not as a species of *Magneuptychia*. These results suggests that all morphological similarities of *M. flavofascia* with other *Magneuptychia* are either convergences or plesiomorphies of the "*Splendeuptychia* clade" (*sensu* Peña et al. 2010).

In summary, the purpose of this report is to provide biological information for this recently described species, and we hope that the information may be useful and encourage further studies of this butterfly endemic to the Brazilian Cerrado.

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