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LARVAL POLYCHROMATISM IN THE NEOTROPICAL HAIRSTREAK *STRYMON BUBASTUS* (STOLL) (LYCAENIDAE, THECLINAE, EUMAEINI) ASSOCIATED WITH TWO NEWLY DOCUMENTED HOST PLANTS IN THE ATACAMA DESERT

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ABSTRACT. Host-associated larval polychromatism is described for the first time for the Neotropical hairstreak *Strymon bubastus* (Stoll, 1780) (Lepidoptera, Lycaenidae, Theclinae, Eumaeini) based on larvae collected in the Atacama Desert of northern Chile on inflorescences of *Phyla nodiflora* (L.) Greene (Verbenaceae) and *Waltheria ovata* Cav. (Malvaceae) and reared to adult. This is the first record of a host plant in the family Verbenaceae for *S. bubastus*. Although other Malvaceae already have been recorded as its hosts, this is the first record of it feeding on *W. ovata*. Identical sequences (n=19) of the DNA barcode fragment (657 base pairs) of the cytochrome c oxidase subunit I (COI) gene were obtained from larvae collected on the two plants, providing additional support for conspecificity. However, deep divergence (>2%) was found among these sequences and others from geographically distant localities of the Neotropics. Deep divergence could be associated with phenotypic differentiation of *S. bubastus* over its wide geographic range.

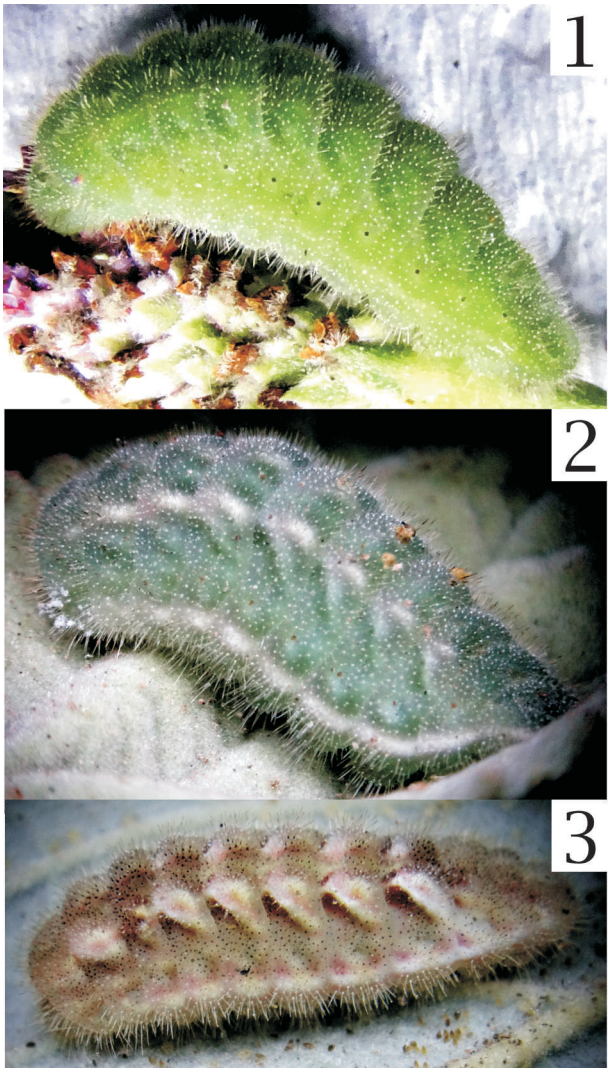
Additional key words: DNA barcoding, florivory, polyphagy, *Phyla nodiflora, Waltheria ovata*

The hairstreak *Strymon bubastus* (Stoll, 1780) (Lepidoptera, Lycaenidae, Theclinae, Eumaeini) is widely distributed in the Neotropical Region, ranging through a greater part of mainland South America and the Caribbean Islands (Robbins & Nicolay 2002, Nicolay & Robbins 2005). Several geographical adult phenotypes are recognized within this range, one of which, previously known as *S. sapota* (Hewitson, 1877), is endemic to the Atacama Desert (Nicolay & Robbins 2005). This phenotype is the only one recorded in Chile, where it is restricted to a few coastal valleys of the Atacama Desert, representing the southern limit for the range of *S. bubastus* along the Pacific coast (Peña & Ugarte 1996).

Despite the extensive geographic range of *S. bubastus*, its biology has been little studied (Silva et al. 2014). The available data are restricted mostly to some host records, including plants of the families Boraginaceae, Convolvulaceae, Fabaceae, Malvaceae and Portulacaceae (Beccaloni et al. 2008, Silva et al. 2011, 2014), suggesting a polyphagous habit for this species.

Polyphagy is common among larvae of Eumaeini, especially in flower-feeding species (Robbins & Aiello 1982, Brown 1993, Silva et al. 2011). For example, Monteiro (1991) recorded 30 host plants belonging to 10 families for larvae of *Rekoa marius* (Lucas, 1857) in a single locality in Brazil, while 44 host plants belonging to 19 families were recorded for *Parrhasius polibetes* (Stoll, 1781) by Kaminski et al. (2012). Some species of *Strymon* Hübner, 1818 also can be highly polyphagous (Robbins & Nicolay 2002, Silva et al. 2011). In addition, larvae of Eumaeini may display cryptic, host-associated color patterns (Ballmer & Pratt 1989, Monteiro 1991, Kaminski et al. 2012, Bächtold et al. 2013, Silva et al. 2014) due to the hypothesized accumulation of carotenoid and flavonoid pigments (Monteiro 1991). As a consequence, a wide range of color patterns is usually displayed by the larvae of some polyphagous species (Monteiro 1991, Kaminski et al. 2012).

Geographic variation in important biological features such as host plant use patterns, has been described for some butterflies (Rodrigues & Moreira 2002, Meister et al. 2015, Vilbas et al. 2015), including species inhabiting the arid landscapes of the Atacama Desert and neighboring areas of the Andes (Vargas 2013, 2014). Such geographic variation may be expected in widely distributed species, as is the case of *S. bubastus*, whose populations are separated from one another in different habitats along its range, where different plants



FIGS. 1–3. Polychromatic final instar of *Strymon bubastus*. **1**) unique color pattern recorded on *Phyla nodiflora*; **2**) dominant color pattern on *Waltheria ovata*; **3**) less frequent color pattern on *W. ovata*.

are available. The host plant records currently known for *S. bubastus* are based on collecting and rearing performed at different localities of its range (Beccaloni et al. 2008, Silva et al. 2011, 2014). However, the hosts of the Chilean populations have been unknown, impeding an understanding of the biology of this hairstreak at the local level.

The objective of this study is to provide the first data on the biology of the larvae of *S. bubastus* in northern Chile, including the first mention of host-associated polychromatism on two newly recorded host plants. In addition, the first sequences of DNA barcodes (sensu Hebert et al. 2003) are provided for the Atacama populations of *S. bubastus*.

MATERIALS & METHODS

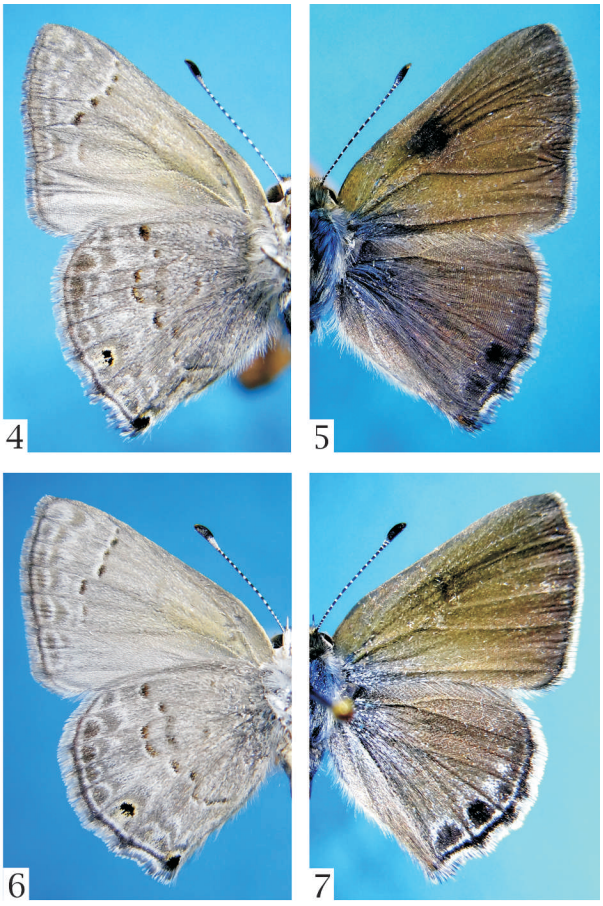
Final and penultimate instars of *S. bubastus* displaying host-associated color patterns were collected on inflorescences of *Phyla nodiflora* (Verbenaceae) and *Waltheria ovata* (Malvaceae) in the Azapa valley, Atacama Desert of northern Chile, between June 2008 and April 2015. The larvae were reared in the laboratory in individual plastic vials with inflorescences of their respective plants, which were changed daily until the larva stopped feeding and prepared for pupation. The adults obtained from the pupae were pinned and spread for species identification. Genitalia of two males and two females obtained from each plant were dissected following standard procedures (Winter 2000). Vouchers are deposited in the Colección Entomológica de la Universidad de Tarapacá (IDEA), Arica, Chile.

In order to assess the possible existence of genetic divergence between larvae displaying the two main color patterns (see below), the DNA barcode fragment (657 base pairs) of the mitochondrial cytochrome oxidase subunit I (COI) gene was sequenced for specimens collected on the two host plants in April 2015. Genomic DNA was extracted from pupae following the procedures described in Huanca-Mamani et al. (2015). PCR amplification and sequencing of the COI DNA barcode were performed by a commercial facility (Macrogen, South Korea) using the primers LEP-F1 (5'-ATTCAACCAATCATAAAGATAT-3') and LEP-R1(5'-TAAACTTCTGGATGTCCAAAAA-3') developed by Hebert et al. (2004). The sequences were aligned following the MUSCLE method (Edgar 2004) as implemented in the software MEGA6 (Tamura et al. 2013) to survey variable sites. A molecular identification was performed in BOLD v.3 Identification System (Ratnasingham & Hebert 2007).

RESULTS

Forty-two final and penultimate instars were collected on inflorescences of the two plants: 20 on *P. nodiflora*and 22 on *W. ovata*. All the larvae were reared successfully to the pupal stage, as parasitoids were not detected in the rearing. Only 23 adults were obtained from the pupae; the remaining 19 pupae were preserved in ethanol 95% to be used in DNA extraction.

Larval color pattern. All the larvae collected on *P. nodiflora*(n=20) were uniformly light green with short white secondary setae (Fig. 1), and six males and five females were reared from them. Twenty larvae collected on *W. ovata* were mostly pale green with one dorsal and one lateral cream white stripe, and with



FIGS. 4–7. Adults of *Strymon bubastus* reared from larvae collected on inflorescences of *Waltheria ovata*. **4**) Male, ventral view; **5**) Male, dorsal view; **6**) Female, ventral view; **7**) female, dorsal view.

short white secondary setae (Fig. 2), and they yielded five males and five females. An additional, less common (n=2) color pattern also was detected on *W. ovata* (Fig. 3), and was mostly pale yellow with the same dorsal and lateral stripes of the previous pattern, but with dark red blotches anteroventral to the dorsal stripe on A1–7, pink blotches dorsally to the lateral stripes on A1–8, and with the base of many secondary setae of the laterodorsal area black. One male and one female were reared from these larvae.

Wing pattern and genitalia. Rearing revealed no correlation between the color patterns of the larvae and the wing patterns or the morphology of the male and female genitalia. All the adults (12 males; 11 females) obtained from the larvae reared in the laboratory displayed the typical wing pattern of the Atacama Desert populations of *S. bubastus* regardless of the host plant on which the larvae were collected in the field, thus adults reared from only one host (*W. ovata*) are presented in Figs. 4–7. As well, no differences were found in the morphology of the male and female genitalia of the adults reared from the two sources.

DNA barcodes. Nineteen DNA barcode sequences (657 bp) were obtained, nine from the pupae reared as ex-larvae on *P. nodiflora* (GenBank accession: KT358184-KT358192), and 10 from pupae reared as ex-larvae on *W. ovata* (GenBank accession: KT358193-KT358202). Divergence among all sequences was 0%, as no variable sites were found. The nearest barcode sequence found in BOLD to the only Chilean haplotype was one "early release" of *S. bubastus* with 97.69% similarity, while the tree based identification tool of BOLD clustered the Chilean haplotype exclusively with *S. bubastus* sequences from Argentina, French Guiana and Puerto Rico.

DISCUSSION

The congruence in the wing pattern and the genitalia of the adults reared from the larvae collected on *P. nodiflora*and *W. ovata* suggests that larvae of the same hairstreak species (*S. bubastus*) feed on inflorescences of at least two plants in the Azapa Valley, and display host-associated color patterns. The only biological observation previously published on the populations of *S. bubastus* inhabiting the extremely arid environments of the Atacama Desert of northern Chile indicated that the adults visit flowers of *Alternanthera halimifolia* (Lam.) Standl. ex Pittier (Amaranthaceae) (Peña & Ugarte 1996). However, a survey of more than 60 plants of *A. halimifolia* plants yielded no larvae of *S. bubastus* in the Azapa Valley between the years 2010 and 2011, suggesting that this plant is used only as a nectar source by the adults, and not as a host by the larvae of this hairstreak.

Host plants. This is the first mention of the family Verbenaceae as a host plant for *S. bubastus*; and, even though Malvaceae already was reported as a host for this hairstreak (Beccaloni et al. 2008), this is the first record of its association with *W. ovata*. In addition, although *S. bubastus* is a polyphagous species (Beccaloni et al. 2008, Silva et al. 2011), *P. nodiflora* and *W. ovata* are the first host plants recorded for its Chilean populations, providing important information for future ecological studies or conservation projects at a local scale. The coastal valleys of the Atacama Desert of northern Chile are under strong anthropological pressures mostly associated with intensive agricultural activities that have strikingly modified the original habitats and restricted the natural vegetation to small, isolated patches (Luebert & Pliscoff 2006). The Chilean range of *W. ovata* is restricted to a few of these

coastal valleys (Muñoz-Pizarro 1966), where its presence is extremely rare, while at the same sites *P. nodiflora* is clearly more frequent. Additional field studies will be required to assess the relative importance of the two species in successfully supporting the local populations of *S. bubastus* under the environmental conditions of the Atacama Desert.

Larval polychromatism. This is the first mention of host-associated polychromatism for larvae of *S. bubastus*. The three color patterns reported here were cryptic on their respective plants. The only color pattern recorded on *P. nodiflora* provides the larva with excellent camouflage because its light green color perfectly matches that of the basal portion of the inflorescences and the leaves of the host. Thus, as penultimate and final instars eat externally with only the head introduced into the inflorescence, the thorax and abdomen are visually confused with the basal portion of the inflorescence and with the adjacent leaves. The commonest color pattern recorded on *W. ovata* is easily confused with flower buds and leaves of the host, enabling the larva to remain camouflaged when it is eating inflorescences at the flower bud stage; the less common color pattern provides the larva with camouflage when it is eating opened flowers. Monteiro (1991) reported similar variation in the larval color pattern of *Rekoa palegon* that was correlated with the age of the inflorescence on *Mikania stipulacea*. In addition, the three color patterns here detected are strikingly different from the one reported by Silva et al. (2014) for a mature larva of *S. bubastus*, which was cryptic on the inflorescences of its host, *Galactia* sp. (Fabaceae) in central Brazil. These findings suggest a great ability of the larvae of *S. bubastus* to display a variety of host-associated color patterns, in accordance with observations reported for other hairstreak species with polychromatic larvae (Monteiro 1991, Kaminski et al. 2012). As suggested by Brower (1958), patterns of host-associated polychromatism similar to that described here for *S. bubastus* break the phytophagous population into several "visual species" providing a useful strategy to avoid bird predation, because the predator bird must be able to learn each of these separate "prey images" before it can search for them.

DNA barcodes. The DNA barcode sequences reported here are the first available for the populations of *S. bubastus* of the Atacama Desert. The absence of variation among the sequences of larvae collected on the two plants indicates that all the sequences belong to the same species, in accordance with the identification based on wing pattern and genitalia morphology. The divergence (2.31%) between the Chilean haplotype and the nearest sequence deposited in BOLD is high compared to intraspecific divergences reported for other Eumaeini species (Faynel et al. 2012, Frye & Robbins 2015). Although deep COI divergences (>2%) may be indicative of unrecognized cryptic species (e.g. Landry & Hebert 2013, Huemer & Mutanen 2015), many cases of deep divergence involving clearly conspecific samples have been reported for Lepidoptera (Wiemers & Fiedler 2007, Hausmann & Huemer 2011, Hausmann et al. 2011, Huemer et al. 2014). Cases of deep divergence in Lycaenidae are mostly associated with samples from distantly located populations of widely distributed species (Wiemers & Fiedler 2007), a scenario similar to that reported here for *S. bubastus*, as the BOLD sequences are from locations extremely distant from northern Chile (Argentina, French Guiana and Puerto Rico). Additional DNA barcode sequences from populations of intermediate locations would be required to verify whether genetic variation is correlated with the geographic phenotypic variation already mentioned along the range of *S. bubastus* (Nicolay & Robbins 2005).

Further remarks. Larvae and pupae of myrmecophilous species of Lycaenidae may be associated with ants at a variable level ranging from facultative to obligate (Fiedler 1991, 1995). The life stages that interact with ants are characterized by the presence of some morphological specializations, such as the dorsal nectary organ (DNO) on the seventh abdominal segment, the pore cupola organs (PCO), tentacle organs or dendritic setae (Ballmer & Pratt 1991, Duarte et al. 2001, Duarte et al. 2005, Silva et al. 2014). The presence of DNO and PCOs was verified for the first time on the larvae of *S. bubastus* in this study regardless of the color pattern. However, attendant ants were not found in the field, suggesting that *S. bubastus* could be a facultative myrmecophilous species, at least at the local level. Ballmer & Pratt (1991) indicated that intra-specific geographic differences in myrmecophily might occur. Such geographic variation is expected in a widely distributed species such as *S. bubastus*, along whose range the ant composition and abundance can vary greatly.

Host plant ranges of the Lycaenidae of northern Chile still are only partially known. Based on the published records, Polyommatinae appear to be restricted to Fabaceae (Benyamini 1995, Vargas & Parra 2009, Vargas 2014), while Theclinae have been reared from Asteraceae (Vargas & Duarte 2014), Fabaceae (Vargas & Parra 2009), Malvaceae, and Verbenaceae (this study). Further studies would be required to reach a better understanding of the biology and evolution of the Lycaenidae of the arid

environments of the Atacama Desert and neighboring areas of the Andes.

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