Original paper

Notes on the life history of *Aguna megacles megacles* (Mabille, 1888) (Lepidoptera: HesperIIDae: Eudaminae) feeding on *Bauhinia* species in the State of Alagoas, Brazil

Notas sobre la historia de vida de *Aguna megacles megacles* (Mabille, 1888) (Lepidoptera: HesperIIDae: Eudaminae) alimentándose de especies de *Bauhinia* en el Estado de Alagoas, Brasil

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ABSTRACT. Eudaminae Mabille, 1877 (HesperIIDae), recognized as subfamily recently, is rich in Brazil. It is along the Neotropical Region where a significant part of the diversity is found, however, information that involves the biology of species is poorly yet. This paper gathers new bioecological data of an *Aguna* species from the municipality of Maceió (Alagoas, Brazil), close to the Environmental Protection Area of “Catolé” and “Fernão Velho”, a remnant of Atlantic Forest. Leaves containing eggs were collected in a peri-urban area (9° 33’ 26” S, 35° 46’ 36” W) and taken to laboratory to observe post-embryonic development. Larvae were also collected from another host plant in an intra-urban area (9° 39’ 40” S, 35° 41’ 58” W). The specimens were identified as *Aguna megacles megacles* (Mabille, 1888) and the two Fabaceae as *Bauhinia pentandra* (Bong.) D. Dietr. and *Bauhinia monandra* Kurz. from exsiccates deposited at the Herbarium. Rearing from the eggs collected on *B. pentandra* exposes a post-embryonic development that lasted 53.4 days for six larval instars individuals, and 46 days for the

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five larval instars specimen. The larvae built a shelter since the first instar and in laboratory conditions they preferred pupate on the base of the cage. This is the first report of development features for *A. m. megacles*, including six and five larval instars, as well as a new locality for the State of Alagoas, and two more host plants of *Bauhinia* suggesting specialist behavior.

Key words: biological relationship; food plants; life cycle

RESUMEN. Eudaminae Mabille, 1877 (Hesperiidae), reconocida como subfamilia recientemente, es rica en especies en Brasil. A lo largo de la Región Neotropical es donde se encuentra gran parte de la diversidad, sin embargo, información que involucra la biología de las especies es poco conocida todavía. Este trabajo reúne nuevos datos bioecológicos de una especie de *Aguna* en el municipio de Maceió (Alagoas, Brasil), cerca del Área de Protección Ambiental de "Catolé" y "Fernão Velho", un remanente de Bosque Atlántico. Las hojas que contenían huevos se colectaron en un área periurbana (9° 33' 26" S, 35° 46' 36" W) y se llevaron al laboratorio para observar el desarrollo postembrionario. También se colectaron larvas de otra planta huésped en un área intraurbana (9° 39' 40" S, 35° 41' 58" W). Los especímenes fueron identificados como *Aguna megacles megacles* (Mabille, 1888) y las dos Fabaceae como *Bauhinia pentandra* (Bong.) D. Dietr. y *Bauhinia monandra* Kurz. desecados depositados en el Herbario. La cría a partir de los huevos colectados en *B. pentandra* expone un desarrollo post-embrionario de 53.4 días para seis estadios larvales y 46 días para el espécimen de cinco estadios larvales. Las larvas construyeron un refugio desde el primer estadio y en condiciones de laboratorio la pupa prefirió la base de la jaula. Este es el primer informe de características de desarrollo para *A. m. megacles*, incluye seis y cinco estadios larvales, así como una nueva localidad para el Estado de Alagoas, y dos plantas huésped más de *Bauhinia* que sugieren un comportamiento especialista.

Palabras clave: interacción bioecológica; planta alimenticia; ciclo de vida

INTRODUCTION

It is estimated that the Lepidoptera order, represented by butterflies and moths, range over 500,000 species (Gaston, 1991; Kristensen *et al.*, 2007). Within this order, Hesperiidae Latreille, 1809, also known as skipper butterflies, comprises over 4,000 species (Warren *et al.*, 2008; Sahoo *et al.*, 2016), and nowadays there are nine recognized subfamilies (Li *et al.*, 2019). It is in the Neotropical Region, about 2,369 species, where it maintains great part of diversity of these butterflies (Duarte *et al.*, 2012).

This group is easily identified through its robust body, the thorax larger than the abdomen, and spaced antennae with a hook in the distal part (Murillo-Hiller, 2008; Triplehorn & Johnson, 2015; Murillo-Hiller *et al.*, 2019). Most larvae are well known for their shelters built with leaves from host plants, which may differ during the ontogeny as also among genera (Greeney & Jones, 2003; Greeney, 2009).

Four subfamilies are well represented in Brazil (Duarte *et al.*, 2012): Eudaminae Mabille, 1877; Pyrginae Burmeister, 1878; Heteropterinae Aurivillius, 1925; and Hesperiinae Latreille, 1809.

Eudaminae includes the genus *Aguna* Williams, 1927 which consists in 27 described species nowadays (Austin & Mielke, 1997; Siewert *et al.*, 2015).

In HesperIIDae, several species go through five instars of larval development (Klots, 1971; Tashiro & Mitchell, 1985; Valentine & Johnson, 2000; Wendt & Carvalho, 2001; Greeney & Warren, 2003; 2009a; b; 2011; Moraes *et al.*, 2012; Sharanabasappa *et al.*, 2016; Lastra-Valdés & Cañamero, 2017; Freitas, 2018), but it may have eight instars as *Elbella luteizona* (Mabille, 1877) (Pyrginae) (Lepesqueur *et al.*, 2017). In addition, its immatures use mainly Fabaceae Lindl., Piperaceae Giseke., Malvaceae Juss., and Poaceae Barnhart as host plants (Duarte *et al.*, 2012).

Despite the richness of species in the Neotropical Region, the knowledge concerning geographical distributions, bioecological features and phylogenetic relationships remains poor in comparison with others Lepidoptera families (Warren *et al.*, 2008), for instance, Nymphalidae (Wahlberg *et al.*, 2005).

Thus, studies on bioecological features become extremely essential because they are keys to understanding of biological relationship amongst the species and the environment which involves them, especially with regard to the food source knowledge; as well as bioecological information not known yet for the vast majority of species (Powers *et al.*, 2021). Therefore, knowledge about host plant associations and locality records of these interactions are important information on bioecology of insects, mainly amongst Lepidoptera (Donahue, 1993), as also for planning biological conservation (Vargas, 2012).

Through this, the aim of this paper is filling the gaps regarding the immature stages, bring new data concerning the host plants association (exotic and native), and report larval behavior features of an *Aguna* species occurring in the municipality of Maceió, State of Alagoas, close to the Environmental Protection Area of "Catolé" and "Fernão Velho", a remnant of Atlantic Forest, one of the greatest hotspots of biodiversity in Brazil (Myers *et al.*, 2000).

MATERIALS AND METHODS

On April 2019, sixty-five eggs laid on leaves of a native *Bauhinia* L. species (Fabaceae) were collected near the Instituto de Ciências Biológicas e da Saúde (ICBS) located in the *Campus* A. C. Simões at the Universidade Federal de Alagoas (UFAL) (9° 33' 26" S, 35° 46' 36" W), municipality of Maceió (Alagoas, Brazil). Also, thirty-eight larvae were also collected on October 2019 from an exotic *Bauhinia* species used on sidewalks in an urban area of Maceió far away from any remaining of Atlantic Forest (District of Ponta Verde, 9° 39' 40" S, 35° 41' 58" W).

Thereafter, they were taken to the Laboratório de Bioecologia de Insetos (LABIN). Soon after, each leaf with egg was examined under Leica EZ4 Stereo Microscope and placed singly into a small capped container (100 mL) with a piece of paper towel covering the inner base, which was changed daily and moistened, as well as the larvae collected in order to observe pupation and adult emergence. They were kept in a temperature ranging daily from 24,1 to 26,4 °C and relative humidity from 59 to 78.04 %. The freshly hatched larvae were kept into these containers until the

fourth instar, when they were transferred to the Bernadete cage (300 mL) (Lima & Carvalho, 2017). Since the eclosion until pupation, the larvae were fed with fresh leaves from the host plant, and both container and cage were clear using 70 % ethanol daily.

Afterwards, the adults were freezing sacrificed, pinned using instructions from Almeida *et al.* (1998), deposited at the collection from LABIN provisionally. Voucher specimens will be deposited at the Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia da Universidade Federal do Paraná (DZUP), Curitiba, Paraná, Brazil. Photos of these specimens were sent to Harold Francis Greeney from the Yanayacu Biological Research Station and Center for Creative Studies, in Ecuador, so as to be identified. Branches from the host plants were led to MAC Herbarium of the Instituto do Meio Ambiente do Estado de Alagoas (IMA-AL) and turned into exsiccated in order to be deposited and identified.

A geographic distribution map was prepared based on the available information from the literature — Mielke, 1971; Biezanko & Mielke, 1973; Austin & Mielke, 1997; Mielke & Casagrande, 1997; Mielke *et al.*, 2008; Núñez Bustos, 2009; Kerpel *et al.*, 2014; Pérez *et al.*, 2017; Melo *et al.*, 2019 —, plotting the points or indicating the country of origin in cases where the collection point was not indicated — Mielke, 1971; Biezanko & Mielke, 1973; Austin & Mielke, 1997; Mielke *et al.*, 2008.

RESULTS

The host plants were identified as the native *Bauhinia pentandra* (Bong.) D. Dietr. (Registration number in Herbarium as MAC 65039) and the exotic *Bauhinia monandra* Kurz. (Registration number in Herbarium as MAC 65225).

The reared hesperiid specimens were identified as *Aguna megacles megacles* (Mabille, 1888) (Hesperiidae: Eudaminae) (Fig. 1), and this is the first report of occurrence in the State of Alagoas (Fig. 2).

From the sixty-five eggs of *A. m. megacles* collected on *B. pentandra* leaves, mostly were laid singly and isolated on the adaxial surface of the leaf. They exhibited a dome-shaped and whitish colour soon after laying. Some days later, near eclosion, they turned to orange (Fig. 3a). Despite having hatched 26 larvae, only six reached the adult stage. Five larvae presented six instars and their post-embryonic development lasted 53.4 days (53.4 ± 4.40) (mean/standard deviation): the first instar lasted 3.6 ± 0.55 (Fig. 3b); the second, 3.6 ± 0.55 (Fig. 3c); third, 5.4 ± 1.14 (Fig. 3d); fourth, 5.6 ± 1.14 (Fig. 3e); fifth, 7.8 ± 0.55 (Fig. 3f); sixth, 11.4 ± 1.52 (Fig. 3g); and pupal stage, 16.2 ± 0.84 (Fig. 3h). The five-instar larva had the following durations: 3; 4; 4; 7; 11 for the last larval instar; and 17 days for the pupal instar, recording the emergency of the adult 46 days after neonate eclosion. Both developments observed in laboratory are shown in the figure 4.

Along the ontogeny, since first instar until sixth instar, all the larvae built only one type of shelter by folding the leaf without any cut, and binding it with silk. During the first and second instars, the larvae folded only a tiny part of the leaf, which was soft and easily to be manipulated,

and the last instars were characterized by folding both two parts of the leaf kept together with silk (Fig. 5). During the pupation process, the preference of all larvae was to pupate in the cage's base, some preferred to stay under the paper towel, while others pupated inside the fallen folded leaf (shelter) from the host plant over the base.

Figure 1. *Aguna megacles megacles* (Mabille, 1888) (Lepidoptera: HesperIIDae: Eudaminae): dorsal view on the left; ventral view on the right.

DISCUSSION

Bauhinia pentandra is a native shrub which occurs in Amazon Rainforest (Silva *et al.*, 1989), Caatinga, as also in vegetation transition zones like Caatinga-Cerrado (Agra *et al.*, 2008). *Bauhinia monandra*, known as orchid tree, is a small tree native from Asia (Connor, 2002), and has the following synonyms (Schmitz, 1973; Fern, 2014): *Bauhinia kappleri* Sagot, *Bauhinia krugii* Urb., *Bauhinia richardiana* Voight, *Caspareopsis monandra* (Kurz.) Britton & Rose, *Bauhinia porosa* Baill., *Bauhinia punctiflora* Baker. Both species are used in traditional medicine (Agra *et al.*, 2008).

Figure 2. Distribution of *Aguna megacles megacles* (Mabille, 1888) (Hesperiidae: Eudaminae).

The genus *Aguna* was revised by Austin & Mielke (1997) being arranged into ten species-groups as well as raising the number of new described species. Austin & Mielke (1997) and Biezanko & Mielke (1973) comment that the species *Aguna megacles* was wrongly spelled as "*megaeles*" in the original description, by Mabille, who soon after corrected his own mistake. This

subspecies, *A. megacles megacles*, occurs in other South America countries (Fig. 2) as Argentina (Mielke, 1971; Austin & Mielke, 1997; Núñez Bustos, 2009), Bolivia, Paraguay, and Uruguay (Mielke, 1971; Austin & Mielke, 1997), while *Aguna megacles malia* Evans, 1952 is only known in Venezuela (Mielke, 1971; Austin & Mielke, 1997). *Aguna megacles megacles* has a wide distribution in Brazil along the five regions (North; Northeast; Center-West; Southeast and South) in the following states: Rondônia, Maranhão, Piauí (Austin & Mielke, 1997); Paraíba (Austin & Mielke, 1997; Kerpel *et al.*, 2014); Pernambuco (Melo *et al.*, 2019); Bahia (Austin & Mielke, 1997); Tocantins, Goiás (Austin & Mielke, 1997); Mato Grosso (Mielke, 1971; Austin & Mielke, 1997); Distrito Federal (Mielke *et al.*, 2008); Mato Grosso do Sul (Mielke, 1971); Minas Gerais, Espírito Santo, Rio de Janeiro (Mielke, 1971; Austin & Mielke, 1997); São Paulo (Austin & Mielke, 1997; Mielke & Casagrande, 1997); Paraná (Austin & Mielke, 1997; Pérez *et al.*, 2017); Santa Catarina (Austin & Mielke, 1997); Rio Grande do Sul (Mielke, 1971; Biezanko & Mielke, 1973; Austin & Mielke, 1997).

Aguna megacles megacles had already been recorded feeding on *Bauhinia* species, for instance: *B. candicans* Benth. (Biezanko *et al.*, 1974); *B. forficata* Link. (Biezanko & Mielke, 1973). Moreover, there is information that confirm others *Bauhinia* species being host plant to seven *Aguna* species: *Aguna albistria albistria* (Plötz, 1880) — *Bauhinia pulchella* Benth. (Diniz *et al.*, 2001) —; *Aguna asander* (Hewitson, 1867) — *Bauhinia aculeata* L. (Janzen *et al.*, 1998), *Bauhinia monandra* (Janzen, 2004), *Bauhinia unguolata* L. (Janzen & Hallwachs, 2009) —; *Aguna aurunce* (Hewitson, 1867) — *Shnella glabra* (Jacq.) [syn. *Bauhinia glabra* Jacq.] (Janzen & Hallwachs, 2009) —; *Aguna claxon* Evans, 1952 — *Bauhinia cookii* Rose (Janzen & Hallwachs, 2009) —; *Aguna coeloides* Austin & Mielke, 1998 — *Bauhinia unguolata*, *Schnella guianensis* (Aubl.) [syn. *Bauhinia guianensis* Aubl.] (Janzen & Hallwachs, 2009) —; *Aguna metophis* (Latreille, [1824]) — *Bauhinia cookii*, *Bauhinia unguolata* (Janzen & Hallwachs, 2009) —; *Aguna panama* Austin & Mielke, 1998 — *Schnella glabra* (Janzen & Hallwachs, 2009). These evidences bring a possibility that the immatures of *A. m. megacles* are specialist herbivores since all available data implies in some *Bauhinia* species being host plant to them as also to other species of *Aguna*.

Over four other hesperiid species present six instars during all larval development (Heitzman, 1965; 1966; Emmel & Emmel, 1990; Cock, 2010). A similar post-embryonic development occurs in *Urbanus acawoios* (Williams, 1926) (Eudaminae) when the specimens need five instars to complete its development (Trevisan *et al.*, 2004; Wendt & Carvalho, 2006), but few larvae may need six instars (Pinto & Carvalho, 2001).

Concerning the shelter behavior, Greeney (2009) observed an *Aguna* species feeding on *Bauhinia* sp. and reported this behavior during the final instars of larval development. This behavior represents a good way to protect themselves against several natural enemies due to the fact that most of these hesperiid larvae waste their time inside these shelters when they are not feeding (Janzen *et al.*, 1998), remaining less vulnerable to parasitoids and predators. For the researchers this information, when well observed, is extremely valuable to be used as phylogenetic characters (Greeney, 2009), as also to gather more knowledge regarding behavioral concerns of several species.

Figure 3. Immature stages of *Aguna megacles megacles* (Mabille, 1888) (Hesperiidae: Eudaminae) on *Bauhinia pentandra* (Bong.) D. Dietr. (Fabaceae): **a)** Egg laid on the adaxial surface; **b)** First instar; **c)** Second instar; **d)** Third instar; **e)** Fourth instar; **f)** Fifth instar; **g)** Sixth instar; **h)** Pupa.

Figure 4. Histogram showing the two post-embryonic developments of *Aguna megacles megacles* (Mabille, 1888) (Hesperiidae: Eudaminae) feeding on *Bauhinia pentandra* (Bong.) D. Dietr. (Fabaceae).

Figure 5. Shelter built by the larva of *Aguna megacles megacles* (Mabille, 1888) (Hesperiidae: Eudaminae) using the leaf of *Bauhinia pentandra* (Bong.) D. Dietr. (Fabaceae).

The behavior related to the pupation process noticed in the laboratory may be an evidence that larvae of *A. m. megacles* pupate inside the leaves from the plant next to the soil, or in the plant litter next to the host plant. When Dam & Wilde (1977) examined the biology of *Urbanus proteus* Linnaeus, 1758, most of the larvae chose pupate in the base of the container under dead leaves. In the case of *Urbanus esmeraldus* (Butler, 1877), the larvae used to leave the host plant and pupate either in soil or in the plant litter (Moraes *et al.*, 2012).

CONCLUSIONS

This is the first record of *A. m. megacles* in the State of Alagoas, Northeast of Brazil, also its immature stages with some aspects of their behavior. Moreover, its host plant range is now expanded by presenting *B. pentandra* and *B. monandra* as two other *Bauhinia* sp., reinforcing the specialized feeding condition for this hesperiid species and many other species of this genus.

Although *B. monandra* is an exotic species, being a feeding plant of a native species as *A. m. megacles* seems to be an important indicator of its adaptive strategies for survival in intra-urban environments. This association seems to contribute to the preservation of native insects in response to the habit anthropogenic alterations.

Regarding larval development, the predominance of six larval instars (86 % of individuals) differs from patterns observed in other species in which extra numerary instars are rarer. These laboratory studies indicate that further examinations are required, especially field observation aiming to confirm these findings. Besides, this paper supports future works to study species which still remain their biology unknown with the purpose to increase more knowledge.

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