LRF JRL
The Journal of Research on the Lepidoptera
Volume 46: 67-74
THE LEPIDOPTERA RESEARCH FOUNDATION, 28 October 2013
ISSN 0022-4324 (print)
ISSN 2156-5457 (online)

Notes on the morphology and larval development of *Methona themisto* (Hübner, 1818) (Lepidoptera: Nymphalidae: Ithomiini) from southeastern Brazil

Karlla V. C. Barbosa[1] and Thiago V. V. Costa[1,2]
[1]Rua Dr. Saul de Camargo Neves, 163, Vila Constância, CEP 03755-100, São Paulo, SP, Brazil.
[2]Museu de Zoologia, Universidade de São Paulo (MZUSP), Avenida Nazaré 481, Ipiranga, CEP 04218-970, São Paulo, SP, Brazil.
barbosa.karlla@gmail.com, tvvcosta@usp.br

Abstract. Little has been published on the natural history, immature stages and development of the Neotropical butterfly genus *Methona* Doubleday, 1847. In spite of being relatively common where it occurs, many aspects of the morphology and development of *Methona themisto* (Hübner, 1818) still are poorly known. Here we describe the morphology and duration of the egg, larval and pupal stages of *Methona themisto* in Southeastern Brazil. A single plant of *Brunfelsia uniflora* (Solanaceae) was chosen by the butterflies to lay their eggs, and larvae and pupae were reared on it. Adults were observed laying single, white eggs mostly on the abaxial surface of fresh leaves. All larval instars were black with 12 yellow bands, except for the first few days after molting, when they were dark brown with green heads and anal plates. The pupa was pendant, with a mean length of 2.24 cm. It was pale yellow with black stripes and the body was slightly bent where the abdomen joined the wing-pad apices. Mean duration of the pupal stage was approximately 14 d. We did not observe any relationship between pupal duration and weather conditions or the time of year.

Key words: *Methona*, larvae, pupa, butterfly, Nymphalidae, Ithomiini, *Brunfelsia uniflora*, Solanaceae

Introduction

The Neotropical genus *Methona* Doubleday, 1847 comprises seven species of medium-sized (wingspan about 8 cm), colorful nymphalid butterflies distributed from Costa Rica in southern Central America throughout most of South America, reaching their southernmost limit in southern Brazil, Argentina and Uruguay (Mielke & Brown, 1979; Hill & Tipan, 2008). Despite often being common where they occur, some *Methona* species are relatively poorly known. Available information on larval development of the genus is based mainly on studies of *M. confusa* Butler, 1873, *M. curvifascia* Weymer, 1883, and *M. themisto* (Hübner, 1818) (Brown, 1987; Drummond, 1976; 1986; Drummond & Brown, 1987; Willmott & Freitas, 2006; Hill & Tipan, 2008). *Methona themisto* is a relatively common butterfly, occurring in subtropical latitudes of South America and inhabiting forest edges, open areas and gardens in urban regions. The species is known mainly due to the habits of its larvae, which eat exclusively the leaves of species of the genus *Brunfelsia* (Pohl), a very common ornamental representative of Solanaceae locally known as 'Manacá' (Biezanko, 1960; Drummond, 1976; 1986; Brown, 1987; Drummond & Brown, 1987; Brown, 1992). Thus, larvae of *M. themisto* are considered pests because they may cause much damage and even total defoliation of single plants (Figueiredo-Junior, 1939; Silva *et al.*, 1968; Ruszczyk & Nascimento, 1999).

The life cycle of the species has been studied by Berg (1886), Lordello (1951), Brown & Freitas (1994) and Willmott & Freitas (2006). However, its immature stages and larval development still are poorly documented and none of these works presents a detailed morphological description of the eggs, larvae and pupae, neither makes comparisons between *M. themisto* and the other species of the genus. Here we describe details of egg morphology, and morphology and duration of the larval instars and the pupa of *Methona themisto* in southeastern Brazil, and provide notes on its natural history.

Received: 1 July 2013
Accepted: 5 October 2013

Materials and methods

We made all observations from July 2012 to April 2013, in the municipality of São Paulo (23°30'32"S, 46°29'57"W), southeastern Brazil. During the first phase, between July and September 2012, we studied the 5th instar and pupa of *M. themisto* found on a single plant of *Brunfelsia uniflora* (Pohl) (Solanaceae) located in an urban garden, to record morphological aspects and natural duration of these stages. The larvae were observed directly on the plant, until they matured and ceased feeding, and wandered off in search of pupation sites. At this time they were transferred to a plastic observation box protected from potentially damaging rain and wind, where plant branches and leaves previously cut off were included. In the second phase, from November 2012 to April 2013, we observed the plant carefully for signs of oviposition, noted the morphology and duration of the egg stage and the 1st, 2nd and 5th instars, and as well made observations of larvae that we transferred to and reared in plastic boxes. These boxes were maintained under the same conditions and were cleaned and had leaves replaced daily. Observations were recorded daily and head capsules and exuviae were collected. Over the whole study, we examined, photographed or videotaped five oviposition events, 12 eggs, four 1st instars, two 2nd instars, 61 5th instar and 61 pupae. All measurements are given as mean ± 1 SD. To record images we used a Sony HX9 Digital Camera and a GoPro Hero 2 Video Camera (all videos and photos are available from the authors on request).

Results

Brunfelsia uniflora (Pohl) (Solanaceae), known locally as 'Manacá', was used as host plant by the larvae of *M. themisto*. All eggs and larvae were found on a single plant individual.

Oviposition. Oviposition (n=5, Fig. 6C) was observed between 11am and 1pm on sunny days, when the adult was most active and flying around the host plant. During oviposition, the female perched on the leaf, curved her abdomen below the leaf and laid the eggs mainly on the opposite side of the leaf. On a few occasions we observed the female ovipositing on the same leaf surface as where it was perched, i.e., the upper, adaxial surface. The process of oviposition lasted no more than six seconds from perching, laying and departing for another leaf. The female laid a single egg on each leaf. On a few occasions more than one egg was recorded on a single leaf.

Egg. Mean diameter 0.93 ± 0.2 mm (n = 12), height 1.40 ± 0.10 mm (Fig. 1). The upright egg is of ovoid shape, the chorion ornamented with parallel ribs and numerous regular dips in between. It is white and glistening, gradually darkening to buff, particularly after the 4th day. A few (2–4) days before hatching eggs became translucent, with the embryo visible inside as a dark spot (Fig. 1D).

Larva. First instar (n=4, length: 9 ± 2 mm). Head round, pale green just after hatching, became black at the second day. Body roughly round in cross section, dark green with 12 pale yellow thin bands on the first day, turning brown with yellow bands on the second day, and black with yellow bands from third day onwards. Anal plate pale green at the first day, became brown at the second day. Thoracic legs, prolegs and crochets pale brown at first day, became dark brown at the second day (Figs. 2A, B & C).

Second instar. As described for first instar, but black with thin yellow bands (n=2, Figs. 2D, E & F). Head rounded, green on the first day, becoming black the following day. Anal plate pale green on the first day, becoming brown the second day. Thoracic legs and prolegs green on first day, becoming brownish green in the second day, and black from third day onwards.

Fifth instar. Head capsule width: 4 ± 1 mm (n=4), body length just before pupation: 37.4 ± 0.8 mm (n=61). Like most of previous instars, the body was black, with thin, pale orange bands (Fig. 3). Anal plate, thoracic legs and prolegs black, crochets grey. Within a few hours before pupation, the stripes became paler (Fig. 3F). Feeding damage by all instars consisted of circular or ovoid perforations, either on the margin or in the middle of the leave blades (Fig. 3D).

Pupa. The molt to the pupa takes place quickly, roughly 6 seconds from splitting of the larval cuticle to the process of compressing it towards the cremaster when the exuvia is discarded. Pupa (Figs. 4 & 5) pendant, slightly bent where the wing pad apices meet the abdomen, mean length 22.4 ± 3 mm (n = 8). Fresh pupa entirely pale yellow with black cremaster (Fig. 5A). After about 30 min a few black stripes appear on the last segment before the cremaster, and after roughly 120 min black stripes and rows of black dots appear throughout the pupa (Figs. 5B & C). The pupa has two dorsal longitudinal rows of elongate spots, which are larger and more rounded closer to the cremaster. The extent of dark markings was variable among individuals, some having heavier markings than others (Figs. 5E & F for comparison). The pupa became brown roughly three days before adult eclosion, and darkened further 24 h before eclosion, with its dorsal part slightly transparent (Fig. 5G). After adult eclosion, pupal exuviae were totally translucent except for the black markings described above (Fig. 5H).

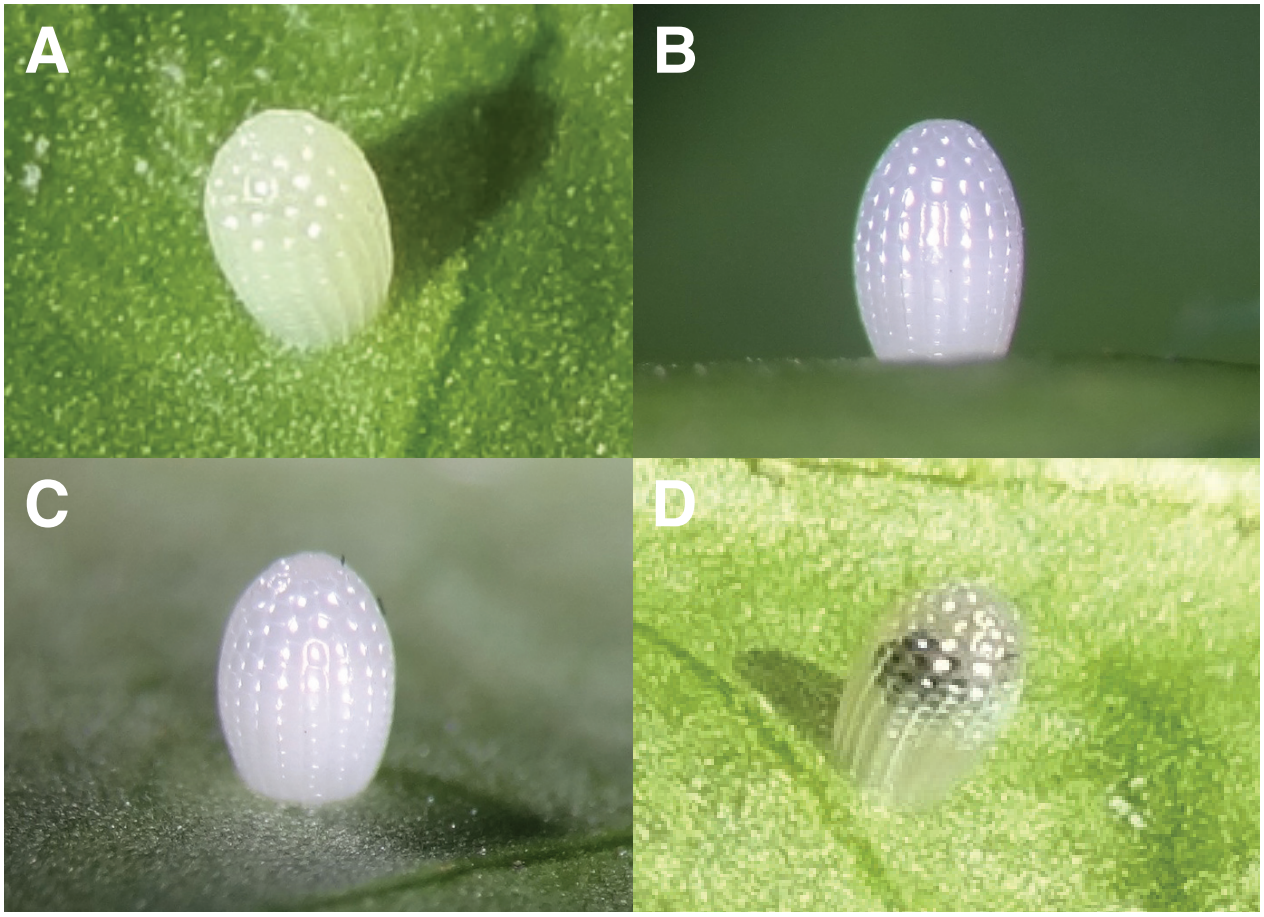

Figure 1. Eggs of *Methona themisto*. **A**. Just after oviposition. **B** & **C**. six days after oviposition. **D**. Shortly before hatching, blackish head capsule of larva discernible.

Mean duration of the pupal stage was 14.21 d (range: 9–23 d). Metamorphosis was not observed in 14 of the 61 individuals, but morphology was examined for all 61. Of the remaining 47 individuals, 13 (*ca* 20%) did not complete metamorphosis due to unknown reasons. In most of these cases (n=10), a small, hollow area (diameter 0.5 mm) appeared in the pupa, with internal liquid suspended from the inside, suggesting either predation by some arthropod, albeit no predation event was observed directly, or, more likely, that the pupa died for some reason and the internal tissue was broken down by bacteria. A single mature larva, already attached to a small twig and ready to pupate, detached and fell to the floor of its box. There it remained motionless for three days, as if dead. Surprisingly, it then transformed to a pupa, although not hanging from any branch and lying in direct contact with the sandy floor of the box. Morphology and color of this pupa was the same as the other individuals. After 14 days as a pupa, the imago eclosed, but with seriously deformed wings. After a day of trying to walk around the container, moving its wings and trying to fly, this individual took short flights and left the box, but was unable to perform normal flight.

Discussion

Although studies on Neotropical Lepidoptera have increased during the last few decades, for the majority of butterflies of the region still basic information on their life-cycles is lacking (Vane-Wright & Ackery, 1989; Moraes *et al.*, 2012). Despite being common where it occurs, this also applies to *Methona themisto*. The species is known mainly due to the habits of its larvae, which eat exclusively the leaves of *Brunfelsia uniflora* (Pohl), a very common

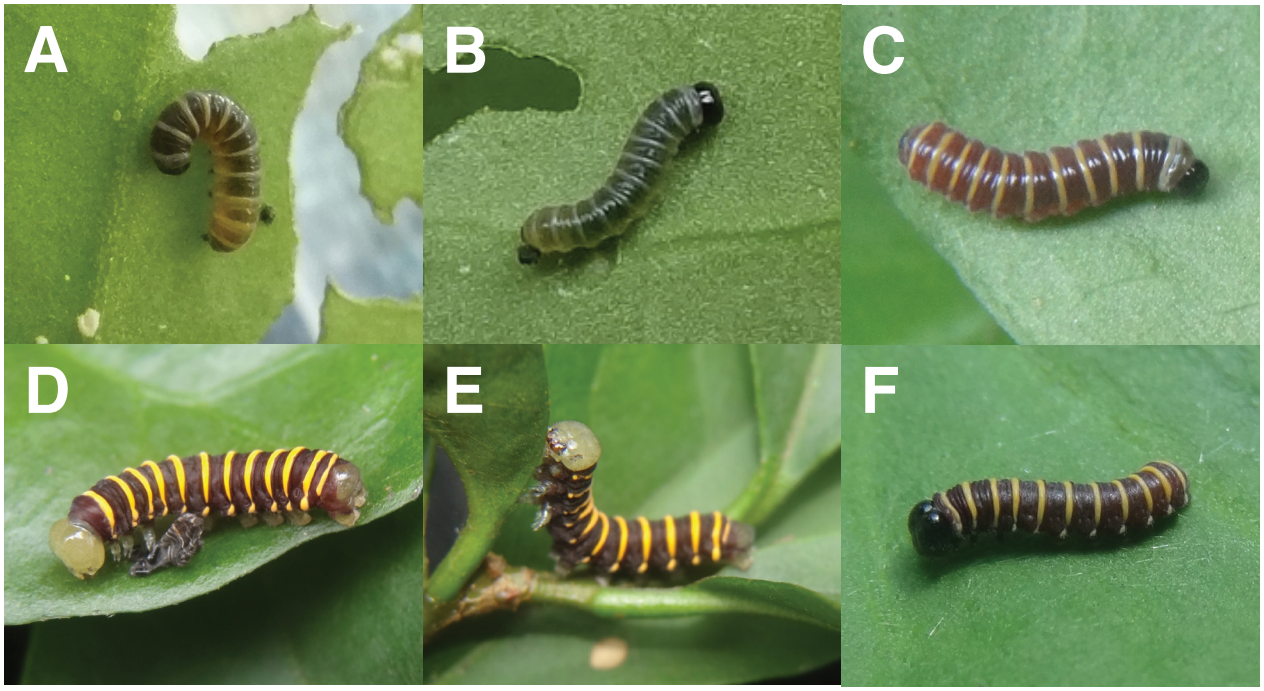

Figure 2. Larvae of *Methona themisto*. **A**. First day of first instar. **B**. Second day of first instar. **C**. Third day of first instar. **D** and **E**. First day of second instar. **F**. Second day of second instar.

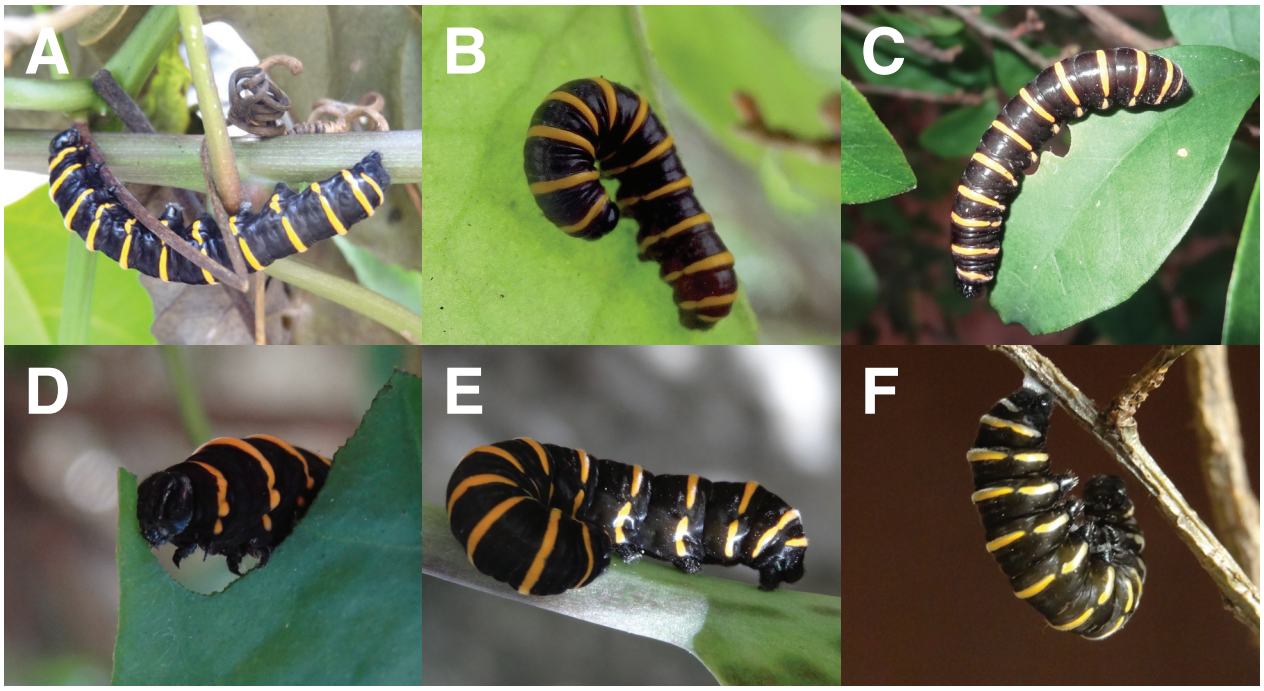

Figure 3. Fifth instar of *Methona themisto*. **D**: larva while feeding; **F**: just after fastening for pupation.

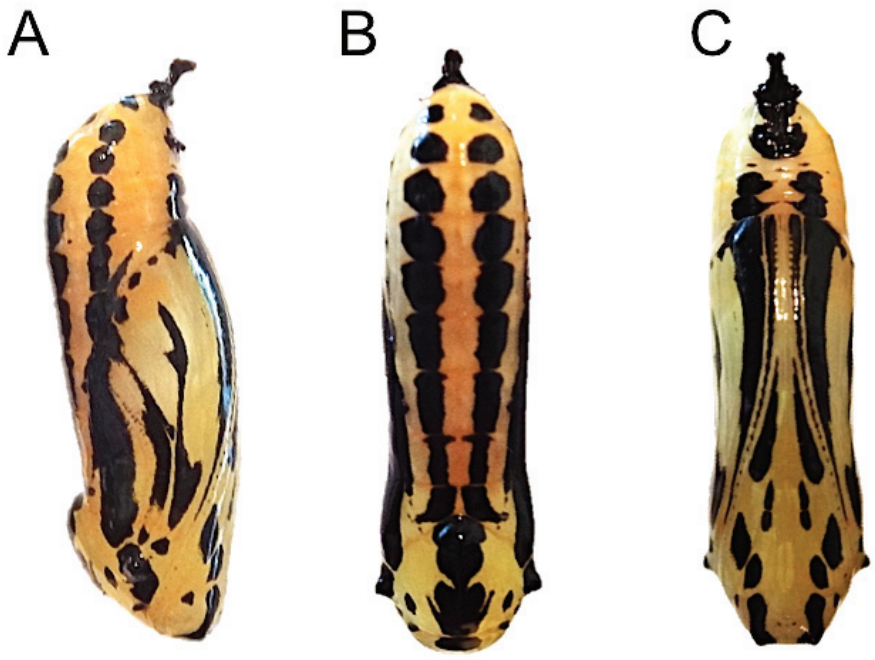

Figure 4. Pupa of *Methona themisto*. **A**. Lateral view. **B**. Dorsal view. **C**. Ventral view.

ornamental Solanaceae plant (Biezanko, 1960; Brown, 1992). The larvae of *M. confusa* and *M. curvifascia* are also known to feed on another species of that genus, *B. grandiflora schultesii* Plowman, and both these *Methona* species can even be found together on a single plant (Hill & Tipan, 2008).

The leaves of the larval host plant where eggs and larvae were found varied greatly in size and age. Eggs were observed mostly on the abaxial side of the leaves, even though a few eggs were recorded on the adaxial surface. Only single eggs were encountered on the host plants and observed during oviposition. We found no evidence of eggs laid in clusters by *M. themisto*. In just one case we recorded two eggs on the same leaf, however they were a few centimeters apart. Most species of Ithomiini are known to lay single eggs, although a few have been reported as cluster-laying, such as species in the genera *Mechanitis*, *Hypothyris*, *Episcada*, *Ithomia*, *Pteronymia* and *Methona* (Hill & Tipan, 2008). However, intraspecific variation in oviposition behavior has been reported for some ithomiine species, *e.g., Mechanitis menapis* and *Hypothyris euclea* are known to lay eggs in clusters in Costa Rica, but single eggs in Ecuador (Gilbert, 1969; Drummond, 1976; Hill & Tipan, 2008). Among the species of *Methona*, only *M. confusa* is known to lay eggs in clusters in some parts of its range (Hill & Tipan, 2008), but a record of a solitary larva from Venezuela suggests that it may also lay single eggs (Brown, 1987; Hill & Tipan, 2008). It would be interesting to examine whether the single-egg pattern is consistent throughout the entire range of *M. themisto* or whether it varies according to hostplant quality, geographical region or season.

Concerning larval development, our observations provide details on the morphology of many stages in the development of *M. themisto*, mainly concerning the colors and patterns of larval head, segments, legs and prolegs, as well as on the pupae. Most instars of *M. themisto* are quite similar in morphology to those of congeneric species, such as *M. confusa* and *M. curvifascia* (Hill & Tipan, 2008). Nevertheless, the larvae of *M. themisto* are easily distinguished from those of *M. confusa* and *M. curvifascia* mainly by the color and width of the yellow bands, which are narrower and darker, tending to orange, in the former, and paler and wider in *M. confusa* and *M. curvifascia*, whose larvae are very similar to each other (see Figs. 2 and 3 in Hill & Tipan, 2008). Regarding the first instar, the color of head capsule, thoracic legs and prolegs is similar between *M.*

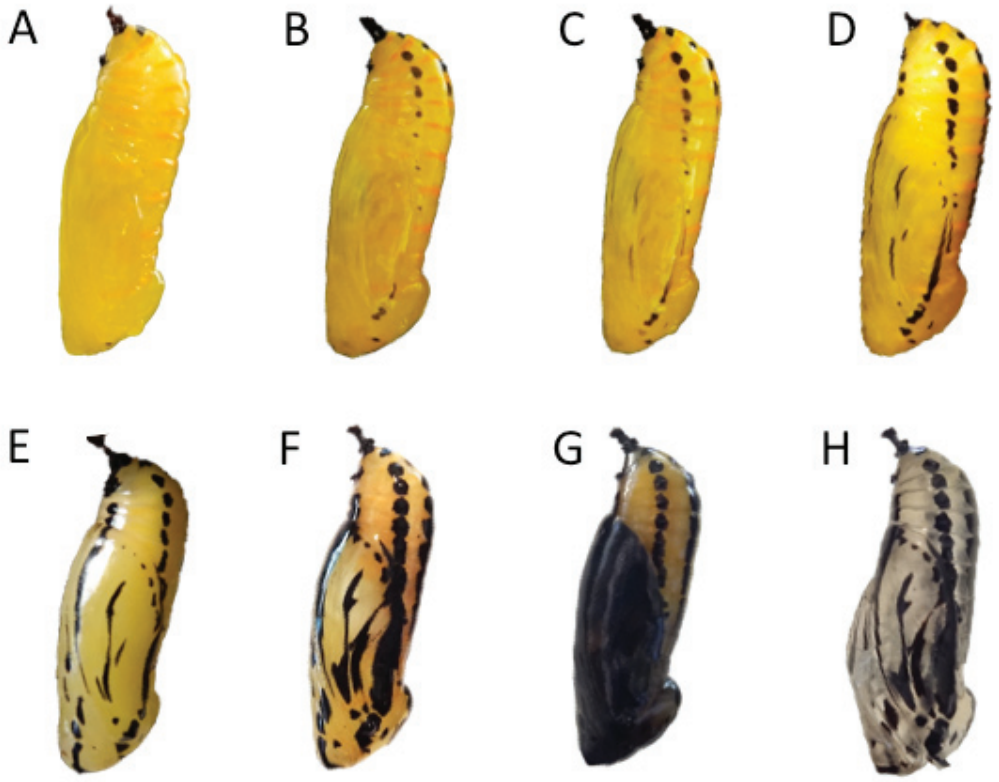

Figure 5. Lateral view of pupa of *Methona themisto*. **A**. Just after pupation. **B**. 30 min after pupation. **C**. 60 min after pupation. **D**. 120 min after pupation. **E**. 24 h after pupation. **F**. Three days after pupation, showing an extremely heavily marked pattern of spots and stripes. **G**. 24 h before eclosion. **H**. pupal exuvia.

themisto observed here and *M. confusa* and *M. curvifascia*. However, the color of the head and anal plate in the first day of *M. themisto* was pale green, instead black as reported in first hatched larvae of *M. confusa* and *M. curvifascia* (Hill & Tipan, 2008). Furthermore, the anal plate, thoracic legs, prolegs and crochets in the first instar of *M. themisto* were dark brown, not black as reported for the other two *Methona* species. The same was observed in the second instar, which had green head and anal plate on the first day and became black and dark brown, respectively, in the second day. The fifth instar is very similar in morphology among *M. themisto*, *M. confusa* and *M. curvifascia*. On the other hand, larvae of *M. megisto* are strikingly distinct from the remaining *Methona* species by the large width of the yellow bands in their lateral portion, just above the thoracic legs and prolegs (see Willmott & Freitas, 2006).

The larvae of *M. themisto* have 12 transverse yellow bands, including one on segment A9, which is also present in *M. megisto* and *M. confusa* (Brown, 1987; Brown & Freitas, 1994, Hill & Tipan, 2008). This pattern is considered a synapomorphy of the genus by Willmott & Freitas (2006). However, *M. curvifascia* lacks the band on A9, presenting 11 bands in total (Hill & Tipan, 2008), indicating that this character varies within the genus. Thus, from a systematic view, either the lack of a transverse band on A9 can be a plesiomorphic condition in that species, and the evolution of an extra-band occurred after the divergence of that species from the rest of the group. Or, the presence of the band on A9 in the ancestor was followed by a secondary loss in *M. curvifascia*. A molecular study places *M. curvifascia* as the basal *Methona* species (Hill & Tipan, 2008), what may indicate that the former hypothesis is more likely, *i.e.*, the lack of an extra-band as a plesiomorphic character in *Methona*. From the same perspective, assuming *M. curvifascia* as the most basal *Methona* species, the width and color of the bands, which are extremely similar in *M. confusa* and *M. curvifascia*, are likely to be plesiomorphic in these two species.

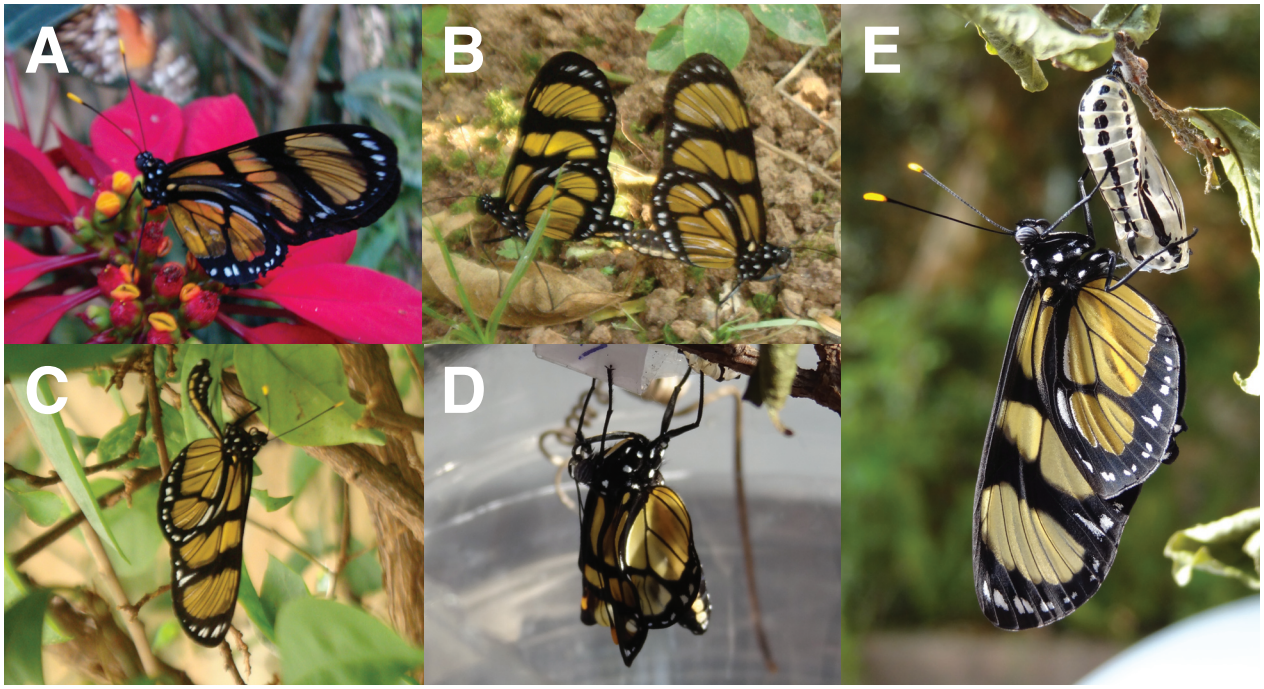

Figure 6. Imagos of *Methona themisto*. **A.** Foraging. **B.** Copulating. **C.** Ovipositing, egg being laid on the adaxial (upper) face of the leaf. **D.** Adult just after eclosion from the pupa. **E.** Adult 2 h after eclosion.

The traits observed here in *M. themisto* are highly congruent with the following characters of *Methona* larvae listed in Willmott & Freitas (2006), with their character states indicated in parentheses: 22(1) absence of subdorsal filaments in the last instar; 49(1) presence of a conspicuous colored ring on A9; 54(0) a straight pupa; 55(1) presence of a pronounced curve on the dorsal edge of abdomen in posterior half to cremaster; 56(0) a slightly indented dorsal edge of abdomen at thorax-abdomen suture; and 59(0) smooth dorsal edge of abdomen at third abdominal segment. Nevertheless, Willmott & Freitas (2006) reported that *M. megisto* and *M. themisto* lay eggs preferentially at the border of leaves, listing this as a synapomorphy for the genus (p. 26, character 9:1). Even though we observed eggs being laid by *M. themisto* in the border of some leaves, oviposition location did not seem confined to leaf edges, but instead it appeared to occur at random on the leaf surface. In the same way, *M. curvifascia* does not seem to lay eggs preferentially near the leaf border, and *M. confusa* lays eggs in clusters covering a large portion of the leaf (Hill & Tipan, 2008). Thus, the existence of different patterns of oviposition and their systematic importance must be re-evaluated within the genus *Methona*.

Extensive intraspecific variation was observed in the shade and extent of the black markings on the pupae of *M. themisto*. The extent of the black markings in this species appears to be more similar to *M. confusa* than to *M. curvifascia*, which has smaller black stripes and spots (Hill & Tipan, 2008). The range of variation in the black markings in the pupae of *M. themisto* was comparable to, or even larger than reported for *M. confusa*. It needs to be evaluated if the shape and extent of these markings can be useful for taxonomic studies. Duration of the pupal stage varied from 9 to 23 d, with a mean of 14.21 d, comparable to that reported for *M. confusa* and *M. curvifascia*, in which the length of the pupal stage varies from 11 to 12 days (Hill & Tipan, 2008).

The data presented here, especially concerning the morphology and other detailed aspects of the larval development of *M. themisto*, contribute to fill some gaps on the knowledge of the genus *Methona*. The intraspecific variation in many aspects of the species' development could be a fruitful area for investigation, and this study underscores the importance of continuing research that focuses on the immature stages in *Methona*, as well as other ithomiine species, in order to better understand their biology, systematics, and conservation.

Acknowledgements

We thank the staff of the Museu de Zoologia da Universidade de São Paulo (MZUSP), especially Prof. Dr. Marcelo Duarte, for permitting us to visit the collection under their care. We also are very grateful to Dr. Keith R. Willmott, Dr. Annette Aiello and Prof. Dr. Konrad Fiedler for their helpful comments on the submitted manuscript.

Literature cited

Berg, F. W. K. 1886. Observaciones sobre los estados preparatorios de algunos lepidópteros argentinos. Anales de la Sociedad Científica Argentina 21(6): 277–281.

Biezanko, C. M. 1960. Satyridae, Morphidae et Brassolidae da Zona Sueste do Rio Grande do Sul. Arquivos de Entomologia Série A (3–4): 1–13.

Brown, K. S., jr. 1987. Chemistry at the Solanaceae/Ithomiinae interface. Annals of the Missouri Botanical Garden 74: 359–397.

Brown, K. S., jr. 1992. Borboletas da Serra do Japi: diversidade, hábitats, recursos alimentares e variação temporal, pp. 142–186. *In*: Morellato, L.P.C. (ed.). História Natural da Serra do Japi: Ecologia e Preservação de uma Área Floretal no Sudeste do Brasil. Universidade Estadual de Campinas, Campinas, Brasil.

Brown, K. S., jr. & A. V. L. Freitas. 1994. Juvenile stages of Ithomiinae: overview and systematics (Lepidoptera: Nymphalidae). Tropical Lepidoptera 5(1): 9–20.

Drummond, B. A. 1976. Comparative ecology and mimetic relationships of Ithomiinae butterflies in eastern Ecuador. PhD thesis, University of Florida, 361 pp.

Drummond, B. A. 1986. Coevolution of ithomiine butterflies and solanaceous plants, pp. 307-327. *In*: D'Arcy, W.G. (ed.), Solanaceae: Biology and systematics. New York, Columbia University Press.

Drummond, B. A. & K. S. Brown, jr. 1987. Ithomiinae (Lepidoptera: Nymphalidae): summary of known larval foodplants. Annals of the Missouri Botanical Garden 74: 341–358.

Figueiredo-Júnior, E. R. 1939. Notas sobre *Thyridia themisto* Huebn., 1823, praga do manacá. Boletim Biológico de São Paulo 4(3): 512–515.

Gilbert, L. E. 1969. Some aspects of the ecology and community structure of ithomid butterflies in Costa Rica, pp. 61-92. *In*: Organization for Tropical Studies Report, Organization for Tropical Studies.

Hill, R. I. & L. A. Tipan. 2008. Description of the immature stages of *Methona confusa* Butler, 1873 and *Methona curvifascia* Weymer, 1883 (Nymphalidae, Ithomiinae) from Eastern Ecuador. Journal of the Lepidopterists' Society 62(2): 89–98.

Lordello, L. G. E. 1951. Contribuição ao conhecimento de alguns Ithomiidae brasileiros, predadores de Solanáceas (Lepidoptera, Rhopalocera). Anais da Escola Superior de Agricultura Luiz de Queiroz 8: 99–100.

Mielke, O. H. H. & K. S. Brown, jr. 1979. Suplemento ao Catálogo dos Ithomiidae Americanos de R. Ferreira d'Almeida (Lepidoptera) (Nymphalidae: Ithomiinae). Curitiba, Universidade Federal do Paraná.

Moraes, A. R., H. F. Greeney, P. S. Oliveira, E. P. Barbosa & A. V. L. Freitas. 2012. Morphology and behavior of the early stages of the skipper, *Urbanus esmeraldus*, on *Urera baccifera*, an ant-visited host plant. Journal of Insect Science 52:1–18.

Ruszczyk, A. & E. S. Nascimento. 1999. Biologia dos adultos de *Methona themisto* (Hübner, 1818) (Lepidoptera, Nymphalidae, Ithomiinae) em praças públicas de Uberlândia, Minas Gerais, Brasil. Revista Brasileira de Biologia 59(4): 577–583.

Silva, A. G. A., C. R. Gonçalves, D. M. Galvão, A. J. L. Gonçalves, J. Gomes, M. N. Silva & L. Simoni. 1968. Quarto catálogo de insetos que vivem em plantas do Brasil: seus parasitos e predadores. Rio de Janeiro, Ministério da Agricultura e Laboratório Central de Patologia, 622 pp.

Vane-Wright, R. I. & P. R. Ackery. 1989. The biology of butterflies. New Jersey, Princeton University Press, XIII + 429 pp.

Willmott, K. R. & A. V. L. Freitas. 2006. Higher-level phylogeny of the Ithomiinae (Lepidoptera: Nymphalidae): classification, patterns of larval hostplant colonisation and diversification. Cladistics 22: 297–368.